Evaluation Of Tasco® As A Candidate Prebiotic In Broiler Chickens

by

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Abstract

Tasco® made of sun dried brown seaweed (*Ascophyllum nodosum)* by Acadian Seaplants Ltd., has displayed prebiotic like properties with ruminants and may be an alternative to antibiotic growth promoters. Tasco® was fed to male broiler chickens for 35 days in a series of three trials which compared Tasco® to the prebiotic inulin and an antibiotic and determined Tasco®'s optimal inclusion level for broilers. Trials investigated Tasco® fed at 2.0% for 14 days only and examined its effects in a 45 day trial and when subjected to microbial challenge. Tasco® enhanced growth comparatively to inulin and the antibiotic virginiamycin. Alteration of physiological variables in all three trials supported the possibility of microflora changes in the gut as a mode of action. Low levels of Tasco® (0.25% and 0.5%) were consistently effective at improving growth. Microbiological profiles, currently under way, will aid in final determination of Tasco®'s qualifications as a prebiotic.

List of Abbreviations and Symbols Used

Antibiotic Growth Promoter……..……..AGP

Ascophyllum nodosum……...ANOD

Ascophyllum nodosum extract…….……..….ANE

Body Weight…….……..BW

Body Weight Gains……..……BWG

Brilliant Green……...……..BG

Carbohydrate…….....CHO

Degree of Polymerization……...…….DP

Feed Intake…….……...FI

Fructooligosaccharides……..FOS

Fucose Containing Polysaccharide……FCP

Gastrointestinal Tract…….……GIT

Gut Associated Lymphoid Tissue…….…….GALT

Glucooligosaccharides…….…….GOS

Immunoglobulin……..……...Ig

Isomaltooligosaccharides……..IMO

Lipopolysaccharides……...LPS

Lysine Iron Agar…….....…...LIA

Mannosoligosaccharides…….……..MOS

Microbial Associated Molecular Patterns……...MAMPs

Most Probable Number……..…MPN

Pattern Recognition Receptors…….…...PRRS

Registered Trademark ……..…….®

Rappaport-Vassiliadis Soya……..……RVS

Short Chain Fatty Acids ……..……...SCFA

Surface Area……...…….….SA

Tetrathionate……..……..….TT

Transgalactooligosaccharides……..……..….TOS

Volatile Fatty Acids……...……...…VFA

Xylooligosaccharides……..……...…….XOS

Xylose Lactose Tergitol™ 4……...……...….XLT4

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Chapter 1. Introduction

In the past, antibiotics have played a large part in animal agriculture as a means of increasing growth performance. Recently however there has been increased concern regarding development of antibiotic resistant bacteria which could lead to reduced antibiotic effectiveness under human health applications (Wray and Davies 2000). Antibiotic growth promoters (AGP) have already been banned in the European Union and consumer demand for antibiotic free meat is increasing in North America (Janardhana et al. 2009). This has led to a search for alternatives to AGP use.

Two of the most promising areas of research are prebiotics and probiotics which, like AGPs, act to alter gut microbial populations. These populations play a large role in gut health, pathogen resistance, and dictating the amount of energy and nutrients derived from food (Gibson and Roberfroid 1995; Buddinton 2009). Antibiotics act to decrease all microbial populations, with the aim to reduce host competition with bacteria for nutrients (Lu et al. 2008). Probiotics and prebiotics on the other hand aim to selectively increase beneficial populations such as *Bifidobacterium* and *Lactobacillus* in order to decrease binding sites for pathogens and increase beneficial fermentation products like short chain fatty acids (SCFA)(Gibson and Roberfroid 1995).

Tasco® is a product made of sun dried brown seaweed (*Ascophyllum nodosum*) by Acadian Seaplants Ltd. which has been shown to decrease *Salmonella* in the excreta of broiler chickens (F. Evans personal communication) and decrease *E. coli* O157: H7 on the hides of feedlot steers (Allen et al. 2001). These results indicate that Tasco® may act as a prebiotic and may be a viable alternative to AGPs. Most research with Tasco® has

been conducted in ruminant species and its effects in monogastrics are therefore largely unknown. The monogastric nature of the poultry digestive system, combined with the short lifespan of the birds, makes broilers ideal simple monogastric models with which to study the potential of Tasco® as a feed additive.

Chapter 2. Literature Review

2.1 The Gastrointestinal Tract in Immunity and Nutrient Absorption

The gastrointestinal tract (GIT) is the most anatomically diverse organ system (Klasing 1999). Although the specific anatomy changes from species to species its function remains universal. These functions include digestion of feedstuffs, osmoregulation, endocrine regulation of digestion and host metabolism, immunity and defense against pathogens and harmful substances, and detoxification of toxic molecules from the environment or host (Buddington 2009). The poultry digestive system is characterized by several uniquely specialized components, including the mouth, esophagus, crop, proventriculus, gizzard, intestines, paired ceca, rectum, and cloaca (Klasing 1999; Józefiak 2004). These GIT components act in a sequence of grinding, acidifying, hydrolysing, emulsifying, and transporting end products in order to process ingested feedstuffs (Klasing 1999).

2.1.1 Function and Structure of the Upper Digestive Tract

The beak, tongue, and oral cavity grasp food and act in mechanical processing, lubrication, and movement of the feed down the eosaphagus. From the eosaphagus, food travels towards the proventriculus via peristaltic contractions. The eosaphagus contains longitudinal folds in the mucosa which allow it to expand to accommodate various food bolus sizes. This ability to expand is particularly utilized in the crop, a region of esophageal widening just prior to reaching the thoracic cavity. This area is used to store food and can collapse or expand according to the amount of ingested food present. The lining of the crop is congruent with that of the esophagus, as it is a continuation of that organ. In the distal portion of the crop the esophagus narrows once more until reaching

the proventriculus where digestive enzymes are added to aide in digestion (Klasing 1999).

The main function of the proventriculus is in digestive enzyme production (Damron 2006). Pepsin and HCL are secreted from gastric glands in the proventriculus (Klasing 1999) creating an environment with an acidic pH, which has been recorded from 2.14 (Angel et al 2010) to 4 (Damron 2006). Feed passes quickly through the region (Damron 2006) and so the enzymes do not act upon it until reaching the gizzard (Klasing 1999).

Within the gizzard, feed is ground down in order to reduce size and increase surface area (SA) available for the secreted proventricular enzymes to act upon (Klasing 1999). To aid in its function, the gizzard is composed of two pairs of smooth muscles that are asymmetrically aligned in order to optimally mix and grind the feed. The lumen of the gizzard is lined with a hard cuticle composed of rod – like projections formed from secretions of the tubular glands which line the organ. The cuticle both aides in grinding the feed and acts to protect the lumen from the HCL and pepsin. Once feed is sufficiently ground it is released into the small intestine through a pyloric fold which separates the upper digestive tract from the lower (Klasing 1999).

2.1.2 Function and Structure of the Small Intestines

The small intestine is the primary site where enzymatic digestion occurs and nutrients are absorbed (Klasing 1999; Ewing and Cole 1994). This region begins with the duodenal loop which encircles the pancreas and continues through the jejunum and into the ileum. Hepatic and pancreatic ducts join up with the intestines in the duodenal loop

(Ewing and Cole 1994). Pancreatic enzymes secreted through these ducts hydrolyse lipids, proteins, starches, and nucleic acids in feed to smaller oligomers within the lumen of the small intestine (Klasing 1999). The duodenum is therefore a major site of feed breakdown. When feed enters into the jejunum, digestive enzymes continue to act upon it. It is here where the absorption of nutrients begins to take place (Damron 2006). Oligomers which had been hydrolyzed by pancreatic enzymes are further broken down to monosaccharides, free amino acids, and nucleotides before being absorbed at the enterocyte brush border (Klasing 1999). Once digesta enters the ileum most nutrient absorption has already occurred, though some does take place in this region. The function of the ileum is instead one of transition from the small intestines to the large intestines (Damron 2006). Throughout the small intestine populations of microflora are present which aid in fermentation of the feed. The majority of this presence is found in the region closest to the large intestine (Ewing and Cole 1994).

2.1.3 Function and Structure of the Lower Gastrointestinal Tract

What feed is not absorbed in the small intestine moves on towards the large intestine. At the juncture of the small and large intestine the paired ceca are found, into which smaller particles in the digesta are pushed by peristaltic movement of the large intestine (Duke 1986). Selection of digesta for entry into the ceca occurs via a meshwork of villi present at the cecal entrance which exclude larger particles (Duke 1986). Within the ceca some of the carbohydrate (CHO) content of the digesta is degraded by the plentiful cecal microbial populations, and some vitamin synthesis occurs (Coates et al. 1968; Jorgensen et al. 1996; Józefiak 2004). Further functions of the ceca include water absorption, fat digestion and absorption, and degredation of nitrogenous compounds

(Józefiak 2004; Klasing 1999). In addition to increased nutrient availability bacterial fermentation in the ceca allows harmful substances to be detoxified (Moran 1982; Csordas 1995). From the ceca feed enters the short colon where high levels of fermentation also occur (Klasing 1999), though less than that observed in other monogastric species (Flickinger et al. 2003). This region absorbs and secretes electrolytes and water and stores and secretes waste material (Gibson and Roberfroid 1995).

2.1.4 The Microstructure of the Gastrointestinal Tract

Beyond the basic GIT structures is a complex system of microstructures which include villi, microvilli, and their corresponding crypts. These structures increase absorptive SA for nutrients. Further still within the crypts and villi are goblet cells which secrete mucus (Klasing 1999), tight junctions which are complexes of epithelial cells (Chichlowski et al. 2007c) that regulate movement of solutes and ions, and paracellular pathways which also control movement of nutrients (Rehman et al. 2009a)

The main role of the crypts is cell generation. In addition to regenerative cells responsible for producing mucus and new epithelial cells for the villi, absorptive cells and goblet cells are also contained within the crypts (Ayabe et al. 2000; de los Santos et al. 2007).

Villi act in nutrient digestion and absorption. They contain rich capillary beds where absorbed nutrients, like CHO and amino acids, enter the blood and are transported to the portal blood vessels (Klasing 1999). Villi are observed in several different shapes, such as flat and straight, curved and convoluted, tongue – shaped, or ridge – shaped. Shape of the villi affects how they interact with the digesta and how much of the

epithelium is able to interact with nutrients. Stage of development and epithelial cell turnover can both affect which villi shapes are present (van Leeuwen et al. 2004).

The turnover of enterocytes in the villi and crypts is an important process which reflects the conditions of the gut and determines the extent of energy use by the intestine, as well as how efficiently nutrients are absorbed. Intestinal epithelial cells are synthesized in the crypts. They then travel along the villi surface towards the tip. Cells are sloughed off into the intestinal lumen within 48 to 96 hours (Potten 1998; Imondi and Bird 1966). The rate at which this progression occurs determines small intestinal cell turnover (Pluske 2001). There are two ways in which turnover is regulated; alteration of the number of crypts which produce cells or alteration of the cell production rate within each crypt (Sakata and Inagaki 2001). Increased crypt cell production typically occurs along with deeper crypt depths when villi are being shortened due to increased cell loss (Pluske 2001). In this situation there is high cell turnover due to normal sloughing or inflammation from bacterial pathogen or toxin presence (Yason et al. 1987). High cell turnover rates are associated with increased protein and energy requirements (Rebolé et al. 2010). If crypts are also affected by adverse conditions in the gut or lack of nutrients then a decreased rate of cell renewal occurs and villus atrophy results (Pluske 2001). When villi height is increased due to the buildup of epithelial cells it allows for increased absorptive area which enhances digestive and absorptive functions. It is also associated with increased expression of brush border enzymes and nutrient transport systems (Pluske et al. 1996; Caspary 1992).

Several protective barriers are present as part of the intestinal microstructure. The lamina propria contains connective tissue within the mucosa which supports the villi

enterocytes. This structure provides a barrier to pathogens which might infiltrate the intestines and is an important part of the immune system (Gartner and Hiatt 2006). Tight junctions are found in between epithelial cells. They selectively regulate passive diffusion of ions and other small solutes through highly permeable paracellular pathways thereby preventing uptake of intact macromolecules (Stoidis et al. 2010; Rehman et al. 2009a). Tight junctions are also responsible for allowing only dead bacteria or bacterial components to be translocated across the intestinal wall for sampling by the immune system, rather than viable organisms which could invade the tissues (Stoidis et al. 2010). Due to importance in regulating movement across the mucosa, when these intestinal barriers are compromised it allows antigenic and toxic substances to gain access to systemic circulation (Rehman et al. 2009a).

2.1.5 Influence of Environmental Factors on Gastrointestinal Microstructure

Several factors can influence microstructures of the gut. These include the diet, presence of pathogens in the gut, stressors, and/or beneficial microflora composition. Toxins can result in high tissue turnover leading to short, thin villi and a low villi height to crypt depth ratio, which are both associated with diarrhea, decreased disease resistance, and poor performance (Yason et al. 1987; Awad et al. 2006; Xu et al. 2003). Diet composition can influence the shape of the villi. For example, highly methylated pectin in the diet was found to decrease zig zag shaped villi and increase ridge- shaped villi. These alterations correspond with lower performance (van Leeuwen et al. 2004). On the other hand, increased glutamine in the diet increased zig zag shaped villi, which was associated with increased performance (van Leeuwen et al. 2004). Temperature too can

influence the microstructure. Exposure of birds to high temperatures has been shown to reduce crypt depth (Burkholder et al. 2008).

2.1.6 Environment of the Gastrointestinal Tract

Beyond the physical structure of the GIT, gut environment plays a large role in nutrient absorption and health, though it is highly influenced by outside factors. Gut pH is affected by fermentation products such as SCFA, the composition of non- digested material (Lahaye 1991; Cummings and Macfarlane 1991), and feed particle size (Svihus et al. 2004; Huang et al. 2006; Scott et al. 2008). pH in turn alters microbial populations and nutrient digestion. Diet can also alter transit time, with diets that slow down passage rate prolonging fermentation which allows increased amounts of metabolites beneficial to gut integrity, such as SCFA (Dunkley et al. 2009).

2.1.7 The Role of Mucus in Gastrointestinal Tract Immunity and Function

Within the crypts are found goblet cells which aide in epithelial cell repair when the mucosa is damaged (Ikeda et al. 2002). Goblet cells secrete polymeric mucin glycoprotein which forms the mucus that becomes a gel on the mucosal surface (Sklan 2004). This substance is the first line of defense against bacteria and other pathogens (Forstner and Forstner 1994; Van Klinken et al. 1995) and is the largest interface between an organism and its environment (Rozee et al. 1982). It protects the mucosa from irritants like bile salts, digesta, and digestive enzymes (Klasing 1999). Mucin producing goblet cells are present in birds as early as 3 days before hatch (Uni et al. 2003). As an animal matures the mucus layer thickens and becomes increasingly colonized by microflora from the gut (Rozee et al. 1982).

The ability of the mucus to protect against pathogens is vitally important (Lewis et al. 2010). Mucus not only provides a barrier but it may also act as an antibacterial. Oligosaccharides of mucins contain compounds which specifically adhere to mannosyl, allowing competitive binding to type 1 fimbria of gram- negative pathogens in order to prevent their attachment to the intestinal wall (Sajjan and Forstner 1990). This allows the mucus to trap and remove pathogens from the intestine (Belley et al. 1999). Mucus can aide in the proliferation of desirable bacterial species by providing an environment optimal to their growth due to mucus's high CHO content (Deplancke and Gaskins 2001).

Mucus is composed of mucin and trefoil factor peptides. The mucin component can be separated into acidic and neutral mucins, which each differ in terms of their physio- chemical characteristics (Kiernan 1990; Forstner and Forstner 1994; Fontaine et al. 1996). Acidic mucins can then be further subdivided into sulphated and sialylated mucins (Kiernan 1990; Forstner and Forstner 1994). Sialylated mucins contain a sialic acid as the terminal sugar of the mucin glycoprotein while sulfated mucins contain a sulphate on their glucosamine residues (Rhodes 1989).

Different mucin types have different actions in the gut and are produced in response to differing gut environmental conditions. The quantity and composition of the mucus in the small intestine is most influenced by the diet fed while in the large intestine it is a matter of intestinal flora present (Sharma and Schumacher 1995). The composition of the mucus found will vary according to the region of the GIT. For example Forder et al. (2007) found greater numbers of goblet cells producing acidic mucins in the ileum of broilers compared to the jejunum.

High levels of sulphated and sialyated mucins are thought to indicate a matured intestinal barrier (Fontaine et al. 1996) due to their presence making the mucus more acidic and viscous, thus increasing mucosal resistance to bacterial enzymes and increasing protection against translocation (Fontaine et al. 1996; Robertson and Wright 1997). However, a high degree of sulfation alone is associated with immature goblet cells (Turck et al. 1993). Some bacteria secrete enzymes which are able to degrade sulphated mucins so the presence of sialyated mucins as well as sulphated in a mature intestine is likely a defense mechanism against such degredation (Forder et al. 2007).

Due to their different characteristics, alterations in the mucus could cause changes in the ability of pathogenic, as well as commensal microflora, to attach to the gut wall (Deplancke and Gaskins 2001). Neutral mucins contain mannose residues to which some bacteria can adhere (Firon et al. 1984). Type 1 fimbria were found to be able to adhere to ileal mucus but not to mucins attached to goblet cells. On the other hand AC/I fimbria, a less common adhesion found on avian pathogenic *E. coli,* could adhere to mucins attached to goblet cells but not to ileal mucus. It was thought that this was due to differing compositions of the mucins in these two areas. Thus different bacteria would be able to adhere to various mucin types according to their compositon (Edelman et al. 2003).

2.1.8 Development of the Gastrointestinal Tract

2.1.8.1 Development of the Intestines

The GIT growth rate is much faster than that of the rest of the body in the prehatch and early posthatch periods. Due to this, it is large and functionally developed

by the time the chick has hatched (Klasing 1999). During the first week of age the small intestine continues to develop at a faster rate then the rest of the body organs (Uni et al. 1998a; 1998b; 1999). One of the key stimulators of its further development is physical exposure to feed (Shira et al. 2005) and therefore early feeding improves initial gut development (Uni and Ferket 2004).

It is during the period when the GIT is developing at such an accelerated rate that the chick is switching from receiving nutrients via the yolk sac to receiving nutrients via the feed (Uni and Ferket 2004). The yolk stalk connects the yolk sac to the GIT and provides a one –way passage for material into the intestines (Peebles et al. 1998). This is found in between the jejunum and ileum and is composed of connective tissue and mucosa lined with glandular epithelium (Kar 1947). During the first five days posthatch it was found that there was an increase in the amount of material moving into the intestine from the yolk sac (Peebles et al. 1998). By the fifth day posthatch approximately 85% of the material from the yolk sac was absorbed (Noble and Ogunyemi 1989). Body weight (BW), relative weights of the intestine, liver, gallbladder, and yolk stalk have all been demonstrated to increase between day 0 and 5 posthatch while the weight of the yolk sac decreases due to the transition that is occurring during this time as the chick becomes relient on feed for nutrition (Duke 1986).

2.1.8.2 Development of the Microstructure

As the GIT tract is growing in size so too are the microstructures within growing and developing, stimulated by exposure to feed (Uni et al. 1999; Aptekmann et al. 2001; Gartner and Hiatt 2006; Sklan 2001). The first indications of villi appear in the small intestine between day 14 and 17 incubation with a regular zig zag pattern of pre – villus

ridges. This is followed by crest cells appearing on the top of the ridges and two rows of either finger – shaped or plate – like villi (Lim and Low 1977; Bayer et al. 1975). Villi development involves a succession of villi shapes as the birds grow. The most significant of these changes were found to occur in the middle and distal parts of the small intestine. In a one day posthatch chick villi are mostly cylindrical with coned tops (van Leeuwen et al. 2004) and crypts are beginning to form (Uni et al. 2000). Within 48 to 96 hours posthatch the intestinal crypts have become defined. By day 5 the villus – crypt axis is developed (Uni et al. 2000). From day 7 onwards the percentage of villi that are classified as tongue – shaped decreases and the percentage that are classified as ridge-shaped increases. The villi then continue to broaden in the middle and distal portions of the small intestine from day 7-28 posthatch, possibly via fusion of previously separate villi (van Leeuwen et al. 2004).

2.1.9 Structure and Function of the Gastrointestinal Immune System

As the GIT provides such a high amount of exposure to the environment, and therefore to potential pathogens, the immune defenses of the GIT must be expansive and developed enough to protect from this threat. The immune system of the gut involves several lines of defense. Innate immunity is present in the form of the physical barrier of the gut wall, mucus secretions, tight junctions, low gastric pH, rapid transit, and competitive beneficial microflora (Patterson and Burkholder 2003). Active immunity is present in the form of the Gut Associated Lymphoid Tissue (GALT) (Neish 2009).

The GALT is the largest immune organ in poultry. It is made up of several components. These include Peyer's patches, cecal tonsils, the bursa of fabricius,

lymphoid cells within the lamina propria, intra – epithelial lymphocytes, and other aggregated and solitary lymphoid nodules (Figure 1) (Kajiwara et al. 2003; Muir et al. 2000; Yasuda et al. 2002; Neish 2009; Stoidis et al. 2010).

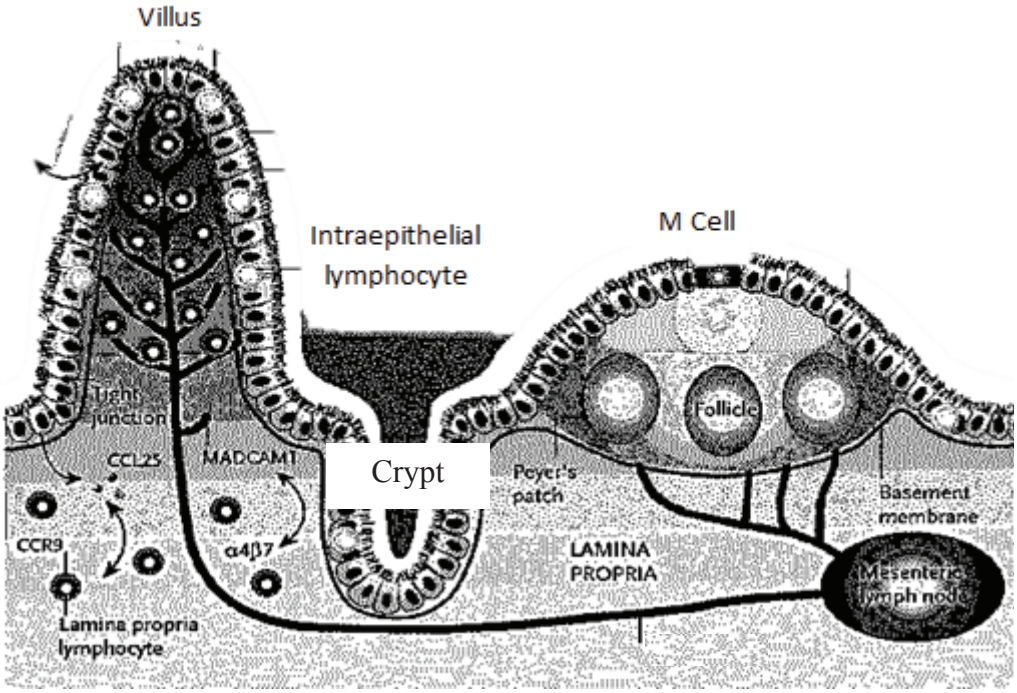


Figure 1: The Gut Associated Lymphoid Tissue (Modified from Mehandru (2007)).

The image displays all the cellular, structural, and chemical components of the GALT. MADCAM1, α4β7, CCL25, and CCR9 are immune signaling molecules which act to direct lymphocytes to the epithelium. M cells and laminia propria lymphocytes are immune cells involed in antigen sampling and processing. The Peyer's patch provides a region developed specifically for sampling antigens from the luminal contents. The lymph nodes provide immune cells such as lymphocytes to process antigens and fight infection.

The GALT becomes even more important when it is noted that avian species do not have lymph nodes throughout the body as mammals do. The GALT, therefore, provides not only local protection to the GIT but also systemic protection (Kajiwara et al. 2003; Muir et al. 2000; Yasuda et al. 2002). Any immune response initiated therein is able to be transferred to the systemic immune system to prevent body wide infection (Buddington 2009).

2.1.9.1 Gastrointestinal Immune Defense Components

Several regions are contained within the GALT, each of which aids in a different area of protection for the GIT. The bursa is located near the cloaca and as such is adapted to respond to rectal antigens (Shira et al. 2005). The cecal tonsils produce effector immune cells which migrate to the intestinal mucosal surface (Muir et al. 2000; Befus et al. 1980). Peyer's patches are mucosal lymph nodes that aid in antigen sampling (Neish 2009).

Found within each of these regions are a number of different immune cells and components, including dendritic cells, macrophages, T cells, B cells (Sasai et al. 2000), and specialized epithelial cells called M cells which cover the Peyer's patches (Neish 2009). Other important players in GIT defense are the secreted Immunoglobulins (Ig), particularly IgA. IgA is secreted into the gut lumen and protects the apical surface of the brush border. It therefore has the ability to alter the microbial populations there by targeting those which the immune system has deemed pathogenic (Lewis et al. 2010) as well as commensal bacteria to prevent microflora overgrowth (Fagarasan and Honjo 2003).

2.1.9.2 Function of the Gastrointestinal Immune System

The GALT acts in constant surveillance of the gut environment including microbes present, both beneficial and pathogenic, and feed antigens (Janardhana et al. 2009). M cells take up food antigens and bacterial cells from the gut lumen and these are then transferred to dendritic cells within the Peyer's patches. Dendritic cells are able to recognize a large range of microbial specific molecular patterns via their pattern recognition receptors (PRRs). These PRRs are transmembrane or intracytoplasmic

receptors that are able to recognize and bind specific MAMPS, which are microbial – associated molecular patterns such as lipopolysaccharides (LPS), flagellin, and peptidoglycans (Neish 2009). PRRs are able to distinguish between pathogenic and commensal bacteria (Buddington 2009), possibly via detection of tissue damages associated with pathogen presence (Lewis et al. 2010). If dendritic cells recognize an antigen as pathogenic it is presented to T cells which then are able to differentiate and initiate the appropriate immune response of the cell mediated or humoral immune system (Neish 2009; Lewis et al. 2010). One such possible response is to activate the formation of small antimicrobial peptides that form pores in the bacterial cell walls (Neish 2009).

Translocation is another process of environmental sampling and involves the passage of viable bacteria or inert particles and antigenic macromolecules from the GIT across the mucosa and into mesenteric lymph nodes and other internal organs. This process allows the GIT to sample antigens within the lumen so that the immune system can keep them away from the internal environment (Stoidis et al. 2010). As the immune system so closely interacts with the microbial populations of the gut, if any component of the GALT is compromised it results in altered microbial populations and possible detrimental effects on host health (Buddington 2009).

2.1.9.3 Development of Gastrointestinal Immunity and the Trough of Immunity

GALT development has been described as gradually increasing until a plateau is reached at maturity (Siegrist 2001; Reese et al. 2006). The GIT at hatch is completely sterile (Klasing 1999); however, immediately following hatch the chick is exposed to adult- type microflora through foraging and their surrounding environment. Due to this,

the instant development and immunological function of the GALT is critical for survival (Bar-Shira et al. 2003). In fact, the GALT develops concurrently with the intestinal tract and functional interactions have been observed between the intestinal contents, enterocytes, intraepithelial leukocytes, and lamina propria leukocytes (Hamzaoui and Pringault 1998; Iijima et al. 2001; Kedinger et al. 1998; Perdue 1999; Pitman and Blumberg 2000).

The GALT at hatch contains functionally immature T and B lymphocytes which attain full function within the first two weeks (Miyazaki et al. 2007). On day 4 posthatch the expression of mRNA for proteins involved in immune function, such as proinflammatory cytokines and antimicrobial peptides, are increased in the GALT (Bar-Shira et al. 2003).

As the GALT is not completely developed at hatch very low of levels of pathogens in the environment, that would not affect an adult bird, are able to have a severe effect in young birds (Nurmi and Rantala 1973). During this time, chicks are being exposed to any bacteria present in the environment causing them to be at risk for infection (Friedman et al. 2003; Noy et al. 2001; Sklan 2001; Uni et al. 2000). When Ask et al. (2007) developed a mathematical model for immunocompetence in chicks by measuring maternal and baseline acquired immune factors it was observed that between day 4 and 8 posthatch the chicks were particularly vulnerable as it was a time of decreasing maternal immunity without acquired immunity optimally functioning. Any encounter with pathogenic antigens at this time leads to an increased rate of degredation of maternal antibodies leaving the chicks even more vulnerable (Kaleta et al. 1972;

Siegrist 2001). There is therefore a trough of immunity in this period when any outside assistance in preventing infection to chicks would be most helpful (Figure 2).

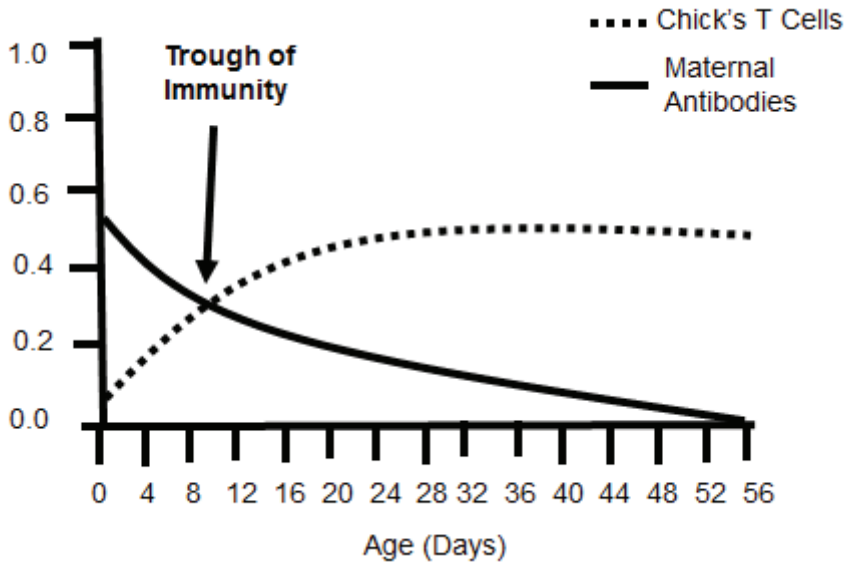


Figure 2: The Trough of Immunity Modified from Ask et al. (2007)

T cell counts and maternal antibody counts of broiler chicks were measured starting at day 0 posthatch displaying a gap in immune protection between day 4 and 12 posthatch.

2.2 Microbial Populations of the Gastrointestinal Tract

Microbial populations play an important role in the gut and have a large presence there. Approximately 90% of cells in or on the body are composed of unicellular organisms. Most of these are within the GIT. Included in this group are over 1000 different bacterial species as well as fungi, protozoa, yeast, and bacteriophages (Lewis et al. 2010). These populations enact a number of effects on the host. Beneficial populations improve nutrient uptake, gut health, and protect from pathogens (Gibson and Roberfroid 1995). Pathogenic species are also present which have the opposite effect (Dunkley et al. 2009; Gong et al. 2002a).

2.2.1 Development of Microbial Populations Throughout the Gastrointestinal Tract

When chicks hatch they have a completely sterile environment in their gut (Klasing 1999). It begins to be colonized with bacteria from the surrounding environment through oral and rectal pathways (Clench 1999) and from the diet within 3-6 hours (Mead and Adams 1975; Amit-Romach et al. 2004). Chicks can use spontaneous sucking movements of the vent called cloacal drinking to take up microflora from the environment for colonization of the posterior digestive tract as well (Klasing 1999). By 5 to 6 hours posthatch there are 10^6 to 10^{10} cfu bacteria /g of feces present (Snel et al. 2002). Some of the bacteria that enter the chick are not adapted to the gut conditions and so are killed by digestive secretions, eliminated by the chick's immune system, or cannot attach to the gut wall and are excreted from the system (Klasing 1999). Other bacteria begin to colonize the GIT and establish niches (Lu et al 2008). When chicks are raised in traditional husbandry systems they are immediately exposed to bacteria from their mother's feces, while in intensive systems bacteria in the environment are not as plentiful and so the colonization of the gut is delayed (Nurmi and Rantala 1973).

Microbial populations present in the gut demonstrate a progression in phylotypes and abundance as the bird matures (Lu et al. 2008) which is very similar to that in pigs, calves, and humans (Mackie et al. 1999). Transitional bacterial communities have been found in broiler chicks on day 3-5, day 5-12, and day 12-17 when the diversity, abundance, and bacterial types present are quite different from those in birds of different days of age (Torok et al. 2009). Nava et al. (2009) found bacterial populations to become less varied among individuals in a flock as birds aged. Aerobic and facultative anaerobes such as *Escherichia*, *Klebsiella*, *Enterobacter* (Yoshioka et al. 1983), *Lactobacillus,* and

Streptococcus (Mackie et al. 1999) are the first bacteria that are able to colonize the gut (Dibner and Richards 2005). These bacteria reduce redox potential in the gut environment allowing obligate anaerobes like *Bacteroides*, *Eubacterium*, *Fusobacterium* (Tlaskalova-Hogenova et al. 2004) and *Bifidobacterium* to begin to colonize (Dibner and Richards 2005; Pieper et al. 2010). These obligate anaerobes make up the majority of the adult microflora (Dibner and Richards 2005). Further development of the populations involves establishment of ecological niches in which specialized bacterial species can grow thus allowing bacterial populations to diversify (Pieper et al. 2010).

Microbe populations in the gut are established in the small intestine prior to populations in the ceca reaching a stable dynamic. In the small intestine the typical adult microflora is present after 2 weeks posthatch. However, in the ceca the adult flora does not become established until 14-30 days of age (Barnes et al. 1972; Amit-Romach et al. 2004). The specific species present in these different areas throughout development also differ. In the first few days *Enterobacteriacae* spp*., Enterococcus* spp., and *Lactobacillus* spp. are the main species found in the ceca. Obligate anaerobes, which perform most of the fermentation in this region, begin colonizing the ceca around day 10 (Salanitro et al. 1974; van der Wielen et al. 2000) and within the first two weeks *Bacteroides* spp. and *Eubacterium* spp. are found (Józefiak 2004). *Salmonella*, *Campylobacter*, and *E. coli* have been identified in the ceca of 14 day old chicks (Amit-Romach et al. 2004). In the duodenum and ileum *Enterococcus* and *Lactobaccillus* are the most dominant species present in the first weeks. After the first week *Lactobaccillus* alone becomes the most dominant group in these regions (Dibner and Richards 2005). When Amit –Romach et al. (2004) looked at the progression of bacterial species in chicks

they found that in young chicks *Lactobacillus* was the only species consistently detected throughout the GIT. As the chicks matured *Lactobacillus* remained the predominant bacterial species in the small intestine while *Bifidobacterium* became more prominent in the ceca.

The progression of microflora species is consistent among similar environments (Apajalahti et al. 1998). It can however, be affected by different husbandry practices. Torok et al. (2009) found differing diets to alter how the ileal microbiota developed for example. In addition, if the chicks are in an environment where they are not exposed to normal facultative anaerobes then they may instead establish populations of unusual species, usually of the genera *Bacteroides*, *Clostridium*, or *Staphylococcus (*Kelly et al. 2007). This can also occur if antibiotics are being fed (Lewis et al. 2010). Current intensive practices in the way eggs are incubated and hatched, chicks are reared, and facilities are maintained have made it difficult for the normal transmission of bacteria to occur from parent to chick (Gong et al. 2002b) and so more likely for occurrence of abnormal bacterial successions (Kelly et al. 2007).

2.2.2 Microflora Species and Population Densities

Each region of the gut provides unique ecological niches for the establishment of bacterial populations adapted to the particular conditions. This lends itself to a great diversity of species being present in the GIT of adult chickens and among different regions (Dibner and Richards 2005). In addition to this, there are several different types of bacteria which interact with the host and gut in various ways. Autochonous bacteria are bacteria which actually colonize a region or regions of the gut. Allochtonous bacteria

are bacteria which instead pass through the gut and do not attach to the gut wall (Pieper et al. 2010). Within the autochonous bacterial populations microbes are further categorized as dominating, sub- dominating, and temporary (Józefiak 2004).

2.2.2.1 Regional Differences in Microflora Species

Specialization of bacterial species to a particular region includes substrate adaptation as well as their ability to anchor to the gut wall or mucus layer, tolerate the gut environment, quickly replace bacterial cells lost to sloughing, scavenge minerals, and resist the persistent low – grade immune responses which occurs towards all microbes present in the GIT (Pieper et al. 2010). There are several survival strategies employed by the microflora. These include tolerating local conditions so as to colonize the surface mucosa, establishing the population away from adverse conditions such as within crypts or in the deep mucosa, or multiplying quickly enough to replenish lost cells (Buddington 2009; Dunkley et al. 2009). Differing survival strategies lead to differences in bacterial populations in the lumen and the mucosa (Gong et al. 2002a). Adaption of bacterial species is demonstrated in the various genes which are activated or silent in bacteria of the same species in different regions (Candela et al. 2010). There is the greatest diversity in species found where there are an intermediate amount of adverse conditions to overcome, as populations can flourish but no one species can dominate due to the presence of mild distubances (Buddington 2009).

There is a decreasing oxygen gradient from the proximal small intestine to the colon and a corresponding change in bacterial species found from aerotolerant to anaerobic (Buddington 2009). There is also a decreasing flow rate gradient from the small intestine to the colon which likewise affects bacterial populations in each region. In

the small intestine, the fast flow rate makes it more difficult for populations to adhere to the gut wall for an extended period and so populations are smaller. In the distal ileum, ceca, and colon there is a slower rate which allows bacteria time to ferment digesta and makes it easier for them to establish lasting colonies and therefore establish larger populations (Buddington 2009; Dibner and Richards 2005). Regions also differ in pH, nutrient availability, and electrolyte and antimicrobial peptide levels. The small intestine has high levels of bile acids and antibacterial peptides which cause low bacterial densities and diversity (Buddington 2009). The ceca has a high pH of 5.65-7.8, favouring ample bacterial populations (Jozefiak et al. 2004). Due to the different conditions in each region, populations of microflora seem to become more diverse the farther apart their regions are in the GIT as they adapt to the particular conditions found (Simpson et al. 1999; Hume et al. 2003; Apajalahti et al. 1998).

Ileal bacterial populations have been measured to be between 10^7 and 10^9 cfu/g of digesta (Apajalahti et al. 1998). The species found in the ileum as well as throughout the small intestine are mostly gram – positive and include mainly *Lactobacillus* but also *Streptococcus* and *Enterococcus. Clostridium* also has a large presence, though not consistently (Lu et al. 2003; 2006; Salanitro et al. 1978; Barnes 1979; Mead 1989). *Lactobacillus* were found to make up most of the bacteria in the lumen of the ileum while *Lactobacillus* and *Enterococcus cecorum* make up the majority of the ileal mucosa bacteria (Gong et al. 2002 b).

The ceca contains possibly the highest number of microorganisms in the GIT and the most diverse (Bjerrum et al. 2006; Barnes et al. 1972; 1973; Barnes 1979). Levels of bacteria at 10^{11} cfu/g of digesta have been reported (Flickinger et al. 2003). Populations

consisting mostly of strict anaerobes are present in the ceca (Józefiak 2004) including large numbers of butyric acid and lactic acid producing bacteria (Bjerrum et al. 2006). *Faecalibacterium prausnitzii*, *Ruminococcus, Clostridium*, *Enterococcus cecorum*, *Bacteroides*, *Peptococcus*, *Streptococcus*, *Bifidobacterium*, *E. coli*, and *Clostridium welchii* were identified as the most common bacterial groups in the ceca of the 200 species found there (Barnes 1979; Gong et al. 2002 b; Flickinger et al. 2003). *Lactobacillus*, mainly of the species *L. reuteri*, *L. oris*, *L. acidophilus*, *L. crispatus*, and *L. salivarius*, have also been found to inhabit the ceca (Selim 2006).

2.2.2.2 Microbial Species of Interest

The bacterial microbiota in the adult GIT are a mix of facultative and obligate anaerobes. These populations are subject to changes in environment, diet, and stress resulting in a great amount of bird to bird variation in microbial populations present according to the particular conditions experienced by the bird (Zhou et al. 2007). However, there are several bacterial phyla which are of interest universally. These include both beneficial and pathogenic species which seem to be of either great prominence or great threat. Main beneficial species in the gut include *Lactobacillus* and *Bifidobacterium* (Bjerrum et al. 2006) while species of concern for the poultry industry and/or food production include *Salmonella*, *Coccidian*, *Clostridium*, and *E. coli* (Flickinger et al. 2003).

Lactobacillus and *Bifidobacterium* are two of the most prominent autochonous species of beneficial microflora. *Lactobacillus* are non-motile gram- positive, non –spore forming rods. *Bifidobacterium* are gram – positive, non- spore forming rods with club – shaped morphologies (Fooks and Gibson 2002). Many *Lactobacillus* species have been

found throughout the GIT, suggesting their importance in the microbiota ecosystems (Guan et al. 2003; Gong et al. 2002 b; Knarreborg et al. 2002). *L. aviarius* and *L. salivarius* are two of the most common (Dhama et al. 2008).

Both *Lactobacillus* and *Bifidobacterium* enact effects in the gut which prevent pathogen growth and promote host health. In their fermentation processes *Lactobacillus* and *Bifidobacterium* species produce lactic and acetic acids which in turn leads to a reduced pH in the gut unfavourable for pathogen growth (Bjerrum et al. 2006; Fuller 1977; Gibson and Roberfroid 1995). *Lactobacillus* and *Bifidobacterium* are also thought to secrete antimicrobial compounds (Havenaar and Huis in't Veld 1992; Dhama et al. 2008; Gibson and Roberfroid 1995) and competitively exclude pathogens (Edelman et al. 2003; Collins et al. 2009). In a study of *Lactobacillus* and *E. coli* found on the epithelium of the chicken ileum it was reported that *L. crispatus* and *E. Coli* 0789 were both able to bind to the same receptors but that *L. crispatus* bound with higher affinity thereby inhibiting *E. coli* 0798 (Edelman et al. 2003). Additionally, *Lactobacillus* are able to increase expression of MUC2 and MUC3 genes for intestinal mucins which further allows them to prohibit attachment of pathogenic bacteria (Kelly and King 2001). *Lactobacillus* increase nutrient digestion by secreting amylase, protease, and lipase (Dhama et al. 2008), while *Bifidobacterium* produce B vitamins as well as digestive enzymes such as casein phosphatase and lysozyme (Gibson and Roberfroid 1995). In addition, *Bifidobacterium* are able to increase growth of butyrate producing species which then further digest nutrients (Steed and Macfarlane 2009). *L. casei* lowers urease activity in the small intestine, decreasing production of toxic compounds like non-protein nitrogen, uric acid, ammonia, and urea (Dhama et al. 2008). *Lactobacillus* further

increase health by stimulating cell mediated immunity and production of Ig, increasing production of interferons, and activating dendritic cells (Dhama et al. 2008; Sato et al. 2009).

Salmonella are gram- negative bacteria and belong to the *Enterobacteriaceae* family (Dunkley et al. 2009). *Salmonella* is a large concern in animal agriculture as it causes gastroenteropathy in humans who consume it (Davies and Wray 1996; Reeves et al. 1989; Bjerrum et al. 2006). Approximately 95% of these cases are of foodborne origin (Mead et al. 1999) and are usually associated with poultry products (Byrd et al. 1997). Sixty percent of the foodborne illnesses are caused by either *S. typhimurium*, *S. Enteritidis*, *S. Newport*, or *S. Heidelberg*. In poultry, *Salmonella* only causes asymptomatic chronic infections in adult birds. However, in birds less than two weeks of age it can cause mortalities (Dunkley et al. 2009) by invading intestinal epithelial cells through adherence to mannose receptors with their type 1 fimbriae and then moving within macrophages to other organs (Barrow 2000; Thomas et al. 2004). Chicks can be exposed to *Salmonella* via deposition on the egg shell or feces in the environment (Dunkley et al. 2009).

Clostridium perfringens is a major pathogen of concern currently in the poultry industry. It is found in human and animal intestinal tracts and throughout the environment (Brandt et al. 1999). It is a gram – positive anaerobic spore- forming bacteria (McDonel 1980). *C. perfringens* is responsible for necrotic enteritis in broilers (Van Immerseel et al. 2004) via the production of extracellular toxins which cause damage to the intestine (Hein and Timms 1972; Long 1973; Shane et al. 1984). While normally present at low levels, any disturbance in the normal flora allows *C. perfringens*

to proliferate rapidly and induce disease (Kondo 1988). *C. perfringens* infection can cause temporary depression in the numbers of *Lactobacillus* present in the gut (Feng et al. 2010).

Other pathogenic species of importance include *Coccidian*, *Campylobacter*, and *E. coli*. *Coccidia* is a parasite of the genus Eimeria. It produces tissue damage which results in reduced growth of the host (Cook 1988). Infection of birds with *Coccidia* is thought to be a pre- disposing factor to development of necrotic enteritis (Al-Sheikhly and Al- Saieg 1980; Baba et al 1997). *Campylobacter* is an enteric pathogen which causes acute diarrhea (Bjerrum et al. 2006). It can cause campylobacterosis in humans (Rosenquist et al. 2003; 2006; Smith et al. 2007) and is currently the most important pathogen source of gastrointestinal illness (Ganan et al, 2012). *Campylobacter jejuni* is found in the chicken GIT, deep in the crypts, and within the mucosal layer (Beery et al. 1988). *E. coli* can be both commensal and pathogenic. In humans pathogenic *E. coli* strains cause enteric infections (Dozois et al. 2003). In addition to infection, some pathogenic bacteria such as *E. coli*, *Clostridium*, *Steptococcus fecalis*, and *Proteus* can produce toxic compounds like ammonia, amines, nitrosamines, phenols, and estrogens (Flickinger et al. 2003).

2.2.3 The Microflora – Host Relationship

The microbiota and their hosts have coevolved over time to result in a mutually beneficial relationship. This evolution has resulted in the microbiota being able to ferment energy sources consumed by the host, such as plant polysaccharides, and colonize the environments provided by the host. The host has become able to utilize

fermentation products produced uniquely by the microbiota, such as SCFA, and gains protection against pathogenic species by the commensal bacteria (Candela et al. 2010; Neish 2009). Microbe populations are kept in check by the host's immune system which regulates their diversity as well as their population sizes (Neish 2009), though it must be exposed to the bacteria during development for commensals to be correctly recognized as non – harmful (Pieper et al. 2010). Even the placement of the bacteria throughout the gut has evolved to most benefit each party. The host keeps bacterial densities low in the small intestine with peristalsis and secretions from the pancreas and intestine in order to fully utilize the highly digestible feedstuffs there. In the more distal intestine, the environment allows bacteria to flourish where they can ferment more poorly digested feedstuffs, producing SCFA beneficial for the host (Buddington 2009). The bacteria too can regulate their environment by upregulating gene action of epithelial cells to alter the biochemistry, physiology, and function of the intestinal barrier (Tucker and Taylor-Pickard 2004). There is therefore a delicate balance present between the host creating an environment optimal for its survival and the bacteria doing the same. This balance is very sensitive and alterations in the gut environment or gut flora can easily disrupt it resulting in GIT disorders (Neish 2009).

2.2.4 Interbacterial Interactions

The microflora ecosystem not only involves interactions with the host but also among different bacterial species. These interactions involve competition for space and nutrients, cross – feeding, and quorum sensing (Pieper et al. 2010; Buddington 2009). The process of quorum sensing involves using stimulus and response to alter population densities (Pieper et al. 2010). Cross- feeding occurs between primary and secondary

degraders. The primary degrader ferments a feedstuff digested by the host, usually either non- digestible xylan – pectin or arabinose – containing dietary CHO, which the secondary degraders are unable to. In this process other products are released which the secondary fermenters are able to use (Buddington 2009; Candela et al. 2010; Hübener et al. 2002). For example *Bifidobacterium* can ferment feed to lactate which can then be used by additional anaerobes to produce butyrate and other SCFAs (Buddington 2009). Some bacteria can decrease competition for nutrients with new syntrophic bacterial species by altering their gene expression to either expand the fermentation substrates they are able to utilize or better take advantage of fermentation products of primary fermentors (Candela et al. 2010).

2.2.5 Microflora Effects on Host Health and Nutrition

Beneficial bacteria in the gut exert multiple effects on many of the host's systems, including metabolism, health, and growth (Amit-Romach et al. 2004). They use a variety of mechanisms to enact these effects and the presence of a diverse and mature GIT microbiota is essential for the fulfillment of these functions (Dunkley et al. 2009).

2.2.5.1 Effects on Pathogen Resistance

Microflora use multiple means to exclude pathogens from the gut. The first of these is competitive exclusion for nutrients and for space. A mature microbiota that occupies all niches is effective in preventing pathogens from colonizing the gut (Dunkley et al. 2009). *Bifidobacterium animalis* MB5 and *Lactobacillus* GG were shown to successively inhibit adherence of *E. coli* K88 (Howarth 2010; Roselli et al. 2006) and culture supernatants from lactic acid producing bacteria inhibited the growth and the attachment of *Helicobacter pylori* (Howarth 2010). The microbiota also produce

substances which have direct antibacterial effects such as organic acids, acidolin, acidophilin, reuterin, lysozyme, lactoferrin, hydrogen peroxide, lactoperoxidase, and bacteriocins like lactocin and lactocidin (Dibner and Richards 2005; Dhama et al. 2008). Bacteriocins have bactericidial effects against several enteropathogens (Lasagno et al. 2002; Dhama et al. 2008). Acidolin is able to inhibit invasion of gram – positive pathogens, while reuterin is able to inhibit bacteria, yeast, and fungi (Howarth 2010; Dhama et al. 2008). *L. bulgaricus* and other beneficial species produce anti- enterotoxins which neutralize pathogen produced enterotoxins (Dhama et al. 2008).

Microbiota can have a direct affect on the host's own defenses as well to fortify them against pathogens. They alter the intestinal barrier by enhancing epithelial cell turnover and angiogenesis and reducing transepithelial permeability (Candela et al. 2010). Manipulation of the environment through products of fermentation, such as reducing pH with the production of SCFA, is thought to play a role in pathogen inhibition (Dunkley et al. 2009). The final mechanism by which native microflora are thought to inhibit pathogens is through stimulation of the immune system. Microbiota stimulate innate immunity by enhancing development of the mucus layer, the epithelium, and the lamina propria (Dibner and Richards 2005). The presence of the microbiota aides in keeping the acquired immune system primed for a fast reaction towards the presence of pathogens (Candela et al. 2010). This involves maintaining a state of chronic low – grade inflammation in the gut via PRR sampling (Neish 2009). Some bacteria, such as *Bifidobacterium* are able to stimulate macrophages to secrete cytokines and reactive molecules against pathogens (Fukata et al. 1999; Sun et al. 2005).

2.2.5.2 Effects on Nutrition

The microbiota increase nutrient absorption, vitamin synthesis, and lipid, protein, and CHO metabolism. Certain microbes are able to synthesize essential vitamins such as B12 and K (Lewis et al. 2010). Microorganisms are able to increase energy storage of the host by increasing glucose uptake from the intestine and glucose and insulin levels in the serum, leading to enhanced lipogenesis in the liver (Candela et al. 2010). *Bacteroides*, *Clostridium*, *Enterobacterium*, *Lactobacillus*, and *Streptococcus* are all able to produce amines from protein decarboxylation. The amine histamine is then able to alter blood flow to the mucosa (Pieper et al. 2010). Beneficial bacteria such as lactobacilli and bifidobacteria enhance the development of the gut which increases early gut efficiency (Palmer and Rolls 1983; Furese et al. 1991).

Microbial fermentation plays a large role in enabling full utilization of feedstuffs by the host. Substrates which are not able to be fermented via intrinsic digestive functions such as resistant starches, non- digestible CHO, oligosaccharides, proteins, and mucins are able to be broken down by the microbiota (Gibson 2004). However, CHO are the most frequent fermentation substrate (Gibson 2004). CHO such as lactose, raffinose, stachyose, and fructooligosaccharides (FOS) are able to reach the lower intestines intact and so are readily available for bacterial fermentation (Gibson and Roberfroid 1995). Of these CHOs soluble fibers are most fermentable as they form gels in the GIT that increases area available for attack by bacterial enzymes (Gibson 2004). Dietary CHO are fermented into SCFA, CO_2, H_2, NH_3, H_2S, CH_4, lactic acid, and branched chain fatty acids (Pieper et al. 2010). The SCFA, with other fermentation products, make up about 10% of the total metabolic energy requirements of the host (Buddington 2009).

Bacteria mostly ferment dietary CHO (Gibson 2004). There are several categories which bacteria fall into in terms of substrates used for fermentation. Bacterial species are able to adapt to ferment new substrates if diets change; however, most species are selective in what type of substrates they are able to ferment (Candela et al. 2010). Saccharolytic species are able to use CHO and include *Bifidobacterium*, *Ruminococcus*, *Eubacterium*, *Lactobacillus*, and *Clostridium* (Gibson and Roberfroid 1995). The main end products of saccharolytic fermentation are SCFA. Proteolytic species produce nitrogenous metabolites as their end products (Gibson 2004). Some species rely on cross- feeding for fermentation substrates. Other bacterial species, such as nitrogen utilizers and gas metabolizers, ferment various unused components of the diet (Gibson and Roberfroid 1995).

2.2.5.3 Effects of Microflora Short Chain Fatty Acid Production

SCFA are very important fermention end products. They not only contribute to the energy requirement of the host but also have roles in pathogen resistance and gut function (Tuohy et al. 2009). Included in SCFA are organic acids, acetate, propionate, butyrate, valerate, isovalerate, and isobutyrate (Dunkley et al. 2009). Shifts in SCFA present are indicative of the substrate available.

The SCFA profile produced changes over time as the chick matures and alterations occur in the diet. At 3 days posthatch acetate can be found in the chicks' GIT while butyrate and propionate are not observed until 12 days posthatch (Jozefiak 2004). Acetate concentrations in the GIT increase until 15 days posthatch when it reaches a stable level of 70 μM/g, while levels of propionate and butyrate become stable at around 8 and 24 μM/g respectively 12 days posthatch (van der Wielen et al. 2000). Though

stable under consistent conditions the levels of each SCFA can be altered according to environmental or diet changes (Van Immerseel et al. 2003).

SCFA influence metabolism via several different mechanisms which vary according to the acid in question. In general, SCFA contribute about 5-15% of the daily requirements for a broiler's maintenance energy (Annison et al. 1968; Gasaway 1976a; 1976b). Some SCFA are used directly by intestinal epithelial cells for cell maintenance energy while others are transported to various host tissues. Butyrate is an important source of energy for colonic cells (Candela et al. 2010) while propionate, L- lactate, and acetate are used by the liver. Acetate is also utilized in the muscle and various other peripheral body tissues (Gibson and Roberfroid 1995). Propionate participates in ATP production in the liver (Gibson 2004). Acetate, propionate, and butyrate which are not utilized by hepatocytes are transported in the blood to other tissues for further metabolism (Patterson and Burkholder 2003; Wu 1997). Various SCFA can have differing functions in the same tissues. For example, acetate in the liver contributes to lipid and cholesterol synthesis but the presence of propionate inhibits this reaction (Candela et al. 2010) and instead promotes gluconeogenesis (Scott et al. 2008). Thus a balance of all three SCFA is most beneficial for host health (Scott et al. 2008). In addition to contributing directly to host energy, SCFA acidify the gut environment which in turn promotes nutrient uptake by improving digestive enzyme activity and increasing microbial phytase (Dibner and Buttin 2002). Pancreatic secretions are increased by the ability of dissociated SCFA to diffuse into enterocytes and interact with cytoplasmic receptors (Dibner and Buttin 2002). SCFA also enact effects on mineral absorption.

Acetate and propionate for example have been shown to increase absorption of Ca^{+2} in humans (Trinidad et al. 1993).

SCFA have been found to alter histomorphology of the intestinal epithelium. They stimulate epithelial cell proliferation and villus size which results in an increased absorptive SA (Dibner and Richards 2005). Butyrate is used by colonic cells as an energy source and thus supports their proliferation (Bosscher 2009). SCFA were found to increase the mass of the mucosal and submucosal tissues as well as crypt cell production (Sakata and Inagaki 2001). Butyrate in particular has been shown to increase the height of villi, increase villi SA, and increase crypt depth when fed to rats (Dibner and Buttin 2002). These effects lead to more efficient nutrient absorption (Dibner and Richards 2005).

SCFA exhibit both direct and indirect antibacterial effects. The main indirect effect is a lowering of the gut pH (Dhama et al. 2008). Some microbes are tolerant of this decrease while others, such as *E. coli*, are not (Russell and Diez-Gonzalez 1997). SCFA have been shown to have direct bactericidial effects on pathogenic species like *Salmonella*, *C. perfringens*, and *E. coli* (Van Immerseel et al. 2003) but not commensal species (van der Weilen et al. 2000). A 50-80% reduction in *Salmonella typhimurium* has been demonstrated in the presence of SCFA (Jozefiak 2004). Butyrate decreases *C. jejuni* at a concentration of 12.5 mM while propionate and acetate decrease it at 50 mM. When Van Immerseel et al. (2003) exposed *Salmonella* to media with propionate and butyrate preincubation it resulted in decreased ability of the *Salmonella* to invade an intestinal epithelial cell line. SCFA affect immune function as well. They suppress inflammatory cytokine secretion and so may aid in the host's ability to tolerate the

commensal microorganisms (Neish 2009). SCFA may also bind to leukocyte receptors to alter their functions (Watzl et al. 2005; Seifert and Watzl 2007).

pH of the environment can influence how effectively the SCFA prevent pathogen growth. SCFA were found to decrease *C. jejuni* at pH 6.0 but at 7.5 they had no effect (Van Deun et al. 2008). A similar observation was made with *S. enteritidis* (Van Immerseel et al. 2003). While at pH 6 preincubation of *Salmonella* with formate or acetate had no effect on its invasion into the intestinal cell line, at pH 7 it was found to in fact increase *Salmonella's* invasion (Van Immerseel et al. 2003). These differences are due to the nature of SCFA as acids. At low pH the acids are in undissociated form and so are lipophilic which allows them to diffuse across the bacterial cell membranes. Once in the bacterial cells they encounter a higher pH of the cytoplasm and dissociate which causes the pH of the cytoplasm to reduce by increasing the inward proton leak and thus disrupting cellular activites (Figure 3) (Dibner and Buttin 2002; Cherrington et al. 1991; Kashket 1987). SCFA are able to inhibit bacterial growth in this way as the bacteria must use their energy to try and maintain its internal pH (Russel and Diez – Gonzalez 1997).

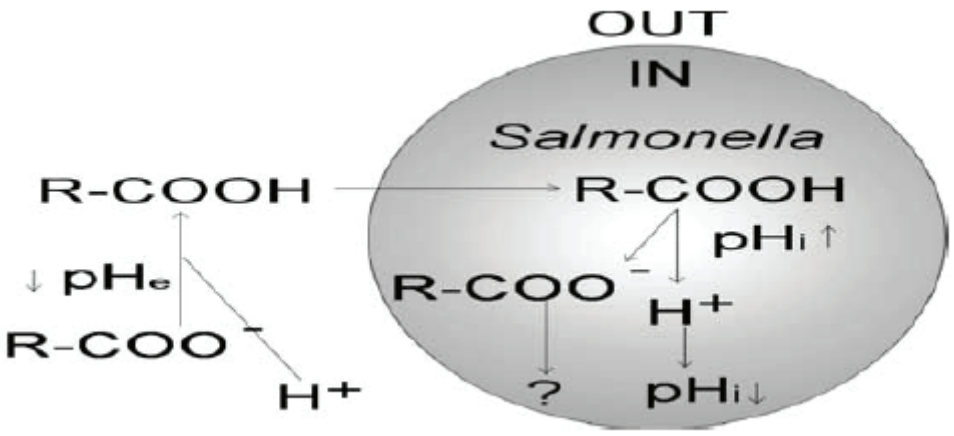


Figure 3: Mechanism of Short Chain Fatty Acid Toxicity to *Salmonella* from Jozefiak (2004)

Short chain fatty acids work by becoming undissociated at low pH's such as those found in the lower gastrointestinal tract. In this form they diffuse across bacterial cell membranes to the cytoplasm where the pH is higher causing them to dissociate. The hydrogen ions released by this dissociation lower the pH of the cytoplasm. The bacterium then needs to use an increasing amount of its energy to maintain internal pH.

2.2.5.4 Effects on Mucus Production

Some of the microflora alter the mucosa in order to better provide binding sites for themselves. These bacteria are able to change sialyated acids on mucins to fucosylated oligosaccharides and thereby create more attachment sites (Pieper et al. 2010). However, Forder et al. (2007) saw a shift from goblet cells producing sulphated mucins to sialyated mucins in the ileum and jejunum of birds raised under conventional conditions on day 4 posthatch. Birds raised in a low bacteria environment displayed no such shift. This change was a result of the host reacting to microflora presence to protect mucins from degredation. Bacteria can alter other mucin characteristics as well. For example, *Lactococci* can increase trefoil factor peptide production which leads to an increase in mucin viscosity (Steed and Macfarlane 2009).

2.2.6 Germ Free Birds

The influence of the microflora on health and growth of the host can best be demonstrated when germ free animals are compared to those raised with normal exposure to bacteria. Most importantly, germ free animals are more susceptible to pathogenic invasion than conventionally grown animals (Dibner and Richards 2005). Intestinal mass is lower in germ free birds (Humphrey et al. 2002) and expression of proteins involved in tight junction formation is decreased (Pieper et al 2010). Intestinal epithelial cell turnover is slower in germ – free rats (Sakata and Inagaki 2001). There are fewer neutral and sulphated mucins but a greater number of sialyated mucins (Meslin et al. 1999; Sharma and Schumacher 2001). Fewer goblet cell numbers and smaller goblet cell sizes have also been demonstrated (Kandori et al. 1996). The immune systems of germ free animals, such as lymph nodes, lymphoid follicles, and Peyer's patches are underdeveloped and antibody diversity decreased. However, if even a single commensal species is introduced into the germ free animal then IgA secretion is stimulated (Dibner and Richards 2005). Conversely, some positive attributes have been observed in germ free birds. Coates et al. (1963) showed germ – free chicks to grow more quickly then those housed in conventional growing conditions. This observation is due to some of the more detrimental effects of commensal bacteria on growth.

2.2.7 Detrimental Effects of Microflora Presence

While the microflora provide the host with a number of beneficial effects they also compete with the host for nutrients and induce effects which increase the host's energy expenditure such as rapid turnover of epithelial cells, increased mucus secretion,

and stimulation of a chronic inflammatory response (Dibner and Richards 2005). In fast growing broiler chickens this increased energy demand may lead to decreased growth performance (Yang et al. 2009). Microflora can also produce toxic compounds through fermentation, such as ammonia and H_2S (Cummings et al. 1979).

2.2.8 Influence of Environment on the Gut Microflora

The microbiota found in the gut can vary throughout the life of an animal according to the conditions it experiences. Feed composition has a huge impact on what species are found (Collins and Gibson 1999). The abundance of key bacterial groups can be altered according to what substrates are available for fermentation and which bacterial species are equipped with the enzymes necessary to ferment them (Scott et al. 2008). The energy content of the feed, type of substrate, and amount of dietary fiber in the feed all have an impact on which species will flourish as well as which metabolites will be produced (Buddington 2009; Scott et al. 2008). Populations of bacteria and concentrations of SCFA have been found to differ according to whether the broilers were fed a corn or wheat –based diet (Mathlouthi et al. 2002) and between a corn or wheat/rye based diet (Hübener et al. 2002). Dietary CHO can also affect the transit time and pH of the gut and thereby influence the gut environment present and change the environmental niches available. In culture, a lower pH resulted in a shift in production of butyrate and propionate to acetate and lactate. This is because bacteria which produce butyrate are most efficient at competing for substrate when the pH is mildly acidic. If the pH is too low, such as 5.2, lactate and acetate producers dominate instead (Scott et al. 2008).

Several factors can affect the delicate microflora ecosystems and balance of cooperation between the host and the microflora. Stressors are one of the main disruptors of this balance (Dunkley et al. 2009). Stressors can cause changes in the intestinal microflora and the intestinal structures which greatly increases the ability of pathogens to colonize (Burkholder et al. 2008). Disruption of feed for example can have a huge impact on the microbe populations by upseting the ecosystems and thereby leaving the animal vulnerable to pathogens (Pieper et al. 2010). Other stressors such as heat stress or increased stocking density can also increase vulnerability (Rigby and Pettit 1980; Mulder 1995; Isaacson et al. 1999; Poppe 1999; Jones et al. 2001).

2.3 Antibiotic and Antibiotic Alternative Use in Animal Agriculture

2.3.1 History of Antibiotic Use in Animal Agriculture

Antibiotics have in the past been commonly used to promote growth in animal agriculture. Their effects were first observed in the 1940s when dried mycelia of *Streptomyces aureofaciens*, which contained chlortetracycline residues, was found to improve growth when fed to animals (Castanon 2007). In 1994 however vancomycin-resistant *Enterococcus* were isolated from farm animals in Great Britain and it was suggested that farm animals could be providing a reservoir for the development of these bacteria (Bates et al. 1994). Following this discovery, Denmark slowly decreased the number of antimicrobials legal for use, with avoparcin being the first banned in 1995 (Dibner and Richards 2005). It was thought that avoparcin's use in animal agriculture would give cross – resistance to vancomycin which is used to treat enterococcal infections in intensive care units (Wray and Davies 2000). In 2000 all non – therapeutic antimicrobials were banned in Denmark (Dibner and Richards 2005). The European

Union followed this with an AGP ban in 2006 (Castanon 2007). In the United States there is now a push to eliminate the use of the AGP flouroquinolone as it is similar to human medicinal drugs (Dibner and Richards 2005). In addition, use of cephalosporins antibiotics for animals was recently restricted by the FDA (Harris 2012). There are outside demands on the North American industry as well. Consumer demands for AGP free birds have increased (Janardhana et al. 2009) and birds must be AGP free in order to be sold to the European Union. In addition, the World Health Organization has suggested that national governments should work towards eliminating AGP in animal agriculture and using them only for therapeutic use (Dibner and Richards 2005). Due to this there is a need for effective antibiotic alternatives to use in animal agriculture before a full AGP ban is put in place.

2.3.2 Effects of Antibiotic Use on Animal Health and Growth

The GIT has been confirmed as the region of AGP activity as some of the antibiotics used are not able to be absorbed (Dibner and Richards 2005). It is also known that AGP exert their effects by acting on the microbes found within the gut as they have no effect on germ-free animals (Dibner and Richards 2005). AGP use can enact several types of changes in the microflora ecosystem. These include reducing the total bacterial load in the gut, reducing pathogen colonization, increasing the growth or metabolism of beneficial bacteria (Lu et al. 2008), and/or homogenizing ileal microbial populations which promotes growth and uniformity of growth (Collier et al. 2003).

Antibiotics act via several mechanisms. Antibiotics like Bacitracin are thought to act by interfering with bacterial cellular processes (Pollock et al. 1994) such as inhibiting

the ability of bacteria to synthesize the cell wall (Stone and Strominger 1971). Others distort the mucosal barrier to alter ability of pathogens to attach (Sklan 2004). Ionophores such as Salinomycin alter the transport of ions across biological membranes (Augustine and Danforth 1999). Virginiamycin blocks protein synthesis by interfering with ribosomes (Chinali et al. 1981).

Antibiotic types not only vary in their mode of action but also in which bacterial populations are affected. For example, Bacitracin/Virginiamycin, and Monensin were found to reduce diversity of ileal bacterial communities and increase those that were rich in *Clostridium*. Bacterial species such as *Lactobacillus* were supressed (Lu et al. 2008). Avilamycin and Salinomycin were found to alter bacterial communities without affecting diversity. They most affected *Lactobacillus* and *Clostridium perfringens* (Knarreborg et al. 2002). Salinomycin has been shown to decrease total bacteria in the ileum (Chichlowski et al. 2007 b). Bacitracin decreases *Clostridium perfringens* (Engberg et al. 2000), *Enterococcus*, *Lactobacillus*, and *Staphylococcus* populations (Barnes et al. 1978; Dutta and Devriese 1981; Devriese 1980).

The alterations in microbial patterns that are caused by AGPs' actions result in a number of indirect effects as well. As microflora levels are decreased competition for nutrients and production of microbial metabolites that decrease growth such as ammonia, amines, phenols, and indoles produced from protein fermentation are also reduced (Dibner and Richards 2005). Fewer microbes in the gut also decreases the subclinical immune response to their presence (Dibner and Buttin 2002). In addition, antibiotics reduce gut size by lowering the SCFAs produced by microbial fermentation which results in lower mucosa cell proliferation, thinner villi, thinner lamina propria, and a thinner gut

wall leading to increase efficiency of nutrient use (Dibner and Richards 2005; Niewold 2007). Antibiotic types which act on the mucosal barrier also initiate changes in the barrier that alter absorption of macromolecules and ions (Sklan 2004).

2.3.3 Detrimental Effects of Antibiotic Use on Animal Health and Growth

While AGP are beneficial in their effects on animal growth, there are several aspects of their use which bring their value into question. The main concern with AGP use is the development of resistant bacteria. It is thought that the selective environmental pressure they place on the gut microbiota causes increased incidence of antibiotic resistant genes which can then be transferred horizontally to pathogenic bacteria. Changing the diversity of microbial populations in the gut can also make niches available which allow resistant strains commonly found in the gut to overgrow such as *Clostridium difficle* and *Candida albicans* (Lewis et al. 2010). These changes can also make the animal vulnerable to infection as, while nonharmful to the host at low levels, overgrowth of both of these species can lead to disease (Buck 1990; Songer and Anderson 2006). Alteration of microbial densities can also make the animal vulnerable to pathogenic colonization if infected with pathogens from the environment before the native microflora can re-establish its niches after antibiotic treatments are terminated (Sekirov et al. 2008). In addition to resistance and pathogen colonization, antibiotics can at times be detrimental to growth. For example, Salinomycin was shown to have a low toxicity threshold under clean conditions which resulted in decreased BW and increased energetic demands on the intestinal tissues of broiler chickens (Chichlowski et al. 2007 a).

2.3.4 Current Alternatives to Antibiotic Use in Animal Feed

Since the EU ban on AGPs, and due to threat of a ban in North America, there is an increased demand for alternatives to AGP that can effectively promote the health and growth of production animals. This need can be demonstrated in the 5% increase in the use of antibiotics therapeutically in Denmark since the ban and the steady increase in the use of the anticoccidial Salinomycin, possibly indicating an increase in the need to control Necrotic Enteritis in the European Union (Dibner and Richards 2005). There has been increased susceptibility to intestinal Spirochaetosis, Avian Colibacillosis, and Necrotic Enteritis in the European Union since the ban (Collins et al. 2009).

Any alternatives studied should be done so with the view that they must improve feed efficiency, gain, and livability to the same degree as antibiotics (Dibner and Richards 2005). Currently a large number of alternatives are being investigated. Vaccinations have been developed for *Campylobacter* and *E.coli* 0157 (Wray and Davies 2000). Organic acids such as butanoic acid and lactic acid have been widely used and have been found to alter microbial populations in the gut (Nava et al. 2009; Dibner and Richards 2005) by killing acid sensitive bacteria and creating a gut environment that is detrimental for pathogenic growth (Dibner and Buttin 2002; Verstegen and Williams 2002). Enzymes are used to increase feed digestibility and decrease intestinal viscosity thereby limiting substrate availability for the microflora in the ileum and decreasing pathogenic load (Preston et al. 2001; Sun et al. 2005). In addition to the aforementioned alternatives, directly feeding benefical microbes and/or directly feeding them substrates to improve their growth in the form of probiotics, prebiotics, and synbiotics has been a

field which has shown promise in providing a usable alternative to AGP (Buddington 2009; Gibson and Roberfroid 1995).

2.4 Probiotics

2.4.1 The Probiotic Concept

Probiotic supplementation uses the knowledge obtained about the benefits of the native gut microflora populations to attempt to influence this microflora in such a way as to allow the host to obtain optimal effects from the relationship. Probiotics are defined as "live microbial feed supplement[s] which beneficially affect the host animal by improving its intestinal microbial balance" (Fuller 1989). Probiotics, also known as direct fed microbials, are composed of beneficial species such as *Lactobacillus* and *Bifidobacterium* (Dunkley et al. 2009). Their use has resulted in increased metabolism, lower ammonia levels, enhanced immunity, increased gut maturation and integrity, increased epithelial cell survival, and increased feed intake (FI) in poultry (Dunkley et al. 2009; Stoidis et al. 2010). These effects are attained through the alteration of gut microflora composition, competition with pathogens, pathogen displacement from the gut, decreased pH of the gut environment, and increased production of antimicrobial substances (Collins and Gibson 1999; Stoidis et al. 2010). In order to enact these effects probiotics must have certain characteristics. They must be able to attach to the intestinal epithelium, establish colonies, secrete antibacterial substances, ferment substrates available from the diet, and/or alter immune function in a way beneficial to the host (Dhama et al. 2008; Lin 2003; Collins and Gibson 1999). However, probiotics are not typically able to adhere to the mucosa for long and so are usually eluted from the gut within a few days if not regularly consumed (Marteau et al. 2004).

2.4.2 Probiotic Types Available for Use

Several different types of probiotic are available. Some of these are single bacterial species while others are composed of mixed – cultures (Dhama et al. 2008). The optimal probiotic products are able to be viably made in a large scale and remain viable through storage and use in animals. They must have the ability to survive the gut environment so as to reach the lower GIT intact and remain animate enough to attach to the epithelium, compete with pathogens, and ferment substrates once there (Gibson and Roberfroid 1995; Dhama et al. 2008). Surviving the upper GIT can be a challenge as most probiotic strains are anaerobic and sensitive to extreme temperatures. To increase a probiotic's vitality in the intestine, bacterial strains are at times encapsulated to reach the lower GIT intact (Gibson 2004). In addition, the probiotic must be non-toxic, non pathogenic, and able to participate in the symbiotic relationship with the host (Dhama et al. 2008). Different probiotic strains and combinations will have different effects. The probiotic used must be catered to the particular conditions within an animal. For this reason probiotic strains used in animal nutrition are different from those commonly used in human nutrition (Pieper et al. 2010).

Commonly used genera in probiotics include lactic acid producing bacteria like *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, and *Bifidobacterium* (Gibson 2004; Roberfroid 2007). Within these genera a variety of species such as *L. sporogenes*, *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. plantarum*, *L. cellbiosis*, *L. salivarius*, *Streptococcus faecium*, *Streptococcus thermophiles*, and *Enterococcus faecium* are used (Roberfroid 2007; Dhama et al. 2008). In addition to bacteria, yeast and fungi are found in probiotic products, such as *Saccharomyces cervisae* (Gibson 2004; Dhama et al. 2008).

In livestock species *Bacillus*, *Enterococcus*, and *Saccharomyces* have been the most common probiotic genera (Owens et al. 2008). In addition, undefined cultures of bacteria are sometimes administered with beneficial effects (Yang et al. 2009).

2.4.2.1 Probiotic Use as Competitive Exclusion Treatments

Included in the concept of probiotics are competitive exclusion cultures which act under the same mechanisms but which are more typically fed during early development and are obtained directly from adult birds' digesta. The discovery of competitive exclusion cultures demonstrates the power of probiotic bacteria. Nurmi and Ranatala (1973) first discovered competitive exclusion when a severe outbreak of *Salmonella infantis* occured among Finnish broiler flocks. The cause of this outbreak was thought to be due to the sanitary conditions in which the broilers were being raised impeding the development of the normal microbiota. To test this theory the researchers injected 0.5 mL of adult bird gut contents into one group of chicks while the other was raised in sanitary conditions. Both of the groups were then inoculated with *S. infantis*. Those given a competitive exclusion culture were 69% free from *S. infantis* while all birds raised under the sanitary conditions were infected. There are several types of competitive exclusion treatments which include defined and undefined cultures. Evidence suggests that the latter is more effective. However, for any competitive exclusion culture to work optimally, it must be applied at hatch (Wray and Davies 2000).

2.4.3 Effects of Probiotic Supplementation on Animal Health and Growth

Probiotics act much in the same way that the normal microflora does to improve host health. Where they differ is the careful selection of probiotics to alter the flora composition in a particular manner to improve the health and growth of the host. This increases control over microflora effects comparatively to if the population had been composed of only those bacteria available in the environment. Probiotics act directly via exclusion of pathogens. Indirectly they also enhance the host's own defense systems, such as the mucosal barrier and immune system, as well as alter fermentation processes and nutrient absorption in the gut (Howarth 2010; Chichlowski et al. 2007a, 2006).

2.4.3.1 Probiotic Effects on Pathogen Resistance

The most basic way in which probiotics decrease pathogen presence is via competition. Viable probiotics which reach the gut begin to adhere to the intestinal epithelium and mucosa. This process then depletes the number of adhesion sites available for pathogens. Once attached, probiotics begin to ferment substrates available in the gut, again limiting the amount of nutrients available for pathogens to utilize. The fermention of substrates by probiotics leads to increased production of SCFA and chemically modified bile acids which then leads to an environment unfavourable for most pathogen growth (Bosscher 2009; Stoidis et al. 2010). Probiotics have been found to increase intestinal mucin gene expression to further inhibit pathogens' ability to adhere to the epithelium (Mack et al. 1999). Pieper et al. (2010) fed *Enterococcus faecium* and *Bacillus cereus* to sows which then transferred the probiotics to their piglets via suckling. This treatment was found to decrease *E. coli* in the gut of the piglets leading to reduced

diarrhea. Similarly, Awad et al. (2009) found lower mortalities for *Lactobacillus* fed broilers compared to the control.

Probiotics further protect against pathogens by enhancing immune function. Bacteria fed for the purpose of mucosal system stimulation are known as immunobiotics (Clancy 2003). They have been found to induce antibacterial defensin secretion by Paneth cells, and increase IgA, T-cell, macrophage, and Th1 cytokine activity. In addition, they have been shown to increase anti-inflammatory cytokines such as IL-10 (Stoidis et al. 2010). *Lactobacillus* increased the relative weights of the spleen, thymus and liver when fed to broilers (Awad et al. 2009). However, which immune systems become activated depends on the probiotic strains fed. *L. paracasei* was found to increase phagocytic activity of cells in the ceca and ileum while *L. plantarum* stimulated antigen specific titres for example (Yang et al. 2009).

2.4.3.2 Probiotic Effects on Intestinal Histomorphology

Probiotics have been shown to alter the microstructures of the GIT and affect cell turnover. A probiotic containing *Lactobacillus*, *Bifidobacterium thermiphilium*, and *Enterococcus faecium* was found to increase jejunal villi height and decrease crypt depth compared to both an antibiotic treatment and the control (Chichlowski et al. 2007b). Primalac, a probiotic composed of several *Lactobacillus* and *Streptococcus* species, was found to increase villi height and perimeter in the jejunum, increase intestinal muscle thickness, and increase crypt depth and goblet cell numbers when fed at 0.3% to broilers. The researchers observed that the mucus layer on the intestines was thinner with the probiotic but that it was more evenly distributed (Chichlowski et al. 2007 b). Conversely, Ikeda et al. (2002) noted an increase in goblet cell number when feeding a probiotic. In

addition to the above effects, probiotics are thought to enhance tight junction integrity in times of inflammation or infection to aid in prevention of pathogen invasion (Montalto et al. 2004; Shen et al. 2005).

2.4.3.3 Probiotic Effects on Animal Nutrition

Probiotics not only act to protect the host from pathogens but also to improve growth and nutrient absorption. They have been found to stimulate cellulytic bacterial growth and increase fiber digestion as well as influence passive nutrient transport (Chichlowski et al. 2006, 2007b; Dhama et al. 2008). How probiotics affect nutrition depends on strains and species utilized. Probiotics designed to reach the ceca or colon have the most influence on metabolism (Neish 2009). These effects, as well as those on the birds' health, act to increase overall growth performance. Awad et al. (2009) found increased BW, average daily gain, and feed conversion ratio in broilers fed *Lactobacillus* spp. compared to the control associated with increased nutrient absorption capacity of the intestines.

2.4.4 Influence of Environment on Probiotic Performance

The beneficial effects observed from any given probiotic product depend on the species found within, their viability, at what level the product is given, how the product is administered, how frequently and for what duration treatment is given, the age of the birds, and environmental conditions (Ewing and Cole 1994; Patterson and Burkholder 2003). Gut environment, which itself is highly influenced by diet, can have a large effect on whether probiotics are able to survive. In the gut, regardless of diet, bacteria must overcome gastric acid, pancreatic enzymes, and competition for space in the large intestine (Collins and Gibson 1999). Even at high doses, probiotic bacteria make up less

than 0.01% of the total bacteria in the GIT or less (Buddington 2009) and so it can be difficult to ensure effects are observed. This limited colonization of probiotic bacteria and requirement for continued high dosages in the feed, in addition to the variation in results with different strains and species, reduces the practicality of probiotic use as feed additives in animal agriculture.

2.5 Prebiotics

2.5.1 The Prebiotic Concept

As it is difficult for probiotics to remain viable and to establish large populations in the gut the concept of prebiotics was introduced as a way of increasing beneficial bacteria populations without administering them directly. As with probiotics, the goal of feeding prebiotics is to increase beneficial bacterial species and so reduce pathogen load and increase host health (Buddington 2009). Prebiotics are defined as "a selectively fermented ingredient that allows specific changes, both in composition and/or activity in the GIT microflora, that confer benefits upon host well being and health" (Roberfroid 2007). Prebiotics offer several advantages over probiotics in that they increase populations of bacteria already present, can affect multiple species of beneficial bacteria at the same time (Yang et al. 2009; Buddington 2009), are cheaper, are easier to include in the diet, and are more likely to reach the lower GIT (Dhama et al. 2008).

2.5.2 Requirements for Definition of a Supplement as a Prebiotic

Inherent in the definition are several requirements which any substance must fulfill before it can be labelled as a prebiotic. A prebiotic must be non fermentable in the upper GIT and reach the lower GIT intact, it must be selectively fermented by a limited number

of beneficial bacteria and stimulate their growth or metabolism, and finally it must alter the microflora populations in such a way that the host's health is increased (Gibson and Roberfroid 1995).

Reaching the lower GIT intact requires feedstuffs to resist gastric acidity, digestive enzymes, and absorption in the small intestine. Fulfillment of this criterion does not require feedstuffs to remain entirely intact all the way to the lower GIT but does require the majority to do so. Candidate prebiotics can be shown to meet the criteria by measurement of their recovery in feces of germ – free rats or killing animals at predetermined time periods after feeding the substrate to measure levels in fecal and GIT contents (Roberfroid 2007). Many foods are able to meet this requirement. Indigestible portions of most feed ingredients include dietary fiber, resistant starches, minerals, polyphenols, and lipids (Saura-Calixto et al. 2000; Cummings and Macfarlane 1991; Garcia et al. 2006; Fleury and Lahaye 1991). These feedstuffs are able to be digested by intestinal microbes as well (Józefiak 2004); however, most of them are not able to fulfill the next requirement of selective fermentation and so are designated as colonic foods rather than prebiotics (Gibson and Roberfroid 1995).

Selective fermentation by beneficial bacteria such as *Lactobacillus* or *Bifidobacterium* is thought to be the most difficult criteria to fulfill (Van der Meulen et al. 2006). Analysis of a candidate prebiotic's ability to meet this requirement involves anaerobic fecal sampling and quantitative microbial analysis of bacteria present, comparing animals fed a control and the test prebiotic. This analysis must include a wide range of bacterial genera. Fecal samples can also be taken and incubated with the prebiotic. The activity of specific bacteria can then be analyzed (Roberfroid 2007).

Demonstration of the last criteria of improving host health is more straightforward and involves measuring animal health in some capacity. This can be a matter of mortality or growth factors. Internal variables such as immune function, nutrient absorption, and histomorphology are also measured as part of this evaluation (Barry et al. 2009). Those substances which are found to meet all three of these criteria can be labeled as prebiotics (Roberfroid 2007).

2.5.3 Effects of Prebiotic Supplementation on Animal Health and Growth

2.5.3.1 Influence of Prebiotic Supplementation on Gut Microflora Populations

Increased beneficial bacteria numbers and/or diversity is one of the required characteristics of prebiotics; however, which microflora species are increased depends on the prebiotic in question. Some prebiotics have been shown to increase *Bifidobacterium* and *Lactobacillus* (Dunkley et al. 2009; Barry et al. 2009; Tuohy et al. 2009). Mannosoligosaccharide (MOS), the candidate prebiotic Astragalus polysaccharide, inulin, and glucooligosaccharides (GOS) have been shown to increase *Lactobacillus* (Baurhoo et al. 2009; Li et al. 2009; Huebner et al. 2007) while MOS, xylooligosaccharides (XOS), arabinoxyoligosaccharides, and transgalactooligosaccharides (TOS) have demonstrated increased *Bifidobacterium* (Baurhoo et al. 2009; Courtin et al. 2008; Li et al. 2009; Ito et al. 1993; Bouhnik et al. 1997). Conversely, none of the oligosaccharides inulin, oligofructose, MOS, short chain FOS, or TOS fed at 4g/kg were found to have no effect on levels of *Bifidobacterium*, *Lactobacillus*, *Clostridium perfringens*, or *E. coli* (Biggs et al. 2007). Huebner et al. (2007) found that different beneficial bacterial species were able to benefit differently

from various prebiotics. Inulin increased *L. paracasei* while GOS increased *L. plantarum* and *L. acidophilus* for example. Thus, effects observed will not only depend on the prebiotic fed, but also on the species present in the gut prior to feeding.

In addition to influencing the bacterial populations' growth, prebiotics can also influence their metabolism. They have been shown to shift bacterial metabolism from proteolytic to saccharolytic in mice, thus favouring production of SCFA (Gibson and Roberfroid 1995).

2.5.3.2 Effects of Prebiotic Supplementation on Pathogen Resistance

One of the desirable outcomes of feeding prebiotics is the inhibition of pathogen presence in the gut. Therefore most prebiotics are evaluated for their ability to reduce pathogenic bacteria. Decreased *Salmonella*, *Coliforms*, and *E. coli* have all been observed with various prebiotics in poultry (Barry et al. 2009). Low levels of MOS were found to decrease both cecal *E. coli* and *Campylobacter* spp.. Birds fed 2.5% of the candidate prebiotic Alcell lignin and challenged with *E. coli* were found to have decreased *E. coli* levels (Baurhoo et al. 2007). The candidate prebiotic Astragalus polysaccharide also decreased *E. coli* in the ileum and cecum (Li et al. 2009).

Some prebiotics are thought to decrease pathogens by binding them directly. Attachment is often by mimicking antigenic binding sites used by pathogens to identify thier hosts (Collins et al. 2009), increasing competition by increasing beneficial species (Steed and Macfarlane 2009), and/or bringing about the production of antimicrobial compounds via their fermentation (Rehman et al. 2009 b; Collins et al. 2009). Another mechanism of pathogen inhibition is priming of the immune system. Prebiotics increase secretory IgA (Agunos et al. 2007), cytokines (Vos et al. 2007), and lymphocyte

populations within Peyer's patches (Manhart et al. 2003). Priming of heterophils to phagocytize invading *Salmonella* in the gut by prebiotics has been reported (Collins et al. 2009). When Li et al. (2009) supplemented chicks with Astragalus polysaccharide, increase in humoral immunity, increase in cellular immunity, and increase in immune organ weights were observed. The cause of these effects is not yet fully understood; however, it is thought to be due to either the increase in beneficial bacteria or SCFAs, or both as either are able to stimulate the immune system in and of themselves (Steed and Macfarlane 2009). In some cases it is the prebiotics themselves which are able to enhance immune function (Akramiene et al. 2007).

2.5.3.3 Effects of Prebiotic Supplementation on Intestinal Histomorphology

Studies of prebiotics commonly show them to have beneficial effects on intestinal microstructure. Alphamune, made of yeast extract, was shown to increase duodenal and ileal villi height in birds (de los Santos et al. 2007). MOS has been demonstrated to increase villi height and goblet cell numbers (Baurhoo et al. 2009). Aspergillus polysaccharide has been shown to increase ileal crypt depth (de los Santos et al. 2005). Birds fed the prebiotic Bio-Mos, containing MOS, were found to have thinner cecal lamina propria which was interpreted as being due to fewer pathogens present (Sun et al. 2005).

2.5.3.4 Effect of Prebiotic Supplementation on Animal Nutrition

By altering bacterial populations, prebiotics are able to alter microbial fermentation and nutrient metabolism. SCFA production increases as the prebiotics are fermented but effects on minerals and lipogenesis have also occured. The higher SCFA levels lead to a decreased pH which increases absorption of cations such as Ca^{+2}. These effects have been

associated with increased bone preservation (Steed and Macfarlane 2009). By interacting with tight epithelial cell junctions there is increased absorption of minerals, such as Mg^{+2}, Fe^{+2}, and Zn^{+2}, via increased paracellular permeability. Iron absorption in the large intestine increased by 23% when prebiotics were fed. Lipogenic enzymes have increased gene expression with prebiotics, leading to decreased serum VLDL (Steed and Macfarlane 2009). Absorption of other nutrients has also been enhanced by prebiotics. Prebiotic fermentation provides energy to bacteria that produce enzymes such as fructosidase, xylanase, and other hydrolases able to increase nutrient availability (Fukata et al. 1999; Sun et al. 2005) thereby releasing fructose, xylose, and various catabolites which can be utilized by the host (Ewing and Cole 1994).

2.5.3.5 Effects of Prebiotic Supplementation on Animal Growth

One of the main goals of prebiotic use in animal agriculture is as a replacement for AGP and as such they are used to increase growth performance. Prebiotics such as FOS and MOS increased FI, improved feed conversion, and increased weight gain (Collins et al. 2009). Bio-Mos improved feed conversion ratios and increased final BW (Sun et al. 2005). The candidate prebiotic ProFeed, made of sugar beet short chain FOS increased body weight gain (BWG) and improved feed conversion ratio (Catalá-Gregori et al. 2008). However, some studies do not find any affect on growth. Baurhoo et al. (2007) for example did not find MOS to alter growth performance at all.

2.5.4 Influence of Environment on Prebiotic Performance

Variations in results of prebiotic research are thought to be due to influence of bird strain, the prebiotic used, and environmental conditions (Geier et al. 2009). Commercial growing conditions present birds with more challenges, such as greater density and

previously used litter, than those found in most experimental situations. Typically when researchers simulate the environment of commercial production situations prebiotic results are greater than those observed in sterile laboratory settings (Janczyk et al. 2010; Catalá-Gregori et al. 2008). The presence of any stressor, for example high temperature, can increase effectiveness of prebiotics (Bailey et al. 1991; Orban et al. 1997).

Other factors are able to influence prebiotic results as well. The birds' diet determines concentration of substrates available to bacteria, in addition to the prebiotic. Fermentability of these substrates, their influence on the gut environment, and the alterations in bacterial populations will have an effect on the prebiotic response (Buddington 2009). Gender of the birds studied can also influence the response. Bozkurt et al. (2009) found liver weight and small intestinal weight to be lowered with prebiotic supplementation in males but not females. Conversely Rehman et al. (2009 b) found oligofructose at 9% and 10% increased BW and improved feed conversion in female, but not male birds. Location of the prebiotic in the gut affects what influence it will have as the conditions prevailing in that particular gut region can alter the physiochemical properties of the fiber (Ohta et al. 1997; Guillon et al. 1993; Hoebler et al. 1998). The final determination of what will result from prebiotic supplementation depends on the bacteria already present in the gut and their concentration when the prebiotics are fed (Roberfroid 2007).

2.5.5 Prebiotic Types Available for Use

Typically, prebiotics contain some sort of CHO such as oligosaccharides, non – starch polysaccharides, or starch (Rehman et al. 2009b). Different types of CHO will

have varying effects and be fermented into a range of end products by the microflora (Dhama et al. 2008). Most researchers of prebiotics have investigated FOS and similar products. Other prebiotic candidates in the literature include TOS, GOS, glycooligosaccharides, lactulose, lacitol, maltooligosaccharides, XOS, stachyose, raffinose, and sucrose thermal oligosaccharides (Patterson and Burkholder 2003). In broiler chickens FOS, inulin, MOS, α – gluco – oligosaccharides, isomaltooligosaccharides (IMO), ketoses, lactose, stachyose, and oligochitosan have been researched (Yang et al. 2009; Rehman et al. 2009b).

The nature of the prebiotic determines where in the gut it will be fermented and how easily. The longer the oligosaccharide chain the slower the prebiotic is fermented and the further it will pass down into the GIT (Van Den Broek and Voragen 2008). More structurally complex prebiotics are fermented more slowly and make it further into the gut (Gibson 2004). When Courtin et al. (2008) tested a variety of prebiotics in broilers it was the least complex, XOS, that was able to increase *Bifidobacterium* populations sooner than the others. Less substituted prebiotic components are more efficiently used by bacteria such as *Bifidobacterium* and so have more of an impact on animal health and growth (Gibson 2004).

Different prebiotics target distinct bacterial species and so may have differing impacts depending on what bacteria were originally present. Geir et al. (2009), comparing the prebiotics MOS and FOS, found that different cecal bacterial profiles were observed when each of the prebiotics was fed. The *Lactobacillus* species profiles present in the ileum were found to be different in particular. Upon closer examination *L. johnsonii* and *L. reuteri* were the species responsible for the varying profiles, with each

responding differently to the substrates. This variation was most likely due to different fermentation enzymes available to each species.

There are several candidate prebiotics which are tested as prebiotics but have not yet been proven to meet all criteria. TOS are composed of oligosaccharides derived from lactose containing β(1,6), β(1,3), and β(1,4) linkages. It has been proven to reach the colon intact but has not been proven to be selectively fermented (Roberfroid et al. 2007). IMO is produced from glucose and contains isomaltose, panose, isomaltriose, and branched oligosaccharides made of glucose. It selectively increases cecal *Bifidobacterium* spp., but can be hydrolyzed in the upper GIT. Lactose promotes lactose-fermenting bacteria, but can be hydrolyzed in the upper GIT as well and so is also not a true prebiotic (Rehman et al. 2009b).

2.5.5.1 Mannosoligosaccharides as Prebiotics

MOS are derived from the cell wall of the yeast *Saccharomyces cerevisiae* and are composed of mannose and glucan (Geier et al. 2009). These CHO are indigestible in the upper GIT and are sometimes labelled as prebiotics (Flickinger and Fahey 2002). This term is debated by some, as MOS do not fulfill the criteria of selective fermentation. Instead of working via this mechanism they instead bind to pathogens possessing type 1 fimbria with their mannose residues and thereby remove them from the gut (Patterson and Burkholder 2003; Yang et al. 2009) and prevent their adherence to the mucin (Newman 1994). Pathogens able to bind to MOS include *E. coli* and *Salmonella* (Rehman et al. 2009b). Through this mechanism, MOS decreased *E. coli* in the litter (Baurhoo et al. 2007) and *Salmonella* in the ceca of chicks (Spring et al. 2000). MOS reduced *Salmonella typhimurium, Salmonella dublin*, *Campylobacter* spp., and *Clostridium*

perfringens infection (Rehman et al. 2009b). While not being selectively fermented, MOS has been shown to increase populations of beneficial bacteria (Baurhoo et al. 2009) such as cecal *Lactobacillus* spp. and *Bifidobacterium* spp. (Rehman et al. 2009b). MOS enact some of the same beneficial effects as prebiotics just not through fermentation. They have been shown to reduce pathogen populations, modulate the immune system, modify intestinal morphology, and alter mucin and brush border enzyme expression (Yang et al. 2009). MOS increased villi height and goblet cell number, and reduced crypt depth (Baurhoo et al. 2009; Iji et al. 2001b). Broilers being fed MOS had increased weight gain, improved feed conversion, and decreased mortality (Hooge 2004).

2.5.5.2 Inulin as a Prebiotic

Inulin is a nondigestible CHO derived mainly from chicory root containing β (2,1) linkages of fructose (Bosscher 2009). Inulin met all three criteria for a prebiotic and so is considered a reference prebiotic (Roberfroid 2009). Inulin altered metabolism, increased immunity, and decreased pathogenic presence (Bosscher 2009). As it is such an important prebiotic it will be covered further in its own section (see section 2.7).

2.6 Synbiotics

Synbiotics are mixtures of probiotics and prebiotics. Their use is an attempt to increase viability of probiotic bacteria when they reach the gut by providing them with substrates which they are specifically adapted to ferment (Awad et al. 2009). Synbiotics have been found to increase levels of *Bifidobacterium* and *Lactobacillus* (Bielecka et al. 2002; Li et al. 2009). The synbiotic Biomin IMBO increased BW and average daily gain, and improved broiler feed conversion ratio over a probiotic alone (Awad et al. 2009). Ghasemi et al. (2010) also found Biomin IMBO to improve feed conversion in birds. A

synbiotic of Astragaulus polysaccharide and probiotics resulted in increased humoral and cellular immune function (Li et al. 2009). Thus, synbiotics can be used to enact equal or better effects than probiotics or prebiotics alone and may present the best answer to antibiotic alternatives.

2.7 Inulin

FOS are hexose based oligosaccharides (Swennen et al. 2006) composed of short and medium length chains of β-δ linked fructans with fructosyl units linked together by β(2,1) glycosidic linkages (Gibson and Roberfroid 1995) (Figure 4). It is the β (2,1) linkages which are not able to be hydrolyzed in the upper GIT and allow the FOS to reach the lower GIT intact (Flickinger et al. 2003; Roberfroid 2005; Niness 1999).

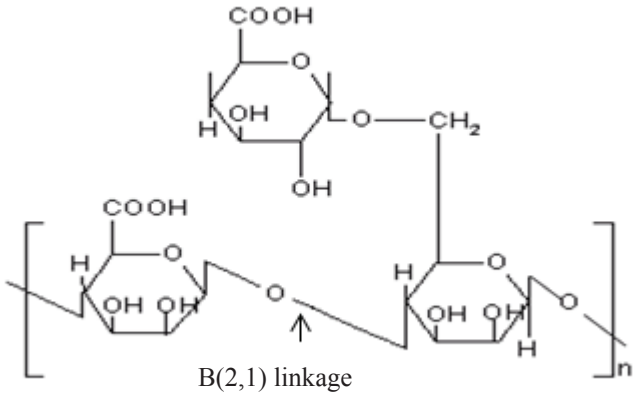


Figure 4: Structure of Inulin from Chourasia and Jain (2003)

FOS are further categorized according to the degree of polymerization (DP) present in their fructan chains. FOS with less than less then 9 DP are typically labelled as oligofructose while FOS with 9 - 60 DP are typically labelled as inulin (Gibson and Roberfroid 1995). Inulin can be further classified into short or long chain with short chain inulin having an average DP of 12 and long chain inulin having an average DP of 25

(Crittenden 1999). However, while there is a determined terminology, inulin in the literature is defined by various chain lengths and terms are often misused and used interchangeably in product labeling and in the literature (Rehman et al 2009b; Flickenger et al. 2003). Most of the FOS used as prebiotics in the animal agriculture industry are derived from chicory root, though FOS is also found in leeks, onion, garlic, wheat, artichoke, and bananas as plant storage CHO (Bosscher 2009; Candela et al. 2010). FOS and its related prebiotics have been highly researched and proven to meet all three criteria for a prebiotic (Gibson and Roberfroid 1995). It is therefore used as a reference to compare candidate prebiotics against and is considered the gold standard of prebiotics (Bosscher 2009).

2.7.1 Effects of Inulin Supplementation on Animal Health and Growth

2.7.1.1 Inulin Supplementation Effects on Beneficial Microflora Populations

FOS are able to be completely fermented by *Bifidobacterium* and *Lactobacillus* in the lower GIT (Hartemink et al. 1997). Bifidobacteria are particularly adept at fermenting FOS as they produce fructosylfructanosidase enabling them to hydrolyze the bond between inulin and oligofructose's fructose moieties (Fooks and Gibson 2002; Roberfroid 2007). Patterson et al. (2010) found inulin of multiple chain lengths to increase *Bifidobacterium* and *Lactobacillus* in the lumen of pigs throughout the small intestine. Rebole et al. (2010) found inulin to increase *Bifidobacterium* and *Lactobacillus* in the ileum and ceca of broilers when fed at 0, 10, and 20g/kg. Deville et al. (2007) found FOS to increase *Lactobacillus* and decrease populations of *Bacteriodes* in culture compared to glucose, indicating a high prebiotic activity. *Eubacteria* and *Roseburia* increased with inulin supplementation (Steed and Macfarlane 2009). However, results are

not always consistent as some studies do not find bacterial populations to be transformed, though some observe the metabolic activities of the bacteria are altered instead (Rehman et al. 2009b).

2.7.1.2 Inulin Supplementation Effects on Pathogen Presence in the Gastrointestinal Tract

The alterations in beneficial bacteria present may lead in turn to lowered pathogen presence. Patterson et al. (2010) found inulin to decrease *Clostridium* spp., *Streptococcus* spp., and *Enterobacteriacae* populations in the lumen and mucosa throughout the small intestine of swine. Reduced susceptibility to infection by *Salmonella* occurred with birds fed FOS (Bailey et al. 1991; Fukata et al. 1999; Donalson et al. 2008). Decreased levels of *E. coli*, *Salmonella* spp., and *Campylobacter* spp. have been demonstrated in broilers fed inulin (Yusrizal and Chen 2003).

2.7.1.3 Inulin Supplementation Effects on Immune Function

FOS aids in pathogen removal by priming the immune system, but is also able to help downgrade chronic inflammation caused by microflora presence in the gut (Patterson et al. 2010). FOS has been found to increase IgM and IgG antibody titers in the plasma, increase B cells, and influence the percentage of T cell and macrophage phenotypes observed; however, it had no affect on proinflammatory or anti-inflammatory cytokines (Janardhana et al. 2009). Yasuda et al. (2009) found inulin to down regulate inflammation genes such as tumor necrosis factor, which is produced in response to the bacterial cell wall antigen LPS (Beutler and Cerami 1989; Ziegler-Heitbrock and Ulevitch 1993). Gram- positive bacteria like *Lactobacillus* and *Bifidobacterium* do not contain LPS, and so it is thought that by increasing their populations inulin is able to down regulate inflammatory genes (Patterson et al. 2010).

2.7.1.4 Inulin Supplementation Effects on Short Chain Fatty Acid Production in the Gastrointestinal Tract

Fermentation of FOS and its derivatives by bacteria results in the production of SCFA. Inulin is fermented into lactic acid, SCFA, and gases. Butyrate is found to increase when inulin is fed due to cross- feeding between bifidobacteria, which does not produce butyrate, and *Eubacteria* and Clostridial species which do, via the conversion of lactate and/or acetate (Bosscher 2009). Rebole et al. (2010) found inulin to increase n-butyric acid and D- lactic acid and increase the ratio of n- butyric: acetic acid when fed to broiler chickens. Rehman et al. (2008) found the proportion of n- butyrate to increase and observed a decrease in n- valerate, though total amounts of SCFA were not affected, when inulin was fed to chickens. The changes in SCFA by inulin were not found to alter cecal pH at all by Rebole et al. (2010). This was thought to be due to presence of calcium or other dietary compounds or due to the buffering capacity of the gut itself (Younes et al. 1996).

2.7.1.5 Inulin Supplementation Effects on Intestinal Histomorphology

Inulin may have further effects on growth by altering intestinal microstructures in a way that improves nutrient absorption (Xu et al. 2003; Pelicano et al. 2005; Rehman et al. 2007). Rebole et al. (2010) observed an increase in villi height:crypt depth ratio when inulin was fed to broilers at 1.0%. Longer jejunal villi and deeper crypts were also observed when inulin was fed at 1.0% (Rehman et al. 2007). In rats, inulin increased epithelial cell number in the colonic mucosa, number of goblet cells, and the length and width of colonic crypts (Steed and Macfarlane 2009).

2.7.1.6 Nutritional Implications of Inulin Supplemenation

FOS has been shown to have a positive effect on mineral uptake in the gut. It is thought that by creating a stable microflora population, decreasing the inflammatory reaction to commensal bacteria, decreasing pH with production of SCFA, increasing absorptive SA, and increasing mineral solubility, inulin is able to improve uptake of Fe^{+2}, Ca^{+2}, Cu^{+2}, and Zn^{+2} (Scholz-Ahrens and Schrezenmeir 2007). Inulin has also been found to reverse decreases in Zn^{+2} absorption observed when phytic acid is present in the diet (Steed and Macfarlane 2009). Ash and Ca^{+2} concentrations in the tibia of broilers was found to increase when inulin was fed at 0.5% and 1.0% (Ortiz et al. 2009). Yasuda et al. (2006, 2009) found iron status to increase in pigs supplemented with inulin and intestinal uptake of Ca^{+2}, Mg^{+2}, and Fe^{+2} increased in rats fed inulin (Gibson and Roberfroid 1995).

2.7.2 Influence of Inulin Chain Length on Performance

The chain length of the FOS product used can substantially influence the outcomes observed as it will dictate where in the gut the prebiotic is able to reach. Patterson et al. (2010) fed inulin of differing chain lengths to swine and found it to increase *Lactobacillus* and *Bifidobacterium* in the proximal gut with short chain length inulin but not until the distal ileum or ceca were increases in bacteria found with longer chain inulin. Type of inulin also affects the strains of bacteria able to use them. Biekela et al. (2002) looked at ability of different bifidobacteria strains to utilize FOS, oligofructose, and inulin of different types. Most of the strains were able to use all the substrates but the amount of their growth on each differed according to their ability to utilize the CHOs. For example some strains that were able to grow well with FOS and

oligofructose as well as low chain length inulin were not able to use inulin that was either highly purified or highly polymerized (Bielecka et al. 2002).

2.7.3 Optimal Levels of Inulin Supplementation in Broiler Feed

The optimal dose of inulin to feed broilers has not yet been determined. It is known however that high levels have a negative effect on growth of broilers (Biggs et al. 2007). Wu et al. (1999) determined that the optimal concentration for FOS was 0.25% to 0.5%. When Biggs et al. (2007) tested FOS at 0.2%, 0.4%, and 0.8% in broilers 0.4% was found to increase average daily gain but levels of 0.2% and 0.8% had no such effect. In addition, FOS at 0.8% was found to decrease metabolizable energy. Rebole et al. (2010) found inulin to increase *Bifidobacterium* and *Lactobacillus* in the ileum at 2.0% and increase *Lactobacillus* in the ceca at 1.0%. Both of these levels produced higher BWG than the control.

2.8 Tasco®

Tasco® is a product made from sun – dried brown seaweed, species *Ascophyllum nodosum* (ANOD), by Acadian Seaplants Ltd. It increased immune function, reduced toxicity when feeding endophyte infected pastures to beef cows, and reduced *Salmonella* in the excreta of broiler chickens (Allen et al. 2001; Saker et al. 2001; Montgomery et al. 2001; Braden et al. 2007; F. Evans personal communication). These effects as well as observations made with other seaweed species have led to a speculation that Tasco® may act as a prebiotic.

2.8.1 Seaweeds as Nutritional Supplements in Animal Feed

Seaweeds are designated as macroalgae and are considered to be rich in dietary fibers (Devillé et al. 2004; Dierick et al. 2009). There are three phyla of macroalgae, classified according to their nutrient and chemical makeup (Ruperez and Saura-Calixto 2001); brown (Wakame), red (Nori), and green (Burtin 2003, MacArtain et al. 2007; El-Deek and Brikaa 2009). The CHO makeup of seaweeds allows them to reach the lower GIT largely undigested and therefore act as substrate for bacterial fermentation. In addition to being a source of fiber, seaweeds also contain minerals, vitamins (MacArtain et al. 2007), protiens, phlorotannins, and carotenoids (Burtin 2003).

2.8.1.1 Nutrient Composition of Seaweeds

Each seaweed type differs in their composition but almost all algal fibers are soluble anionic polysaccharides. Most of these fibers contain sugars unique to seaweeds as well (Lahaye et al. 1993; Lahaye and Keaffer 1997). Total dietary fiber of main seaweed species ranges from 25% to 75% dry weight and 51-85% of this are water soluble fibers (Jimenez - Moreno et al. 2006). Seaweed fibers are mainly classified as either structural or storage polysaccharides. Structural polysaccharides are those which are also found in land plants such as cellulose, hemicellulose, and xylans. Storage polysaccharides on the other hand include fibers not occurring in terrestrial plant sources such as alginates, laminarins, and carrageens (MacArtain et al. 2007). These fibers differ from those in land plants in their fermentability and makeup (Gudiel-Urbano and Goñi 2002). They are mainly composed of uronic acids, fucose, and mannose whereas land plants' fibers are mostly composed of arabinose, xylose, and glucose (Dierick et al. 2009). Red seaweeds contain neutral and acidic polysaccharides such as carrageens while

brown seaweeds tend to have higher dietary fiber (Miscurcova et al. 2010) at around 40% dry matter (Devillé et al. 2007). Brown seaweeds contain mostly acidic polysaccharides like alginates and mannuronic and gluronic acids (Ruperez and Saura-Calixto 2001) as well as cellulose, fucose containing polysaccharide (FCP), laminarin, and mannitol (Dawczynski et al. 2007; Lahaye 1991). Typical minerals contained within seaweeds are Fe^{+2}, Cu^{+2}, Zn^{+2}, and Ca^{+2} (MacArtain et al. 2007)

2.8.1.2 Effects of Seaweed Supplementation on Animal Health and Growth

Seaweeds have demonstrated several prebiotic like effects. Gudiel- Urbano and Goni (2002) fed red and brown seaweeds to rats which resulted in alteration of the composition and metabolic activity of their microflora. The brown seaweed *Laminaria digitata* was found to increase acetic acid, propionic acid, and butyric acid concentrations in the large intestine (Hoebler et al. 2000). In the rats fed red and brown seaweeds cecal pH was found to increase (Gudiel – Urbano and Goni 2002).

Similar to qualified prebiotics, seaweeds affect nutrient digestion. The seaweeds *Porphyra yezoensis*, *Undaria pinnatifida*, *Laminaria japonica*, and *Hizikia fusiformis* bind to bile salts in the gut thereby inhibiting uptake of fats and lowering cholesterol (Wang et al. 2001). Conversely, the seaweed *M. pyrifera* increased ω – 3 fatty acid content in eggs when fed to layers (Carrillo et al. 2008). Red and brown seaweeds have both been found to alter microbial activity so as to decrease enzymatic reactions associated with formation of toxic compounds (Gudiel – Urbano and Goni 2002).

The final indication that seaweeds would be beneficial feed additives is the impact that they have on growth. Supplementation of chicken and duck diets with red seaweed resulted in increased BWG for example (El-Deek and Brikaa 2009; Asar 1972).

2.8.2 Ascophyllum nodosum as a Nutritional Supplement

The brown seaweed ANOD is composed of 8.8% total fiber, 7.5% soluble fiber, 1.3% insoluble fiber, and 13.1% CHO (MacArtain et al. 2007). It contains several bioactive polysaccharides such as laminarin, FCP, and alginates which have beneficial effects on health and growth both on their own and combined within ANOD (Archer et al. 2007; Wang et al. 2006; Lynch et al. 2010; Devillé et al. 2007).

2.8.2.1 Effects of *Ascophyllum nodosum* Supplementation on Animal Health and Growth

Prebiotic – like alterations in microflora populations have been observed when ANOD is supplemented. Dierick et al. (2009) found the *Lactobacillus* to *E. coli* ratio to be enhanced in the small intestine when ANOD was fed at 1.0%. In culture medium simulating digesta, ANOD was found to decrease *E. coli*, *Streptococci*, and total anaerobes, though *Lactobacillus* was also reduced (Dierick et al. 2009). Gardiner et al. (2008) found ANOD extract (ANE) to result in reductions in ileal coliform counts but not coliforms in the colon, ceca, or rectum of swine. *Lactobacillus* was not affected and *Bifidobacterium* levels in the ceca decreased with increasing ANE levels which suggested that ANE was not fermentable by those bacteria (Gardiner et al. 2008). It may be that ANOD is fermented by other bacteria than those normally analyzed for.

ANOD has decreased pathogen presence of *E. coli*, *Pseudomonas*, *Micrococcus*, *Aerobacter*, *Brucella*, *Salmonella*, *Klebsiella*, and *Streptococcus* (Vacca and Walsh 1954). Dierick et al. (2009) observed reduced *E. coli* in the stomach and small intestine of swine with ANOD supplementation. ANOD also decreased *E. coli* in the feces of feedlot

cattle (Barham et al. 2001; Braden et al. 2004; Bach et al. 2008) and on their hides prior to slaughter (Turner et al. 2002).

In addition to effects on microflora ANOD caused alterations in immune function and growth. When Archer et al. (2008) supplemented lambs with ANOD at 30g/day, lambs had increased white blood cell, eosinophil, and lymphocyte counts and decreased IgG and IgM titer. These effects seem to indicate that ANOD increases cell – mediated immunity; however, results are not always consistent as Turner et al. (2002) found no effect of ANOD on GALT function.

ANOD seems to have some function in alleviating detrimental effects of stressors. When lambs were given ANOD prior to being transported, it lowered cortisol levels in the plasma prior to and throughout transport as well as lowered aldosterone levels (Archer et al. 2008). ANOD lowered body temperature during hot periods of transport when fed for two weeks prior to the transport occurring (Archer et al. 2007) and was found to keep temperatures steady during shipping (Archer et al. 2008).

2.8.3 Bioactive Polysaccharides Present in Ascophyllum nodosum

Brown seaweeds are a source of several bioactive polysaccharides such as alginates, FCP, and laminarins (Burtin 2003; Leung et al. 2006; Devillé et al. 2007; MacArtain et al. 2007). More of these types of polysaccharides are found in brown seaweeds than either red or green (Jozefiak 2004). The brown seaweed ANOD contains 28g/100g alginic acid, 11.6g/100g FCP, 4.5g/100g laminarin, and 7.5g/100g mannitol (MacArtain et al. 2007). These polysaccharides are not digestible by digestive enzymes and reach the lower GIT intact (Rioux et al. 2007). They are unique to lower plant species

in their chemical, physiochemical, and fermentation characteristics (Deville et al. 2007). When ability of bacteria to ferment these polysaccharides has been tested varied results are observed. Salyers et al. (1977) found neither *Lactobacillus acidophilus* nor *Bifidobacterium* spp. to be able to ferment alignate, FCP, or laminarin *in vitro*. Conversely, Michel et al. (1993) showed that 80% of laminarin, 57% of alginate, and 12% of fucans fed were feremented by human fecal bacteria after 24 hours. However, both of these studies looked at typical human intestinal microflora and no studies to date have looked at bacteria typical of animal species.

2.8.3.1 Alginate

Alginate is found in the amorphous mucopolysaccharide fraction of brown algae (Kolb et al. 1999). Alginates, acidic storage polysaccharides of seaweeds (MacArtain 2007; Wang et al. 2006), are composed of mannuronic and guluronic acid linked with β (1,4) linkages (Moe et al. 1995). It has been suggested that alginate meets all three criteria for a prebiotic. Wang et al. (2006) found alginate oligosaccharide, which is derived from alginate by bacterial alginate lyase, to resist digestion by enzymes in the upper GIT. It was found to increase fecal levels of *Bifidobacterium* compared to when FOS was fed and to increase *Lactobacillus* as well. *Enterobacteriacae* and *Enterococcus* levels were decreased (Wang et al. 2006).

Other indications of prebiotic activity are in alginate's effects on the gut. Wang et al. (2006) found it to decrease the pH of the ceca comparatively to FOS and Michel et al. (1996) found it to be 65% fermented to SCFA. It decreased post – prandial glycemic response by delaying gastric emptying through increased digesta viscosity (Torsdottir et al. 1991; Ohta et al. 1997). Digestive enzyme levels have likewise been affected with

alginate supplementation. Iji et al. (2001a) found alginic acid to increase both jejunal maltase and sucrase activity.

2.8.3.2 FCP

FCP are sulfated polysaccharides found in ANOD (Hennequart et al. 2004) soluble in water and acid (Rupérez et al. 2002). They contain fucans, uronic acids, galactose, xylose, and sulphated fucose (Rioux et al. 2007). The exact structure which these components form is not yet known (Lynch et al. 2010) though that which comes from ANOD contains mainly fucose linked in α (1,3) and α (1,4) linkages (Chevolot et al. 1999; 2001; Daniel et al. 1999; 2001; Marais and Joseleau 2001). FCP's, like other sulfated polysaccharides from seaweeds, have been found to have special properties. Included in these, are anticoagulation, antioxidation, antiproliferation, antitumoral, anticomplementary, anti- inflammatory, antiviral, antipeptic, and antiadhesive effects (Cumashi et al. 2007; Damonte et al. 2004; de Azevedo et al. 2009).

Like alginate, FCP have been proposed to work as prebiotics. Lynch et al.(2010) found FCP to increase *Lactobacillus* spp. in the proximal and distal colon of swine. The researchers suggested that this proliferation was not only an indication of selective fermentation but that at least some FCP was able to make it to the lower GIT intact. FCP prevented *H. pylori* from attaching to porcine gastric mucin *in vitro* and decreased *H. pylori* infection *in vivo* in gerbils (Shibata et al. 1977).

Alterations in microflora are not the only effects observed from FCP supplementation. They increase cellular immunity while decreasing humoral immunity (Maruyama et al. 2003; Tissot et al. 2003), increase total VFA concentrations in the colon of swine (Mortensen et al. 1988), and decrease colonic pH (Lynch et al. 2010).

2.8.3.3 Laminarin

Laminarin is composed of β (1,3) δ glucan (Zvyagintseva et al. 1999) with β (1,6) branching (Nelson and Lewis 1974). It contains two different chain types; M chains with mannitol in the reducing end and G chains with glucose on the reducing end (Rioux et al. 2007). The exact structure and composition of laminarin differs in degree of branching according to the algal species it originates from (Chizhov et al. 1998). These variations influence solubility. A large number of branches makes laminarin soluble in cold water while low numbers cause it to be soluble only in warm water (Rupérez et al. 2002). Laminarin has been shown to be indigestible by enzymes in the upper GIT (Deville et al 2004).

Selective fermentation of laminarin by *Lactobacillus* and *Bifidobacterium* was not demonstrated when tested by Deville et al. (2007) though Lahaye et al. (1997) found *Lactobacillus* and *Bifidobacterium* to be increased. While Deville et al. (2007) did not show selective fermentation this was possibly due to laminarin being ferementable by other butyrate – producing bacteria like *Clostridium*, *Faecalibacterium*, *Fusobacterium*, or *Roseburia* as butyrate was shown to increase with supplementation (Deville et al. 2007). Laminarin has been observed to reduce *Enterobacterium* spp. in swine (Lynch et al. 2010).

Laminarin has been demonstrated to alter gut environment. Deville et al. (2007) not only showed laminarin to increase butyrate but also total SCFA, acetic acid, and propionic acid. This differs from the results by Lynch et al. (2010) who found a decreased proportion of acetic acid and acetic:propionic acid ratio in swine fed laminarin. Deville et al. (2007) showed laminarin to decrease the presence of neutral mucins in the

jejunum, ileum, and ceca of rats and increase neutral mucins in the colon. Acidic mucins were found to be lower in the cecal wall of the rats fed laminarin. These modifications were considered beneficial to protect the host against bacterial invasion and to be due to laminarin's effects on bacteria and SCFA production; however, alterations in microflora and gut environment by laminarin did not translate into improved growth performance by Deville et al. (2007).

2.8.3.4 Additional Seaweed Polysaccharide Components

Polysaccharides other than those specific to seaweeds are also present in ANOD. Some of these have bioactive properties and so may contribute to ANOD's prebiotic effects. Betaine decreased heat stress affects in poultry (Sheikh-Hamad et al. 1994; Zulkifli et al. 2004) and increased FI and growth in ducks (Wang 2004). It is absorbed mostly in the proximal small intestine and then accumulates in the liver and the intestinal tissues (Kettunen et al. 2001). Betaine is able to spare choline and methionine in poultry diets and can regulate osmotic pressure within cells (Kidd et al. 1997). When supplemented with methionine, betaine increased BWG and improved feed conversion (Zhan et al. 2006). It has increased the villi height:crypt depth ratio in broilers (Kettunen et al. 2001).

Lectins are polysaccharides of ANOD that are able to bind to CHO on bacterial surfaces and thus may hinder their attachment to the gut wall (Fabregas et al. 1989). However, there are few studies on its bioactive effects in broilers.

Phlorotannins are polymerizations of phloroglucinol and occur only in marine brown algae. They have been shown to have selective antibacterial effects by reducing *Fibrobacter succinogens*, *Ruminococcus albus* (Wang et al. 2009), and *E. coli* 0157:H7

(Braden et al. 2004; Bach et al. 2008). Other bacterial populations, such as non-cellulytic bacteria, *Selenomonas ruminatium*, *Streptococcus bovis*, *Ruminobacter amylophilus*, and *Prevotella bryantii* are increased with phlorotannins (Wang et al. 2009). These differing results are thought to be due to dissimilarities in bacterial cell wall structures as the cell wall is the primary site of inhibition by tannins (Jones et al. 1994; McAllister et al. 2005). The studies were performed with rumen bacteria and so different effects may be observed with phlorotannins in mongastric bacterial populations.

2.8.4 Effects of Tasco® Supplementation on Animal Health and Growth

Several studies have been done with Tasco® in agricultural animals. Most have researched its effects in ruminants, though a few have studied Tasco® in swine. No studies have yet been published with broiler chickens; therefore, all current knowledge of Tasco®'s effects in broilers comes from preliminary studies carried out by Acadian Seaplants Ltd.

After steers were inoculated with *E. coli* O157:H7, Bach et al. (2008) fed Tasco® to steers at levels of 1.0% for 14 days, 2.0% for 7 days, and 2.0% for 14 days in addition to a negative control. *E. Coli* detection and concentration were less in environmental samples when Tasco® was fed at 1.0% for 14 days and 2.0% for 7 days. Fecal volatile fatty acids (VFA) and pH were found not to be affected suggesting that creation of a detrimental gut environment was not the method of *E. coli's* inhibition (Bach et al. 2008). When Bach et al. (2008) fed Tasco® to lambs, *E. coli* populations were decreased when Tasco® was fed at 1.0% for 28 days. Fike et al. (2005) fed lambs Tasco® treated endophyte infected pastures or directly fed them Tasco® -Ex, an extract of Tasco®

containing mainly soluble ANOD components. Tasco® – Ex resulted in greater organic matter digestibility than those fed Tasco®- Forage, Tasco® applied directly to the forage. Both Tasco® treatments decreased butyrate concentrations in the rumen. When lambs were exposed to heat stress it resulted in increased VFA concentrations in the Tasco®-Forage fed lambs and the control lambs but had no such effect on Tasco® – Ex fed lambs (Fike et al. 2005).

Stress has been found to alter the response to Tasco®. Kannan et al. (2007) fed Tasco® at 2.0% to goats subjected to transportation stress. VFAs were not altered by Tasco® but rumen pH was lowered. No other variables were found to be affected (Kannan et al. 2007). Allen et al. (2001) placed steers on endophyte infected and uninfected pasture treated with either Tasco®-Ex at 3.4kg/ha or without. The steers were then transported to the feedlot. The endophyte infection altered response to Tasco® in several ways. Tasco® treatment increased phagocytic activity in steers fed infected pastures treated with Tasco® to a level similar to steers that had fed on uninfected pastures. Tasco® increased MHC class II expression in steers on infected pastures but only compared to those that had also grazed infected pastures. Thus, when no challenge was present Tasco® had no effect on immunity. Tasco® was found to reverse the decreased monocyte immune cell function observed when the steers were fed the infected pastures (Allen et al. 2001).

Tasco® has not consistently been found to affect growth in ruminants. Tasco®-Forage treatment had no influence on final BW, though it did cause a decrease in feed:gain in steers fed endophyte infected pastures (Allen et al. 2001). In swine, results have been inconclusive. Turner et al. (2002) found Tasco® to improve average daily

gain, BW, and FI but decrease gain to feed. Gardiner et al. (2008) on the other hand found Tasco® to decrease average daily gain and have no effect on FI or feed conversion.

Early speculation on the effects of Tasco® have suggested that increased bioavailability of trace minerals, vitamins, and/or antioxidants, and alteration of digestibility may play a role in Tasco®'s effects (Coelho et al. 1997; Schmidt and Zhang 1997; Zhang and Schmidt 1999; Fike et al. 2001).

2.9 Areas for Further Tasco® Research

While previous research with Tasco® has shown it to be effective in ruminants and swine and to influence growth and microbial populations, details as to its mode of action, its qualifications as a prebiotic, and its effects on broilers have yet to be determined. Previous studies with prebiotics such as inulin have shown treatments to have a maximum beneficial inclusion level, higher than which detrimental effects are observed on growth and health (Biggs et al. 2007). It is likely that Tasco® has a maximum beneifical inclusion level which would vary according to the animal of interest and would need to be determined prior to more detailed research of the supplement's effects in a species.

Prebiotics have been shown to result in the greatest treatment reponse when stressors are present, as they are in most commercial settings (Bailey et al. 1991; Orban et al. 1997). In accordance with this Allen et al. (2001) and Kannan et al. (2007) found results from Tasco® supplementation to be influenced by either transportation stress or disease challenge. However, it is unknown whether Tasco® may also be influenced by a

stressor when supplemented to broiler chickens.This makes deductions regarding response to Tasco® supplementation in commercial settings difficult.

Ask et al. (2007) found broiler chickens to experience a trough of immunity between day 4 and 12 posthatch when they are vulnerable to pathogen infection. Tasco® has been previously shown to improve immune function in steers (Allen et al. 2001). If it improves immunity in broilers as well then feeding Tasco® during this time period could reduce early mortality and improve overall health of the birds.

Several interesting properties have been observed from Tasco®, and some prebiotics, which have not yet been explored in broiler chickens. Bach et al. (2008) and Allen et al. (2001) have produced results indicating that Tasco® has extended effects past the time when it is withdrawn from the feed. These studies were done with ruminants and so the level of Tasco® which would be effective in this treatment regimen and the duration for inclusion in monogastrics is unknown. Whether this extended response would even occur in monogastrics is also yet to be researched. Baurhoo et al. (2009) noted that MOS did not result in growth changes until the last growing period. It was therefore suggested that longer growth periods may allow prebiotics to have their full effect. If this is also true of Tasco®then an extended growth period, such as is used in the U.S.A., may lead to differentiated results.

A final determination of Tasco®'s potential as an alternative to antibiotics would be a direct comparison with an AGP. No study as of yet has compared Tasco® directly with an antibiotic and so no definitive statement is yet able to be made. Prior to making any claims of this sort a direct comparison study needs to be carried out.

Although Tasco® has been the subject of many research studies in a variety of species, many questions are yet unanswered. First, does Tasco® affect broiler chicken growth and health as a prebiotic? how? at what inclusion levels? and does it compare to inulin? Second, if broiler chickens fed Tasco® are presented with a stressor will there be an increased response, thereby increasing its usefulness in commercial settings where animal stressors are common? Third, does Tasco® display any distinct properties as an additive in monogastrics such as extended response after supplement withdrawal or increased response with longer growing periods? Finally, does Tasco® compare to an AGP as a viable alternative?

Chapter 3. Effects Of Tasco® And Inulin On Growth Of Broiler Chickens In Cages

3.1 Abstract

Tasco® is a candidate prebiotic made of sun dried *Ascophyllum nodosum*. A preliminary study sought to determine Tasco®'s influence on broiler chicken growth, optimal inclusion level, and comparison level by level with known prebiotic inulin. Fourteen dietary treatments of Tasco® or inulin at levels of 0% through 3.0% in increments of 0.5% were fed to 588 male broiler chickens raised in cages to 35 days of age. Tasco® at 0.5%, 2.0%, and 3.0% improved body weight, 0.5% and 3.0% Tasco® improved body weight gain, and 2.5% and 3.0% Tasco® increased feed intake over the whole experimental period compared to the controls ($p \leq 0.05$). On day 7, Tasco® increased villi apprent area and crypt depth over inulin and on day 7 and 21 Tasco® increased villi length over inulin ($p \leq 0.05$). No significant treatment effects were observed on feed to gain, % mortalities, cecal or jejunal pH, relative bursa weight, spleen weight, cecal weight, ileal weight, villi breakage score, mucosal depth, or villi width. Levels of Tasco® of 0.5% and 3.0% were found to be particularly effective at improving growth. Tasco® displayed prebiotic characteristics in increasing villi height, villi apparent area, and deepening crypts compared to inulin. Overall Tasco® was shown to have promise at improving broiler growth and produced results indicative of prebiotic like activity.
Keywords: *prebiotic, seaweed, inulin, Tasco®, poultry*

3.2 Introduction

Currently there have been no published studies with Tasco® fed to broiler chickens; therefore the effects and optimal level for Tasco® in broiler diets are unknown. The exact mode of action of Tasco® is relatively unknown, though previous studies have indicated that it may act as a prebiotic. Comparing Tasco® with the known prebiotic inulin (Bosscher 2009;Gibson and Roberfroid 1995) is therefore of interest and so in this study Tasco® was compared directly level by level with a commercial inulin product. To determine the optimal levels of Tasco® in the feed, Tasco® was fed at seven levels increasing from 0% to 3.0% in increments of 0.5% to broilers housed in cages. Bird growth and physiological data such as organ weights, intestinal pH, ileal histomorphology, and % mortality were measured to determine the influence of Tasco® on broiler growth and examine its mode of action.

3.3 Objectives

Objectives of this trial were to determine the optimal level of Tasco® and inulin in the feed of broiler chickens, compare Tasco® level by level with a commercial inulin product, examine its mode of action, and to determine if Tasco® meets the qualifications of presence in the lower gastrointestinal tract and improvement of host health required for classification as a prebiotic.

3.4 Materials and Methods

In Trial 1, Tasco® was fed at seven levels increasing from 0% to 3.0% in increments of 0.5%. Commercial inulin was also fed at the same level as Tasco®. Tasco®'s suitability as a prebiotic was evaluated by measuring physiological variables

such as pH of the gut contents, intestinal histomorphology, and relative organ weights, as well as growth variables.

3.4.1 Animals and Husbandry

Five hundred and eighty eight male Ross 508 broilers from Clark's Hatchery (Burrts Corner, NB) were used in this trial which took place from April to May 2010. At the hatchery prior to shipping, chicks were vaccinated individually with 0.05 mL of Marek's vaccine (Intervet/Schering –Plough, Kirkland, QC). Chicks were received the day of hatch and upon arrival were randomly placed in eighty four, 60cm x 48cm cages at a stocking rate of 7 birds per cage in two climate controlled rooms at the Atlantic Poultry Research Centre in Truro, NS. Stocking densities were 1.02 kg/m^2 on Day 0 and 23.3 kg/m^2 on day 35. Birds were immediately provided with feed from troughs at the front of the cage and water from nipple drinkers. Feed and water continued to be provided *ad libitum* throughout the trial. Lighting and temperature schedules used are shown in Appendix A, Table A.1. All procedures were carried out in accordance with the Canadian Council on Animal Care guidelines (CCAC 2009).

3.4.2 Diets

Diets were formulated to be isonitrogenous and isocaloric within period. Diets were fed in mash form throughout the trial. Diets were formulated for each of the three experimental periods; starter (day 0-14), grower (day 15-24), and finisher (day 25-35) (Table 1). Diets met or exceeded the NRC (1994) nutrient requirements for birds at these stages of growth.

Table 1: Trial 1 Diet Formulations of the Starter (Day 0-14), Grower (Day 15-24), and Finisher (Day 25-35) Periods with Tasco® or Inulin Fed at the Same Inclusion Levels

	Starter		Grower		Finisher	
Period diet	**Control Diet**	**Additive Diets**	**Control Diet**	**Additive Diets**	**Control Diet**	**Additive Diets**
Ingredients (% as fed)						
Corn	43.58	40.30	50.97	44.71	56.66	50.77
Soybean Meal	39.28	37.32	31.72	32.74	26.56	27.52
Wheat	10.00	10.00	10.00	10.00	10.00	10.00
Poultry Fat	3.41	5.63	3.97	6.20	3.54	5.45
Feed Additive	0.00	3.00*	0.00	3.00*	0.00	3.00*
Ground Limestone	1.63	1.61	1.59	1.58	1.63	1.60
Mono-Dicalcium Phosphorus	0.78	0.82	0.63	0.67	0.59	0.63
Vitamin/mineral Premix†	0.50	0.50	0.50	0.50	0.50	0.50
Iodized Salt	0.43	0.44	0.41	0.41	0.41	0.42
Methioinine Premix‡	0.39	0.39	0.19	0.21	0.10	0.11
Total	100	100	100	100	100	100
Calculated Analysis						
Metabolizable Energy (kcal/kg)	3050	3050	3150	3150	3200	3200
Protein	23.0	23.0	20.0	20.0	18.0	18.0

*The feed additive 3.0% was filled with different percents of the feed additive corresponding to the treatment (Tasco® or inulin) with the remainder filled with corn in the following percentages; 0.5% feed additive and 2.5% corn, 1.0% feed additive and 2.0% corn, 1.5% feed additive and 1.5% corn, 2.0% feed additive and 1.0% corn, 2.5% feed additive and 0.5% corn, 3.0% feed additive and 0% corn. Tasco® was provided by Acadian Seaplants Ltd. (Dartmouth, NS) and inulin was provided by Cargill Inc. (Wayzata, MN) as Oliggo- Fiber™ DS2 inulin (average DP≤10)

†Vitamin/mineral premix contains the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D_3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B_{12}; 29.7 mg niacin; 1.0 mg folic acid,801 mg choline;0.3 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc;25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543mg wheat middlings; 500 mg ground limestone

‡The methionine premix contained 0.5kg/kg DL- Methionine and 0.5kg/kg wheat middlings

Fourteen dietary treatments of Tasco® or inulin were fed at 0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0% of the diet. In each treatment diet, the feed additive replaced corn at the level prescribed (Table 1). Tasco® was provided by Acadian Seaplants Ltd. (Yarmouth, NS) and the commercial inulin product was provided by Cargill Inc. (Wayzata, MN) as Oliggo – Fiber™ DS2 inulin with average DP ≤10. Dietary treatments were blocked by room and randomly distributed among the cages within each room with three replicate cages per treatment per room.

3.4.3 Analysis of Growth Performance

On day 0, 14, 24, and 35 all birds in a cage were weighed and their feed was weighed back and recorded. Feed provided was weighed and recorded each day at feeding. When mortalities occurred, it was recorded, the bird weighed, and the feed of that cage weighed back. Using this data, FI, BW, BWG, and feed:gain ratio on a per bird basis and % mortality were calculated for each period of growth.

3.4.4 Sample Collection

On day 0, prior to cage placement ten birds were chosen randomly, then on days 7, 21, and 35 one bird per cage was randomly chosen and euthanized by cervical dislocation. Each bird was weighed and the ileum, jejunum, ceca, bursa, and spleen were removed. The ileum and ceca were emptied by gentle squeezing then weighed. A 0.5-1.0 cm section was removed from the middle of the ileum and rinsed in deionized water. It was then placed in 10% buffered formalin for storage and subsequent histological analysis. The cecal and jejunal contents were collected and the pH was measured with an Accumet AP62 portable pH/mV meter (Fisher Scientific, Ottawa, ON) according to the

procedure used by Catala –Gregori et al. (2008). The bursa and spleen were also weighed. Relative organ weights were calculated as a ratio of organ weight to BW.

3.4.5 Analysis of Intestinal Histomorphology

Intestinal histomorphological analysis was carried out according to the procedure described by Budgell (2008). In preparation for image analysis, the three slices were cut from each sample then dehydrated in a series of alcohol solutions ranging from 70% to 100%. The three tissue slices were then fixed in a paraffin wax block together after being permeated with xylene. A 0.5 μm slice was cut from the wax block with a microtome and placed on a slide. Each slide was then stained with haemotoxilin and eosin (Drury and Wallington 1980). The clearest of the three slices on a slide was used for measurements. Images were scanned onto the computer using a Nikon Super CoolScan 400ED (Nikon Inc., Japan). Measurements were then taken using SigmaScan Pro 5 (SPSS Inc., Chicago, IL). Villi height was measured from the top of the villi to the start of the crypt. Crypt depth was measured from the bottom end of the villi to the start of the mucosa. Villi width was measured equidistant from the top of the villus to the start of the crypt. Mucosa depth was measured from the end of the crypt to the end of the serosa. Villi apparent area was calculated via the imaging software from the sum of calibrated pixel units within the defined region of each villi. Six to ten measurements were taken per slide and then averaged to give the measurement for each bird. To determine effects of treatment on fragility of villi the amount of broken villi on each slide was also assessed on a scale ranging from 1 (0% broken) to 4 (unreadable) modified from Budgell (2008) to accommodate a greater degree of damage from a scale of 0% to >50% to a scale of 0% to 75%-100% broken villi (Table 2).

Table 2: Breakage Score Scale for Intestinal Villi Modified from Budgell (2008)

Breakage Score	% Broken Villi
1	0-25
2	25-50
3	50-75
4	75-100

3.4.6 Statistical Analysis

The trial was a 2 by 7 blocked factorial design with supplement and level as the main factors and room as the block. Cage was used as the experimental unit. Data was analyzed using ANOVA in SAS 9.2 (SAS Institute Inc., Cary, NC). Growth data and % mortality were analyzed as repeated measures with day as a factor. Where interactions with day were significant (α=0.05) data was sliced by day and analyzed separately. Data on physiological variables were analyzed as a single measurement. Any significant main or interaction effects (α=0.05) were analyzed using Tukeys (Littell et al. 1996) to differentiate the means. When there was a significant supplement by level effect linear and quadratic contrasts were conducted within each dietary supplement at inclusion levels of 0.5%-3.0% to determine relationships among supplement levels (α=0.05).

Statistical Model for Trial 1 Repeated Measures Analysis:

$$\gamma_{ijklm} = \mu + Supplement_i + Level_j + Supplement*Level_{ij} + Day_k + Supplement*Day_{ik} + Level*Day_{jk} + Supplement*Level*Day_{ijk} + Room_l + \epsilon_{ijklm}$$

Statistical Model for Trial 1 Single Measurement Analysis:

$$\gamma_{ijkl} = \mu + Supplement_i + Level_j + Supplement*Level_{ij} + Room_k + \epsilon_{ijkl}$$

Statistical models for experimental design are shown above where γ is the response of the variable being measured, μ is the overall mean response of that parameter, $Supplement_i$ is the effect of supplement for the i^{th} level of supplement (i=1-2), $Level_j$ is the effect of inclusion level for the j^{th} level (j=1-7), Day_k is the effect of the k^{th} level of day(k=1-3), $Room_l$ is the effect of the l^{th} level of the block (room) (l=1-2) , and ϵ is the effects of the uncontrollable factors for the the i^{th} level of supplement, j^{th} level of inclusion level, k^{th} level of day, l^{th} level of the block (room), and m^{th} repetition. The statistical model for single measurement analysis is likewise displayed above where all terms are the same, with the exception that day is no longer a factor.

3.5 Results

The block used in this trial, room, was not found to be significant for any parameter ($p \geq 0.10$). Data was therefore reanalyzed with the block removed.

3.5.1 Growth Performance

Results of ANOVA analysis for each statistical model component are shown in Table 3.

Table 3: ANOVA p- values for Trial 1 Growth Variable Analysis

Growth Variable	Body Weight	Body Weight Gain	Feed Intake	Feed to Gain
ANOVA				
Supplement	0.04	0.56	0.01	0.63
Inclusion Level	<0.0001	<.001	<0.001	0.09
Supplement*Inclusion Level	0.006	0.01	0.03	0.11
Day	<0.001	<0.001	<0.001	<0.001
Supplement* Day	0.82	0.32	0.29	0.005
Inclusion Level*Day	<0.001	0.03	0.003	0.43
Supplement*Inclusion Level*Day	0.09	0.50	0.24	0.05

There was no effect of dietary supplement by experimental period on BW per bird (Table 3). There was however an effect of supplement fed by inclusion level on the BW per bird (Figure 5) when all three growth periods were included.

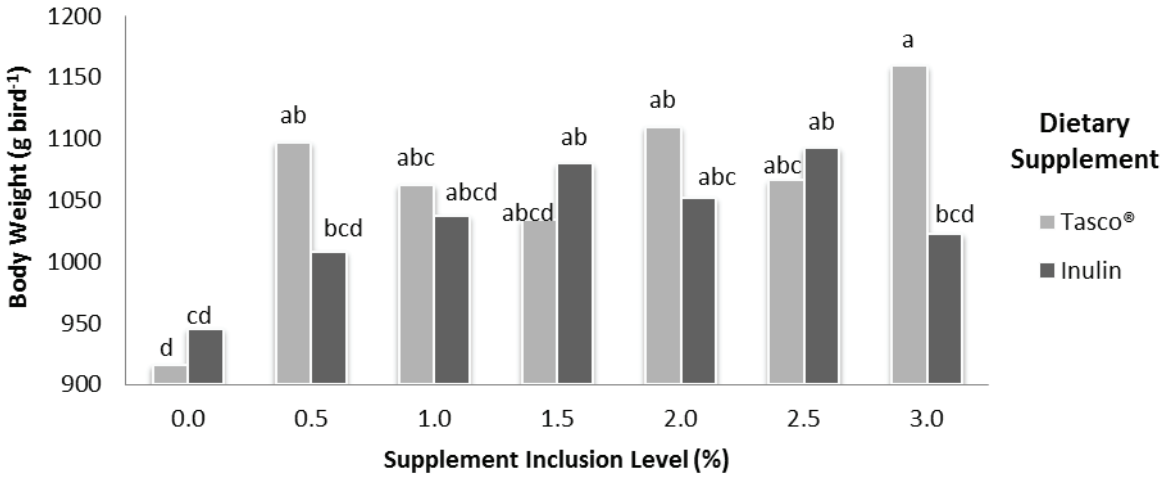


Figure 5: Trial 1 Body Weight (g bird^{-1}) of Broiler Chickens Averaged over Whole Experimental Period with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

$^{a-d}$ means with different letters are significantly different ($p \leq 0.05$)

Feeding Tasco® at 3.0% increased BW per bird over 0.5% and 3.0% inulin. In addition 0.5%, 2.0%, and 3.0% Tasco® as well as 1.5% and 2.5% inulin increased BW over the controls. 1.0% and 2.5% Tasco® and 2.0% Inulin improved BW over the Tasco® control. BW values for each growing period are shown in Appendix B Table B-1. Day 35 BW trends reflected differences seen in the averaged BW values with values for 0.5%, 2.0%, and 3.0% Tasco® and the Tasco® and Inulin controls of 1900.6±35.1, 1892.1±37.8, 1973.9±35.1, 1590.0±37.8, and 1639.5±35.1 g bird^{-1} respectively.

There was a positive linear relationship between Tasco® BW and inclusion level during the grower period ($p=0.03$) (Figure 6). A quadratic relationship for Tasco® BW during the grower and finisher periods ($p= 0.01$ and 0.04 respectively) occurred with response vertex between 1.0% and 1.5% in the grower and finisher periods (Figure 6). There were no linear or quadratic relationships between inulin BW and inclusion level ($p>0.05$).

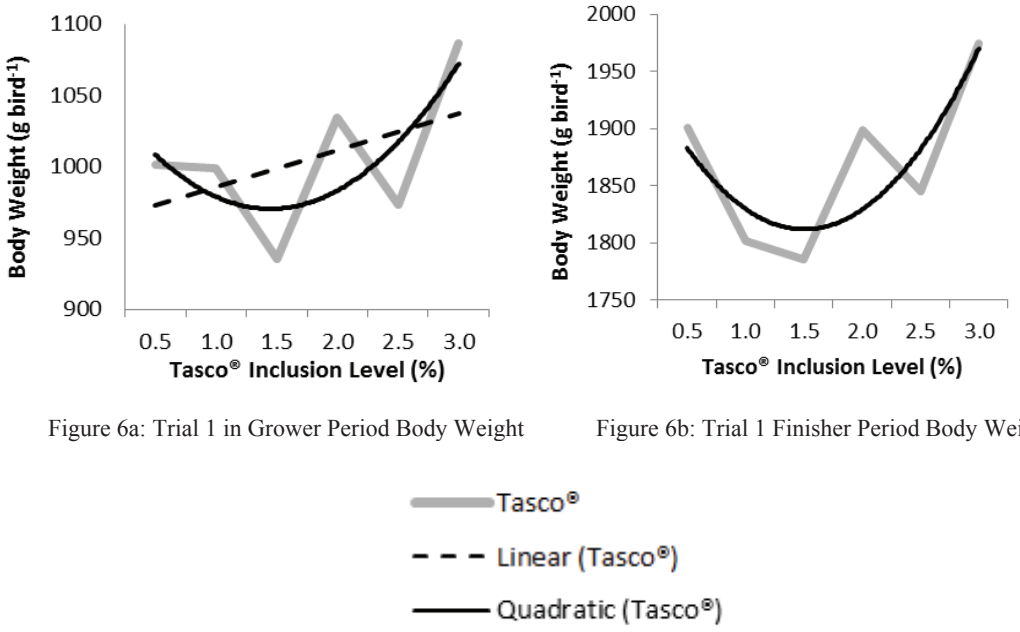


Figure 6a: Trial 1 in Grower Period Body Weight

Figure 6b: Trial 1 Finisher Period Body Weight

Figure 6: Trial 1 Linear and Quadratic Grower (Day 15-24) (Figure 6a), and Finisher (Day 25-35) (Figure 6b) Body Weight (g bird^{-1}) Relationships (p≤0.05) of Broiler Chickens with Tasco® Fed at 0%-3.0% in Increments of 0.5%

There was an effect of inclusion level on BW during all experimental periods (p≤0.05) (Table 3). In the starter and grower periods all inclusion levels, and in the finisher period all inclusion levels except 1.0%, increased BW over the control (Figure 7).

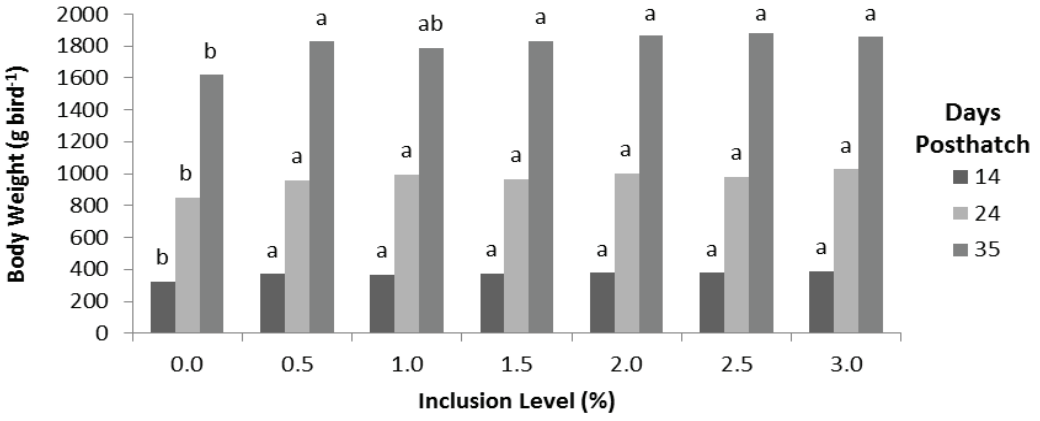


Figure 7: Trial 1 Body Weight (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), and Finisher (Day 25-35) Experimental Periods with Dietary Supplements Fed at 0%-3.0% in Increments of 0.5%

$^{a-b}$ means with different letters within an experimental period are significantly different (p≤0.05)

There was an effect supplement*inclusion level on BWG (Table 3). Feeding Tasco® at 0.5% and 3.0%, as well as Inulin at 2.5%, resulted in improved BWG averaged over all three growth periods compared to the controls (Table 4).

Table 4: Trial 1 Body Weight Gain (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), and Finisher (Day 25-35) Experimental Periods and over Whole Experimental Period with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Days Posthatch			
		0-14	15-24	25-35	Overall Mean 0-35
Dietary Supplement	Supplement Inclusion Level (%)				
Tasco®	0.0	280.2±25.7	511.0±25.7	749.3±28.0	513.5±17.3^{c}
	0.5	346.7±25.7	613.8±25.7	899.0±25.7	619.9±16.9^{a}
	1.0	343.6±25.7	613.3±25.7	802.2±25.7	586.3±16.9^{abc}
	1.5	338.3±25.7	555.2±25.7	850.7±25.7	581.4±16.9^{abc}
	2.0	359.5±25.7	633.1±25.7	838.7±28.0	610.4±17.3^{ab}
	2.5	336.8±25.7	595.1±25.7	871.8±25.7	601.2±16.9^{ab}
	3.0	374.5±25.7	670.1±25.7	887.4±25.7	644.0±16.9^{a}
Inulin	0.0	284.7±25.7	543.2±25.7	769.9±25.7	532.6±16.9^{bc}
	0.5	310.8±25.7	563.0±25.7	846.9±28.0	573.6±17.3^{abc}
	1.0	310.1±25.7	632.7±25.7	883.2±31.3	608.7±17.9^{ab}
	1.5	330.5±25.7	618.4±25.7	882.6±25.7	610.5±16.9^{ab}
	2.0	314.1±25.7	612.5±25.7	861.4±25.7	596.0±16.9^{abc}
	2.5	335.0±25.7	605.0±25.7	932.9±25.7	624.3±16.9^{a}
	3.0	321.4±25.7	604.2±25.7	769.6±25.7	565.0±16.9^{abc}

$^{a-c}$ means±SEM with different superscripts within a column are significantly different ($p \leq 0.05$)

Tasco® fed at 2.0% and 2.5% and inulin fed at 1.0% and 1.5% improved BWG over the Tasco® control (Table 4).

There was a quadratic relationship in Tasco® BWG during the grower period (p=0.01) with response vertex between 1.0% and 1.5% (Figure 8). There were no linear or quadratic relationships between inulin BWG and inclusion level (p>0.05).

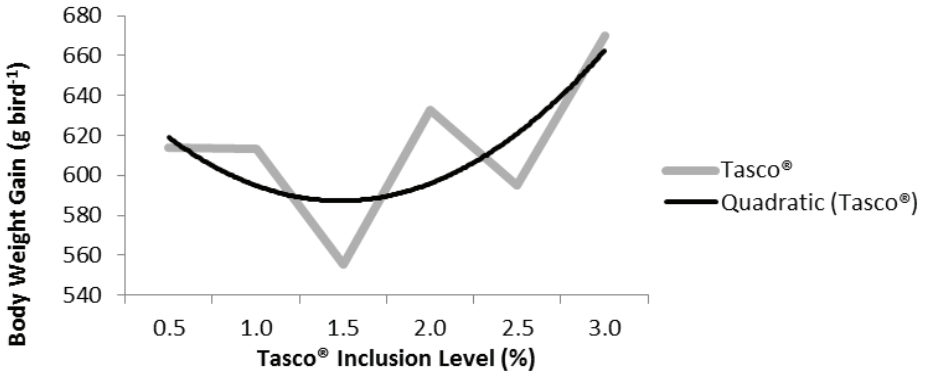


Figure 8: Trial 1 Quadratic Grower (Day 15-24) Body Weight Gain (g bird^{-1}) Relationship (p≤0.05) of Broiler Chickens with Tasco® Fed at 0%-3.0% in Increments of 0.5%

There was an effect of inclusion level on BWG in all experimental periods (p≤0.05) (Figure 9). In the starter period all inclusion levels improved BWG over the control. In the grower period feeding 1.0%, 2.0%, 2.5%, and 3.0% improved BWG over the control and in the finisher period feeding 2.5% improved BWG over the control.

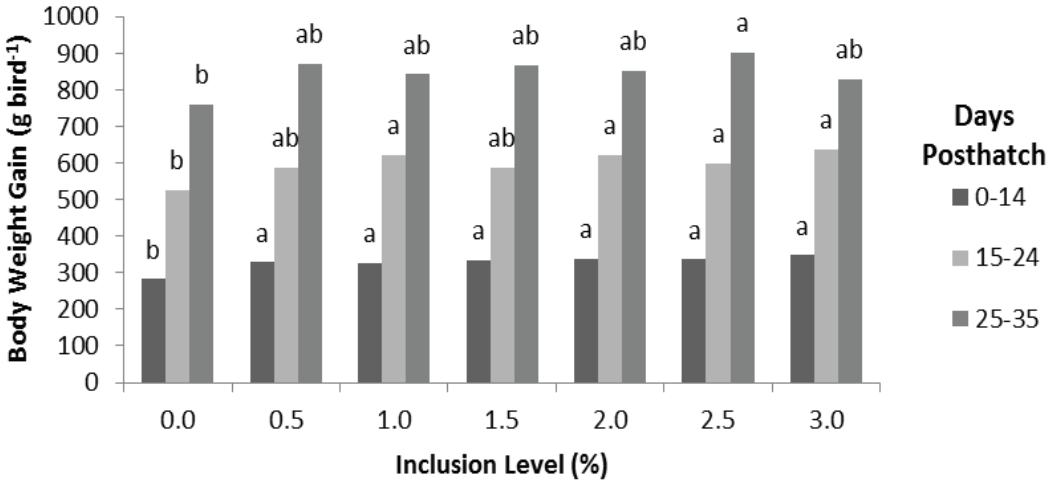


Figure 9: Trial 1 Body Weight Gain (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), and Finisher (Day 25-35) Experimental Periods with Dietary Supplements Fed at 0%-3.0% in Increments of 0.5%

$^{a-b}$ means with different letters within an experimental period are significantly different ($p \leq 0.05$)

There was no dietary supplement by experimental period effect (Table 3; Appendix B Table B-2), though FI averaged over all experimental periods was affected by supplement (Figure 10).

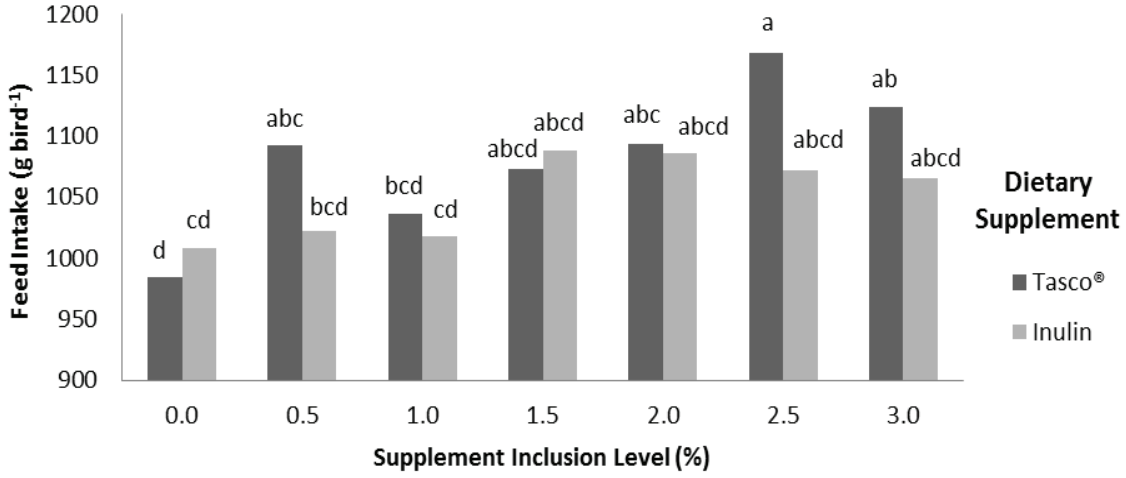


Figure 10: Trial 1 Feed Intake (g bird^{-1}) of Broiler Chickens Averaged over Whole Experimental Period with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

$^{a-d}$ means with different letters are significantly different ($p \leq 0.05$)

Feeding Tasco® at 2.5% improved FI over 1.0% Tasco® and 0.5% inulin. Feeding 2.5% and 3.0% Tasco® improved FI over 1.0% inulin and the controls. Feeding 0.5% and 2.0% Tasco® improved FI over the Tasco® control. There was a quadratic relationship within Tasco® levels for starter period FI (p=0.03) with response vertex between 1.0% and 1.5% and a positive linear relationship within Tasco® levels for the finisher period FI (p=0.01) (Figure 11). There were no linear or quadratic relationships between inulin FI and inclusion level (p>0.05).

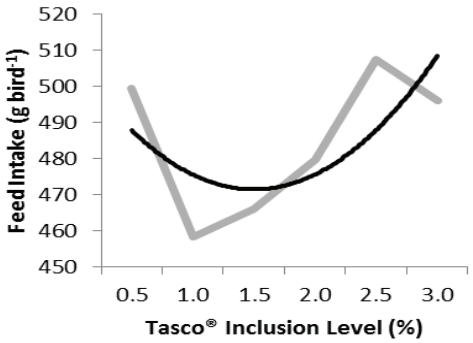


Figure 11a: Trial 1 Grower Period Feed Intake

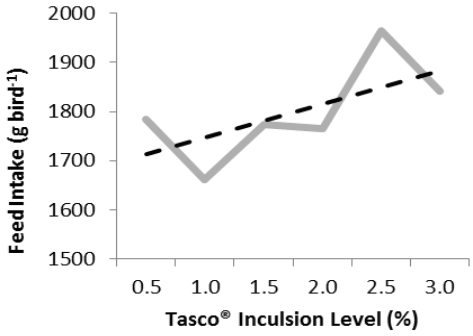


Figure 11b: Trial 1 Finisher Period Feed Intake

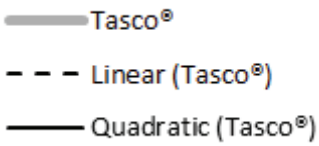


Figure 11: Trial 1 Linear and Quadratic Grower (Day 15-24) (Figure 11a) and Finisher (Day 25-35) (Figure 11b) Feed Intake (g bird^{-1}) Relationships (p≤0.05) of Broiler Chickens with Tasco® Fed at 0%-3.0% in Increments of 0.5%

There was an effect of inclusion level on FI in the grower and finisher periods (p≤0.05) (Figure 12). During the grower period all inclusion levels increased FI over the control while in the finisher period feeding 2.5% increased FI over 1.0% and the control and 1.5% and 3.0% increased FI over the control.

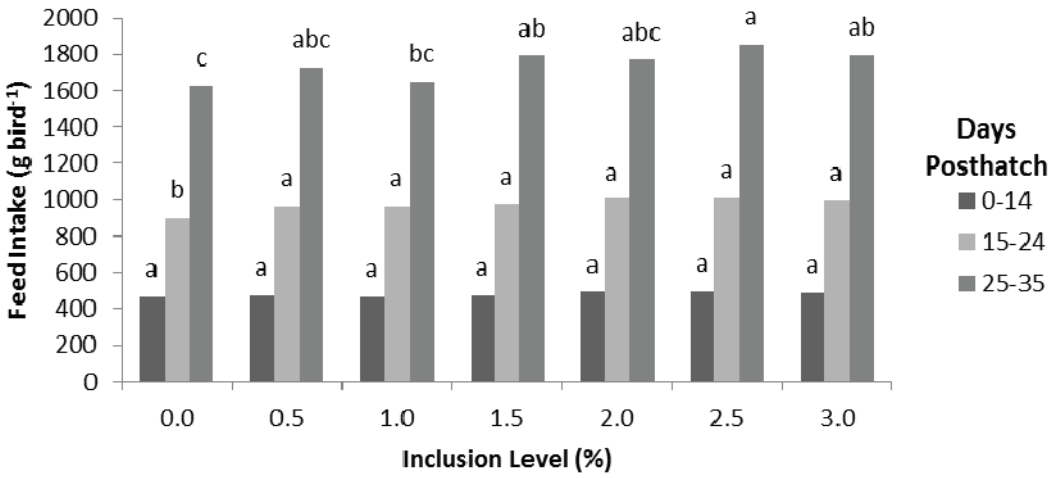


Figure 12: Trial 1 Feed Intake (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), and Finisher (Day 25-35) Experimental Periods with Dietary Supplements Fed at 0%-3.0% in Increments of 0.5%

$^{a-c}$ means with different letters within an experimental period are significantly different ($p \leq 0.05$)

There was an effect of supplement on starter feed to gain ($p \leq 0.05$) (Table 3). Tasco® improved starter feed to gain compared to Inulin (Table 5). However, there was no effect of inclusion level on feed to gain (Appendix B Table B-3).

Table 5: Trial 1 Feed to Gain (g:g) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), and Finisher (Day 25-35) Experimental Period and Averaged over Whole Experimental Period with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

	Days Posthatch		
	0-14	**15-24**	**25-35**
Dietary Supplement			
Tasco®	1.42±0.02^{b}	1.64±0.02	2.04±0.04
Inulin	1.50±0.02^{a}	1.60±0.02	2.12±0.4

$^{a-b}$ means±SEM with different superscripts within a column are significantly different ($p \leq 0.05$)

3.5.2 Bird Health

There were no effects of treatments on mortalities throughout the trial ($p>0.05$) (Table 6). The mortalities ranged from 0.0±2.1% to 8.3±2.1% with most occurring during the finisher period. Cause of mortality was mainly septicemia, varus-valgus deformities of the leg, and ascites, with some dehydration occurring in the early growth period, but were not treatment related.

Table 6: ANOVA p- values for Trial 1 Mortality (%) analysis

	Mortality (%)
ANOVA	
Supplement	0.93
Inclusion Level	0.31
Supplement*Inclusion Level	0.47
Day	0.32
Supplement* Day	0.63
Inclusion Level*Day	0.63
Supplement*Inclusion Level*Day	0.41

3.5.3 Intestinal pH

No differences were found for the pH of the cecal or jejunal contents ($p>0.05$) (Table 7). The pH in the jejunal contents was between 6.0±0.1 and 6.3±0.1 on day 7, 5.6±0.1 and 6.2±0.1 on day 21, and 5.8±0.1 and 6.1±0.1 on day 35. The pH of the cecal contents was between 5.8±0.3 and 6.6±0.2 on day 21 and 6.2±0.2 and 6.8±0.2 on day 35.

The volume of cecal contents from day 7 birds was too low to obtain accurate measurements.

Table 7: ANOVA p-values for Trial 1 Cecal and Jejunal Content pH Analysis

	Cecal Content pH		Jejunal Content pH		
Days Posthatch	**21**	**35**	**7**	**21**	**35**
ANOVA					
Supplement	0.68	0.10	0.29	0.39	0.33
Inclusion Level	0.79	0.57	0.42	0.08	0.70
Supplement*Inclusion Level	0.72	0.86	0.03	0.17	0.17

3.5.4 Organ Weights

No effects of treatment were found for relative bursa weight (Table 8), spleen weight (Table 9), ileal weight (Table 10), or cecal weight (Table 11) ($p>0.05$).

Table 8: Trial 1 Relative Bursa Weight (mg bursa weight: g body weight) of Broiler Chickens on Day 7, 21, and 35 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Days Posthatch		
		7	21	35
Dietary Supplement	**Supplement Inclusion level (%)**			
Tasco®	0.0	1.7±0.3	2.6±0.3	2.8±0.3
	0.5	1.1±0.3	2.4±0.3	2.0±0.3
	1.0	1.1±0.3	2.5±0.3	2.4±0.3
	1.5	1.7±0.3	3.0±0.3	2.7±0.3
	2.0	1.4±0.3	2.8±0.3	2.0±0.3
	2.5	1.4±0.3	2.8±0.3	1.9±0.3
	3.0	2.0±0.3	2.2±0.3	1.7±0.3
Inulin	0.0	1.5±0.3	2.9±0.3	2.4±0.3
	0.5	1.3±0.3	2.4±0.3	2.1±0.3
	1.0	1.1±0.3	2.7±0.3	2.1±0.3
	1.5	1.7±0.3	3.0±0.3	2.4±0.3
	2.0	1.4±0.3	2.9±0.3	2.4±0.3
	2.5	1.4±0.3	2.6±0.3	1.8±0.3
	3.0	2.0±0.3	2.6±0.3	1.9±0.3
ANOVA (p-value)				
Supplement		0.89	0.92	0.34
Inclusion Level		0.28	0.51	0.25
Supplement*Inclusion Level		0.57	0.86	0.11

Table 9: Trial 1 Relative Spleen Weight (mg spleen weight: g body weight) of Broiler Chickens on Day 7, 21, and 35 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Days Posthatch		
		7	21	35
Dietary Supplement	**Supplement Inclusion level (%)**			
Tasco®	0.0	1.3±0.3	1.0±0.1	1.2±0.1
	0.5	2.0±0.3	0.9±0.1	0.9±0.1
	1.0	1.0±0.3	0.9±0.1	0.8±0.1
	1.5	1.8±0.3	0.8±0.1	0.9±0.1
	2.0	1.8±0.3	0.8±0.1	0.9±0.1
	2.5	1.2±0.3	0.8±0.1	0.9±0.1
	3.0	1.1±0.3	0.9±0.1	1.0±0.1
Inulin	0.0	1.4±0.3	1.1±0.1	1.2±0.1
	0.5	1.5±0.3	1.0±0.1	0.8±0.1
	1.0	1.9±0.3	0.9±0.1	1.1±0.1
	1.5	1.8±0.3	1.0±0.1	0.9±0.1
	2.0	0.9±0.3	0.9±0.1	1.1±0.1
	2.5	1.8±0.3	1.1±0.1	0.9±0.1
	3.0	1.2±0.3	0.8±0.1	0.8±0.1
ANOVA (p-value)				
Supplement		0.80	0.24	0.67
Inclusion Level		0.61	0.79	0.15
Supplement*Inclusion Level		0.17	0.54	0.47

Table 10: Trial 1 Relative Ileal Weight (mg ileal weight: g body weight) of Broiler Chickens on Day 7, 21, and 35 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Days Posthatch		
		7	21	35
Dietary Supplement	**Supplement Inclusion level (%)**			
Tasco®	0.0	42.2±3.7	18.0±3.5	25.6±2.3
	0.5	44.1±3.7	18.0±2.8	20.2±2.3
	1.0	44.1±3.7	14.8±4.0	22.4±2.3
	1.5	36.0±3.7	27.5±3.5	19.6±2.3
	2.0	41.6±3.7	18.6±3.1	17.6±2.3
	2.5	40.8±3.7	18.3±3.1	21.6±2.5
	3.0	40.3±3.7	12.3±2.8	18.6±2.3
Inulin	0.0	40.7±3.7	17.0±3.1	20.2±2.3
	0.5	39.5±3.7	19.2±3.1	23.4±2.3
	1.0	44.9±3.7	19.9±3.1	21.9±2.3
	1.5	38.4±4.1	23.9±3.1	18.2±2.3
	2.0	31.6±4.1	16.3±3.1	21.6±2.5
	2.5	37.9±4.1	17.7±2.8	18.4±2.3
	3.0	45.6±3.7	13.9±3.1	18.6±2.3
ANOVA (p-value)				
Supplement		0.46	0.98	0.70
Inclusion Level		0.32	0.02	0.38
Supplement*Inclusion Level		0.53	0.90	0.44

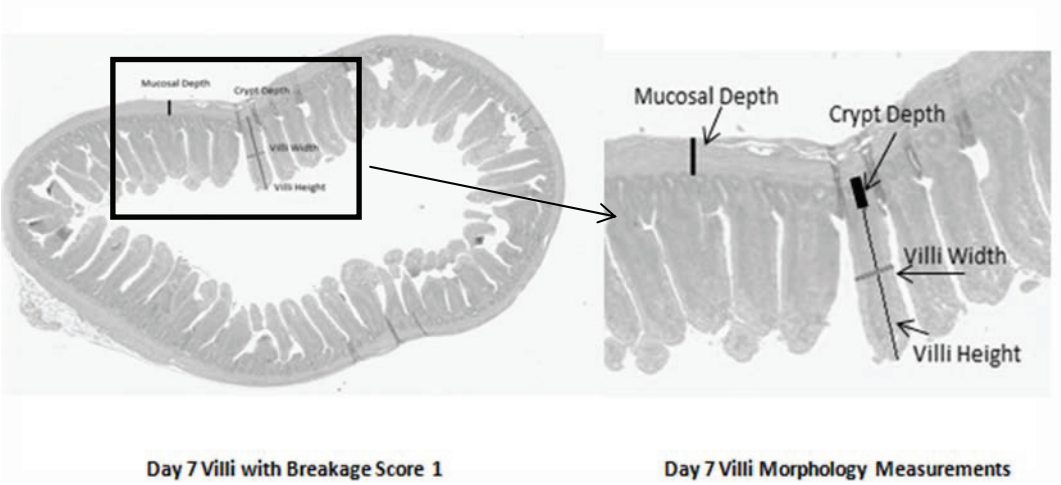
Table 11: Trial 1 Relative Cecal Weight (mg cecal weight: g body weight) of Broiler Chickens on Day 7, 21, and 35 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Days Posthatch		
		7	21	35
Dietary Supplement	**Supplement Inclusion level (%)**			
Tasco®	0.0	13.1±2.6	6.7±1.1	10.8±1.3
	0.5	17.1±2.6	7.8±1.1	8.7±1.3
	1.0	14.6±2.6	11.0±1.1	9.8±1.3
	1.5	16.1±2.6	10.3±1.1	10.6±1.3
	2.0	19.8±2.6	10.3±1.1	8.0±1.3
	2.5	12.0±2.6	8.9±1.1	10.1±1.3
	3.0	15.5±2.6	8.5±1.1	8.3±1.3
Inulin	0.0	19.4±2.6	10.6±1.1	11.5±1.3
	0.5	14.4±2.6	8.6±1.1	9.8±1.3
	1.0	16.1±2.6	9.2±1.1	10.4±1.3
	1.5	16.1±2.6	9.5±1.1	7.9±1.3
	2.0	10.7±2.6	11.0±1.2	9.8±1.3
	2.5	14.9±2.6	9.1±1.1	10.9±1.3
	3.0	19.9±2.6	10.2±1.1	8.5±1.3
ANOVA (p-value)				
Supplement		0.72	0.27	0.62
Inclusion Level		0.83	0.35	0.36
Supplement*Inclusion Level		0.08	0.26	0.72

3.5.5 Intestinal Histomorphology

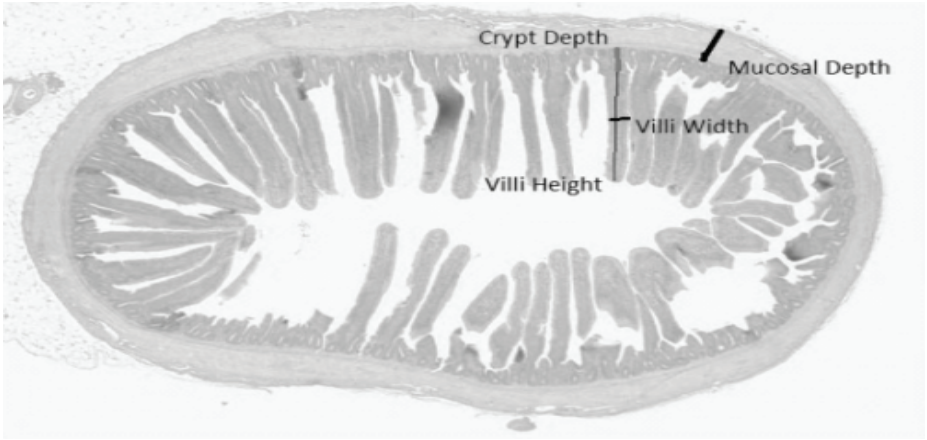
Slides displayed a range of breakage scores (Figure 13); however, treatments did not affect these scores ($p>0.05$). Villi height to crypt depth ratios were not affected on any day and there were no effects on day 21 or 35 villi apparent area or crypt depth or day 35 villi height ($p>0.05$) (Appendix B Table B-4; B-5; B-6).

a)



Day 7 Villi with Breakage Score 1

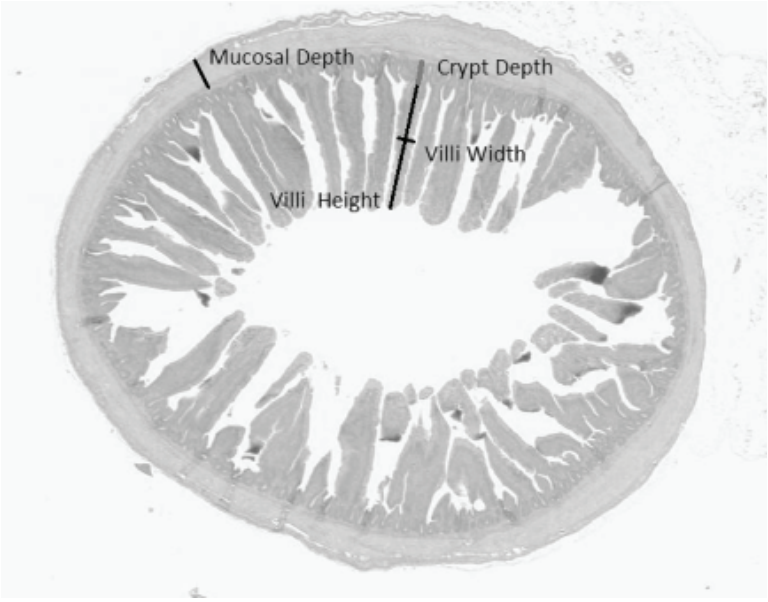
b)



Day 21 Trial 1 Villi with Breakage Score 2

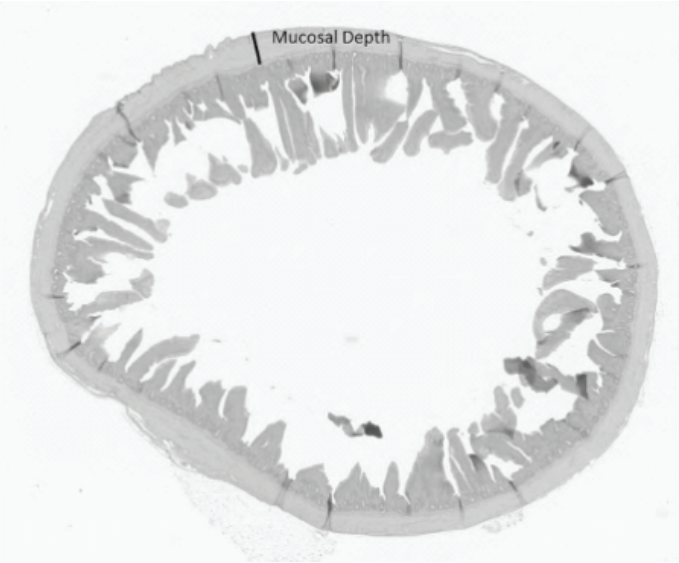
Figure 13: Trial 1 Ileal Villi of Breakage Score 1-4 and Intestinal Histomorphology Measurements

c)



Day 21 Trial 1 Villi with Breakage Score 3

d)



Day 35 Trial 1 Villi with Breakage Score 4

Figure 13: Trial 1 Ileal Villi of Breakage Score 1-4 and Intestinal Histomorphology Measurements (con.)

Mucosal depth, and villi width were not affected by dietary supplementation or inclusion level on day 7 (Table 12), day 21 (Table 13), or day 35 ($p>0.05$) (Table 14).

Table 12: Trial 1 Ileal Intestinal Histomorphology Measurements of Broiler Chickens on Day 7 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Ileal Histomorphology Measurement		
		Breakage Score	**Mucosal Depth(µm)**	**Villi Width(µm)**
Dietary Supplement	**Supplement Inclusion Level (%)**			
Tasco®	0.0	3.0±0.6	157.7±17.8	123.7±13.6
	0.5	3.0±0.4	150.0±12.6	135.3±9.6
	1.0	2.0±0.4	156.5±12.6	120.8±9.6
	1.5	3.0±0.4	182.4±12.6	116.7±11.1
	2.0	3.0±0.4	152.2±12.6	118.0±8.6
	2.5	3.0±0.4	155.3±12.6	129.0±9.6
	3.0	2.0±0.4	155.8±12.6	122.1±8.6
Inulin	0.0	3.0±0.6	194.4±17.8	98.3±11.1
	0.5	3.0±0.4	154.6±12.6	121.9±9.6
	1.0	2.0±0.4	145.3±12.6	120.9±7.8
	1.5	2.0±0.4	147.3±12.6	129.8±7.8
	2.0	3.0±0.4	153.9±13.8	117.4±11.1
	2.5	3.0±0.4	152.7±12.6	117.5±7.8
	3.0	3.0±0.4	166.3±13.8	114.5±13.6
ANOVA (p-value)				
Supplement		0.80	0.93	0.24
Inclusion Level		0.72	0.65	0.79
Supplement*Inclusion Level		0.22	0.36	0.67

Table 13: Trial 1 Ileal Intestinal Histomorphology Measurements of Broiler Chickens on Day 21 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Ileal Histomorphology Measurement		
		Breakage Score	**Mucosal Depth(µm)**	**Villi Width(µm)**
Dietary Supplement	**Supplement Inclusion level (%)**			
Tasco®	0.0	3.0±0.6	212.3±15.2	91.7±10.9
	0.5	3.0±0.4	230.9±12.4	116.2±6.3
	1.0	3.0±0.4	225.6±12.4	123.2±6.9
	1.5	3.0±0.4	217.9±12.4	111.4±7.7
	2.0	3.0±0.4	224.6±12.4	101.4±7.7
	2.5	3.0±0.4	218.4±12.4	118.7±8.9
	3.0	2.0±0.4	226.8±12.4	126.0±6.3
Inulin	0.0	3.0±0.4	214.4±21.5	116.7±7.7
	0.5	3.0±0.4	222.2±12.4	108.1±8.9
	1.0	3.0±0.4	197.1±12.4	112.8±6.9
	1.5	3.0±0.4	214.6±12.4	115.3±7.7
	2.0	3.0±0.4	219.5±12.4	127.6±6.9
	2.5	3.0±0.4	204.5±13.6	111.1±7.7
	3.0	3.0±0.4	219.7±12.4	121.0±6.9
ANOVA (p-value)				
Supplement		0.46	0.21	0.42
Inclusion Level		0.64	0.83	0.38
Supplement*Inclusion Level		0.51	0.95	0.08

Table 14: Trial 1 Ileal Intestinal Histomorphology Measurements of Broiler Chickens on Day 35 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Ileal Histomorphology Measurement		
		Breakage Score	**Mucosal Depth (µm)**	**Villi Width (µm)**
Dietary Supplement	**Supplement Inclusion level (%)**			
Tasco®	0.0	3.0±0.5	270.6±40.5	139.0±12.9
	0.5	3.0±0.3	322.1±23.4	141.9±18.3
	1.0	3.0±0.3	242.3±23.4	153.4±10.6
	1.5	2.0±0.3	332.4±23.4	141.6±8.2
	2.0	3.0±0.3	282.2±23.4	134.7±9.1
	2.5	3.0±0.3	287.9±23.4	133.2±9.1
	3.0	3.0±0.3	270.5±23.4	133.6±10.6
Inulin	0.0	3.0±0.4	283.1±28.7	159.4±10.6
	0.5	3.0±0.3	285.7±23.4	132.0±9.1
	1.0	2.0±0.3	295.8±23.4	138.2±8.2
	1.5	3.0±0.3	290.1±23.4	127.1±12.9
	2.0	3.0±0.3	279.4±23.4	161.9±8.2
	2.5	3.0±0.3	292.6±25.7	172.1±12.9
	3.0	3.0±0.3	317.2±23.4	144.3±18.3
ANOVA (p-value)				
Supplement		0.25	0.71	0.20
Inclusion Level		0.04	0.60	0.63
Supplement*Inclusion Level		0.42	0.30	0.08

There was no effect of supplement*inclusion level on crypt depth (Table 15), villi height (Table 15), ileal villi apparent area (Table 16), or villi height:crypt depth ratio (Table 16) ($p>0.05$) (Appendix B Table B-4, B-5, B-6), however there was an effect of supplement on villi apparent area, crypt depth, and villi height ($p\leq0.05$). On day 7, feeding Tasco® resulted in larger villi apparent area than inulin (Table 18) as well as deeper crypts than inulin (Table 17). Feeding Tasco® resulted in taller villi than inulin when measured on day 7 and 21 (Table 17).

Table 15:ANOVA p-values for Trial 1 Crypt Depth and Villi Height Analysis

	Crypt Depth			Villi Height		
Days Posthatch	**7**	**21**	**35**	**7**	**21**	**35**
ANOVA						
Supplement	0.005	0.93	0.97	<0.001	0.04	0.61
Inclusion Level	0.43	0.07	0.94	0.56	0.04	0.24
Supplement*Inclusion Level	0.75	0.93	0.36	0.72	0.32	0.21

Table 16: ANOVA p-values for Trial 1 Villi Height:Crypt Depth Ratio and Apparent Area Analysis

	Villi Height:Crypt Depth Ratio			Villi Apparent Area		
Days Posthatch	**7**	**21**	**35**	**7**	**21**	**35**
ANOVA						
Supplement	0.85	0.07	056	0.007	0.21	0.16
Inclusion Level	0.11	0.16	0.67	0.99	0.04	0.34
Supplement*Inclusion Level	0.86	0.96	0.66	0.84	0.51	0.30

Table 17: Trial 1 Ileal Intestinal Histomorphology Measurements of Broiler Chickens on Day 7, 21, and 35 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

	Ileal Histomorphology Measurement					
	Crypt Depth (µm)			Villi Height (µm)		
Days Posthatch	**7**	**21**	**35**	**7**	**21**	**35**
Supplement						
Tasco®	131.4±4.0[a]	143.8±4.7[a]	170.9±6.9[a]	519.8±13.0[a]	810.3±23.6[a]	816.3±23.3[a]
Inulin	114.9±3.9[b]	143.3±4.5[a]	171.3±7.0[a]	451.2±13.0[b]	742.9±22.4[b]	833.5±23.8[a]

[a-b] means±SEM with different superscripts within a column are significantly different (p≤0.05)

Table 18: Trial 1 Ileal Intestinal Histomorphology Measurements of Broiler Chickens on Day 7, 21, and 35 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

	Ileal Histomorphology Measurement					
	Villi Height:Crypt Depth (µm:µm)			Villi Apparent Area (mm^2)		
Days Posthatch	**7**	**21**	**35**	**7**	**21**	**35**
Supplement						
Tasco®	4.1±0.1[a]	6.0±0.2[a]	5.0±0.3[a]	0.07±0.003[a]	0.10±0.004[a]	0.12±0.01[a]
Inulin	4.1±0.1[a]	5.4±0.2[a]	5.2±0.3[a]	0.06±0.002[b]	0.09±0.004[a]	0.13±0.01[a]

[a-b] means±SEM with different superscripts within a column are significantly different (p≤0.05)

3.6 Discussion

3.6.1 Growth Performance

Addition of the dietary supplements was found to improve growth. BW, BWG, and FI were improved over the control by addition of supplements as evidenced by the effect of inclusion level on these variables.

Feeding of Tasco® resulted in improved growth using various levels of Tasco®. Level response differed depending on the growth parameter measured. When 0.5%, 1.0%, 2.0%, 2.5%, and 3.0% Tasco® were fed, BW per bird improved. With 0.5%, 2.0%, 2.5%, and 3.0% Tasco® improved BWG, and with 0.5%, 2.0%, 2.5% and 3.0% Tasco® improved FI occured. Feeding 3.0% Tasco® consistently improved FI and BW compared to inulin and the control. The lowest level fed, 0.5% Tasco®, improved three of the growth variables measured indicating that Tasco® at low levels enhanced growth. This observation could aid in the affordability of Tasco® for producers, increasing its feasibility as an antibiotic alternative. Linear and quadratic relationships within Tasco® levels revealed increasing linear relationships with increasing Tasco® levels for BW and FI. This indicates that Tasco® has some effect at higher levels in these variables. Quadratic relationships showed 1.0% and 1.5% Tasco® to be the levels at which poorest BW, BWG, and FI response occurred, indicating the two extreme levels to be those which produce maximum response.

The increased response with increasing levels of Tasco® is somewhat unusual in studies using swine as the monogastric model. Gardiner et al. (2008) found a linear

decrease in average daily gain when feeding ANE to grower – finisher pigs at levels of 0%, 0.3%, 0.6%, and 0.9%. However, the increased linear effect on FI was observed in that study. Turner et al. (2002) found poorer performance with higher ANOD levels, with a decreasing linear effect on gain to feed from levels of 0%, 0.5%, 1.0%, and 2.0% when fed to young pigs. This decreased response with increasing levels may be due to consumption of higher amounts of some of the detrimental components present in seaweeds, such as phlorotannins which have been shown to reduce FI, N retention, N utilisation, and digestion (Lee 1999; Van Heugten 2000; DeLange 2000) and alginates which increase digesta viscosity when present at high levels leading to inhibition of enzyme and substrate diffusion (Ikegami et al. 1990). It is possible that the method with which Tasco® is processed may decrease the presence of these components as seaweed drying method has been found to alter the composition of the final product (Chan et al. 1997). In addition, the dietary supplement used by Gardiner et al. (2008) was an extract of ANOD which may have contained certain components at greater concentrations.

The quadratic response to ANOD supplementation in this study differs from that reported in other research as well. Turner et al. (2002) found quadratic effects on average daily gain and final weights with responses peaking at 1.0% ANOD, whereas in this study BW, BWG, and FI all showed quadratic relationships within Tasco® which dipped between the intermediate levels of 1.0% and 1.5%. Poor response from intermediate levels may be due to the complex ecosystem of microflora and the variety of bioactive polysaccharides present in Tasco®, which may not all require the same inclusion level to have an effect. The exact dynamic which resulted in the poor performance from the

intermediate levels is not certain from the physiological variables measured and may require analysis of microflora populations and a deeper understanding of Tasco®'s mode of action.

It is possible, that in addition to drying method, the varying responses may be due to the different animal models used as well as the variations in growing conditions. In the studies by Gardiner et al. (2008) and Turner et al. (2002) the pigs were housed together in floor pens. This may have given them greater access to bacteria in the environment whereas the broilers in this trial were housed in cages with limited access to bacteria from litter or feces resulting in decreased pathogen exposure but higher stocking densities. It was noted by Gardiner et al. (2008) that the pigs used had high health status and so decreased response may have been due to a lack of pathogenic challenge. Prebiotics and prebiotic candidates, including Tasco®, are believed to have a greater response when some sort of stressor is present (Allen et al. 2001; Bailey et al. 1991; Orban et al. 1997). A final explanation for the variations observed may be that the levels which Gardiner et al. (2008) fed to the pigs were very low compared to this study and Turner et al. (2002), and in much smaller increments as well. This would provide a much narrower picture of Tasco®'s effects at different levels.

Tasco® was not only found to improve growth over the control, but over inulin as well. This study is the first to the author's knowledge that directly compares Tasco® to the known prebiotic inulin. Tasco was shown to improve starter feed to gain compared to Inulin, though individual inclusion levels did not have an effect. In growth variables

where inclusion levels did have an effect for each supplement, Tasco® and inulin did not seem to function optimally at the same levels. While Tasco® was found to have beneficial effects when fed as low as 0.5%, inulin showed improvements in growth at 1.0%, 1.5% and 2.5%, with 2.5% improving both BW and BWG over the controls. This difference may be due to higher concentrations of components responsible for the observed effects from Tasco®. This can not be proven, however, until the exact mechanisms of Tasco®'s actions are revealed.

Similarly to this study, Rebole et al. (2010) found inulin to improve BWG of broilers when fed at 1.0% and 2.0%. Tianxing et al. (1999) however found FOS to increase BW at much lower levels of 0.25% and 0.5% than those showing the improvement in this study. Catala – Gregori et al. (2008) found inulin to improve BWG in broilers, though not FI or feed conversion. Lack of broiler response in FI and feed conversion has commonly been observed from inulin supplementation (Rebole et al. 2010; Geier et al. 2009; Janardhana et al. 2009; Rehman et al. 2008).

3.6.2 Bird Health

Bird mortality was not affected by dietary supplementation in this trial. As prebiotics are most useful in improving bird health under conditions where a challenge is present (Bailey et al. 1991) it may be that there was not sufficient challenge in this trial to result in mortality differences.

3.6.3 Intestinal pH

A decreased intestinal pH is indicative of increased bacterial fermentation and production of SCFA (Dhama et al. 2008). In addition, a lower pH prevents growth of acid- sensitive bacteria such as *E. coli* (Russell and Diez-Gonzalez 1997) while allowing beneficial bacteria, such as butyrate producers, to grow (Scott et al. 2008). There were no effects on pH of cecal or jejunal contents in this study. This is consistent with results of Gardiner et al. (2008) where no effect of ANE on ileal or cecal content pH in grower-finisher pigs occurred and Rebole et al. (2010) with inulin in broilers which found no effect on pH of cecal contents. Though pH was not altered by supplement in this study it does not necessarily indicate that no effect occured on SCFA production as Rebole et al. (2010) found increases in n- butyric acid and D- lactic acid as well as the ratio of n-butyric: acetic acid in broilers fed inulin even though no alterations in cecal content pH were reported.

3.6.4 Organ Weights

There was no response to supplementation in relative cecal weights or ileal weights. This would indicate a lack of effect of supplementation on mucin production and growth of the intestinal mucosa. This corresponds with the absence of effect on intestinal pH, indicating no major increase in SCFA production. The SCFA butyrate is a major source of energy for colonic epithelial cells and so an increase in SCFA of this sort would likely have increased epithelial proliferation (Bosscher 2009). The large decrease in relative ileal weights which occurred from day 7 to day 35 is consistent with that

observed by Lumpkins et al. (2010) of 19.8 g/kg on day 8 posthatch and 10.3 g/kg on day 35. The values in this study are slightly higher however. This is perhaps due to differences in breed used as three different breeds were pooled in that study which most likely would cause differences in growth compared to the Ross 508 birds.

The lack of effect on immunological organ weights seems to indicate that production of Ig and lymphocytes was not increased with dietary supplementation of either fiber. This may be due to a lack of challenge, as the birds were housed in cages and thus more isolated from pathogen presence than in a conventional production setting.

3.6.5 Intestinal Histomorphology

No affect of individual levels of Tasco® were had on intestinal histomorphology measurements in this trial. However, supplement did have an effect. Tasco® increased ileal villi apparent area, increased crypt depth, and increased villi height compared to inulin during early growth periods. This indicates that Tasco® in some way was able to influence the proliferation of intestinal epithelial cells, either by altering bacterial populations or through fermentation into SCFA, or perhaps both. The ileum contains the largest bacterial populations in the small intestine and so any alterations in populations of the small intestine resulting in changes in intestinal histomorphology would be most obvious in this region (Catala – Gregori et al. 2008). Crypt depth and villi height were measured as indications of the amount of turnover occurring in the intestinal cells. An increased villi height, as occurred in this study, is indicative of less turnover and more absorptive SA (Rehman et al. 2007). Deeper crypt depths indicate increased cell turnover

via greater production of epithelial cells (Rehman et al. 2007). It can also be an indication of increased production of goblet cells for mucin production (Ayabe et al. 2000). An increased villi length and deeper crypts, as were observed in this trial, are indicative of a higher production of epithelial cells for the purpose of increasing absorptive area for nutrients. This increased production does increase energy demands on the animal (Rehman et al. 2007), which may be the cause of the lack of improvement of feed to gain. The increased nutrient absorptive area may be responsible for the increased growth performance in birds fed Tasco®. Lack of response in villi height to crypt depth ratio may indicate that the crypt and villi were growing at an equal rate during this time period.

It is interesting to note that the most intestinal histomorphology variables were affected on day 7, then day 21, until on day 35 no affects were observed. On day 7 villi apparent area, crypt depth, and villi height were increased by Tasco®. This may indicate an improvement in nutrient absorption at a time soon after the chicks have switched from being relient on the yolk sac for nutrients to being completely relient on the feed, as 85% of the yolk sac is absorbed by day 5 posthatch (Noble and Ogunyemi 1989). While this effect did not lead to an immediate improvement in growth it may have aided in improved growth variables overall in Tasco® fed chicks. On day 21, while villi apparent area and crypt depth were no longer different among supplements, villi height was still longer in Tasco® fed chicks. This could have given the birds an advantage in nutrient absorption as it would increase the protrusion of the villi into the digesta, enhancing contact with nutrients present. As the crypt depths were no longer deeper, indicating less epithelial cell production, epithelial cell turnover was less and thus the energy

expenditure for cell turnover was also less. It may be that this observation was a lingering effect from Tasco®'s actions on day 7. Another explanation is that as the birds' bacterial populations underwent the transitional period observed on day 12-17 posthatch (Torok et al. 2009), fewer species were present which could ferment Tasco®, resulting in less of an effect on intestinal histomorphology.

3.6.6 Effect of Dietary Supplement on the Trough of Immunity

Chicks experience a trough of immunity between days 4 and 12 (Ask et al. 2007) when they are vulnerable to colonization by pathogens as their acquired immunity becomes active and their gut microbial populations begin to evolve. Tasco® may therefore be of help in ensuring development of a healthy gut microbiota and enhanced development of the immune system. Other studies have indicated that due to the chicks' vulnerability, prebiotic and probiotic use may be of greatest advantage during this time (Sato et al. 2009; Dierick et al. 2009; Barrow et al. 1988; Holt et al. 1999). Birds were sampled on day 7 to determine if physiological variables were altered by Tasco® during early development. In this trial Tasco® increased villi apparent area, crypt depth, and villi length compared to inulin on day 7. These results indicate that Tasco® is initiating effects in the gut which alter birds' physiology during the trough of immunity. The changes enacted on intestinal histomorphology indicate positive alterations which would aid the bird in nutrient absorption. They also indicate that changes may be occurring in the fermentation patterns of microbes or microbial populations present due to Tasco® supplementation.

3.7 Conclusions

Tasco® enacted positive effects on growth at 0.5% and 3.0% in particular. The 2.5% inulin performed optimally of all the inulin levels fed. Tasco® improved both BW and FI over inulin. Comparatively to the control, Tasco® improved BW, BWG, and FI. In addition to improving growth, Tasco® resulted in effects on intestinal histomorphology including increased villi height, villi apparent area, and deeper crypts. These changes suggest that Tasco® may indeed act as a prebiotic. Tasco® shows promise as a prebiotic and antibiotic alternative when fed at levels as low as 0.5%.

Chapter 4. Effects Of Tasco® And Inulin On Growth Of Broiler Chickens Raised In Pens With A Used Litter Challenge

4.1 Abstract

Tasco®, a candidate prebiotic made from sun dried *Ascophyllum nodosum,* was evaluated under production conditions in broiler chickens with a previously used litter challenge compared to known prebiotic inulin. Based on previous results five dietary treatments of 0.5%, 1.75%, and 3.0% Tasco®, a control, and 2.5% inulin were fed to male broilers housed in 60 floor pens in 4 rooms. Two rooms contained pens with a 2:1 mix of previously used to new litter and 2 rooms contained pens with 100% new litter. Diets were formulated for starter (day 0-15), grower (day 16-24), and finisher (day 25-35) experimental periods. Tasco® at 0.5% increased starter body weight over 2.5% inulin ($p \leq 0.05$) and all Tasco® treatments increased grower body weight and body weight gain overall compared to 2.5% inulin ($p \leq 0.05$). Tasco® at 1.75% increased finisher body weight over the control and increased finisher body weight and overall feed intake over 2.5% inulin. Within birds on new litter, 0.5% Tasco® improved finisher feed to gain over 2.5% inulin ($p \leq 0.05$). Tasco® at 3.0% decreased day 21 cecal content pH over 2.5% inulin and the control ($p \leq 0.05$). No intestinal histomorphology effects were observed. Results suggest previously used litter may act as a competitive exclusion rather than stressor resulting in lack of improvement of Tasco® over the control. Tasco® response in the trial suggested possible prebiotic effect on physiological variables in commercial settings, with inconclusive results regarding effects on growth. Inclusion levels of 0.5% and 1.75% had some effect, indicating possible value in the presence of a stronger stressor.

Keywords: *Tasco®, seaweed, prebiotic, litter challenge, inulin, poultry*

4.2 Introduction

In the previous trial, birds were fed Tasco® and a commercial inulin product in a range of levels from 0.5% to 3.0%. Results from that study indicated that both 0.5% and 3.0% Tasco® improved growth optimally out of all the levels. The higher intermediate levels of Tasco® were shown to impart beneficial effects as well. Based on these results, birds were fed levels of 0.5%, 1.75%, and 3.0% Tasco® in this trial. Response from inulin was maximized with an inclusion level of 2.5% in the the first trial indicating this as the ideal level for further study. Prebiotic response has been shown to increase in the presence of a stressor such as pathogen challenge, heat stress, or increased stocking density (Bailey et al. 1991; Orban et al. 1997). Tasco® and inulin at their most advantageous inclusion levels should therefore be tested in the presence of a stressor. Birds were presented with the challenge of placement on previously used litter for Trial 2. While the first trial housed the birds in cages which are not representative of commercial conditions, this trial housed birds on floor pens with the aim of obtaining results more applicable to the industry and the normal group housed production systems.

4.3 Objectives

Objectives of this trial were to determine Tasco®'s response in pelleted diets when birds were challenged using previously used litter, and to compare Tasco® and a commercial inulin product in a floor pen trial setting.

4.4 Materials and Methods

4.4.1 Animals and Husbandry

Twenty one hundred male Ross 508 broilers from Clark's Hatchery (Burtts Corner, NB) were used in this trial which took place from August – September 2010. Prior to shipping, chicks were vaccinated with a 0.05 mL of Marek's vaccine (Intervet/Schering –Plough, Kirkland, QC). Chicks were received day of hatch and upon arrival were randomly placed into sixty 2.13m x 1.40m floor pens at a stocking rate of 35 birds per pen in 4 similar climate controlled rooms at the Atlantic Poultry Research Centre in Truro, NS. Stocking densities were 0.5 kg/m^2 on day 0 and 21.02 kg/m^2 on day 35. Two rooms contained pens with a 2:1 mix of soft wood shaving litter used in a previous trial for 1 flock rotation and new soft wood shaving litter. Two rooms contained pens with all new soft wood shaving litter. Dietary treatments were equally represented with 3 repetitions in each room. Birds were provided with feed via tube feeders and water via nipple drinkers immediately and feed and water continued to be provided *ad libitum* throughout the trial. Upon placement birds were provided feed scattered on 51cm x 43cm cardboard boxes for the first week in addition to feed available from the tube feeders. Lighting and temperature schedules follow that used in Trial 1 (Appendix A Table A-1). All procedures were carried out in accordance with the Canadian Council on Animal Care guidelines (CCAC 2009).

4.4.2 Diets

Diets were formulated to be isonitrogenous and isocaloric within period. Feed was given in crumble form during the starter period and pelleted form during the grower and finisher periods. The diets were formulated for each of the three growth periods; starter (day 0-15) (Table 19), grower (day 16-24) (Table 20), and finisher (day 25-35) (Table 21). Diets met or exceeded the NRC (1994) nutrient requirements for birds at these stages of growth.

Five dietary treatments were fed. Tasco®, provided by Acadian Seaplants Ltd. (Yarmouth, NS), was fed at 0.5%, 1.75%, and 3.0%. The commercial inulin, provided by Cargill Inc. (Wayzata, MN) as the product Oliggo- Fiber™ DS2 inulin with average DP ≤10, was fed at 2.5%.. A negative control diet with no feed additives was also fed. The treatments were randomly distributed among the pens in a room resulting in six replications per litter by supplement treatment combination.

Table 19: Trial 2 Diet Formulations for the Starter Period (Day 0-15) with Tasco® at 0.5%, 1.75%, and 3.0% Compared to Inulin at 2.5%

	Control	0.5% Tasco®	1.75% Tasco®	3.0% Tasco®	2.5% Inulin
Ingredient (% as fed)					
Soybean Meal	40.77	40.96	41.39	41.83	41.66
Corn	40.86	39.81	37.22	34.59	35.61
Wheat	10.00	10.00	10.00	10.00	10.00
Poultry Fat	4.04	4.41	5.33	6.26	5.9
Ground Limestone	1.79	1.79	1.78	1.77	1.77
Mono-Dicalcium Phosphorus	0.72	0.72	0.74	0.75	0.75
Pel- Stik	0.50	0.50	0.50	0.50	0.50
Tasco® *	-	0.50	1.75	3.0	-
Inulin†	-	-	-	-	2.5
Vitamin/mineral Premix‡	0.50	0.50	0.50	0.50	0.50
Iodized Salt	0.44	0.44	0.44	0.45	0.45
Methionine Premix§	0.36	0.36	0.36	0.36	0.36
Total	100	100	100	100	100
Calculated Analysis					
Metabolizable Energy (kcal/kg)	3050	3050	3050	3050	3050.61
Protein	23.0	23.0	23.0	23.0	23.0

*Tasco® was provided by Acadian Seaplants Ltd.(Dartmouth, NS)
†Inulin was provided from Cargill Inc. (Wayzata MN) as Oliggo-Fiber™ DS2 inulin (average DP≤10)
‡Vitamin/mineral premix contains the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D_3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B_{12}; 29.7 mg niacin; 1.0 mg folic acid,801 mg choline;0.3 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc;25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543mg wheat middlings; 500 mg ground limestone
§The methionine premix contained 0.5kg/kg DL- Methionine and 0.5kg/kg wheat middlings

Table 20: Trial 2 Diet Formulations for the Grower Period (Day 16-24) with Tasco® at 0.5%, 1.75%, and 3.0% Compared to Inulin at 2.5%

	Control	0.5% Tasco®	1.75% Tasco®	3.0% Tasco®	2.5% Inulin
Ingredient (% as fed)					
Corn	48.54	47.47	44.85	42.23	43.28
Soybean Meal	32.99	33.17	33.61	34.04	33.86
Wheat	10.00	10.00	10.0	10.00	10.00
Poultry Fat	4.57	4.95	5.88	6.80	6.43
Ground Limestone	1.72	1.71	1.70	1.69	1.70
Mono-Dicalcium Phosphorus	0.58	0.59	0.60	0.62	0.61
Pel- Stik	0.50	0.50	0.50	0.50	0.50
Tasco® *	-	0.50	1.75	3.0	-
Inulin†	-	-	-	-	2.5
Vitamin/mineral Premix‡	0.50	0.50	0.50	0.50	0.50
Iodized Salt	0.42	0.42	0.42	0.42	0.42
Methionine Premix§	0.19	0.19	0.19	0.20	0.20
Total	100	100	100	100	100
Calculated Analysis					
Metabolizable Energy (kcal/kg)	3150	3150	3150	3150	3150.63
Protein	20.0	20.0	20.0	20.0	20.0

*Tasco® was provided by Acadian Seaplants Ltd.(Dartmouth, NS)
†Inulin was provided from Cargill Inc. (Wayzata MN) as Oliggo-Fiber™ DS2 inulin (average DP≤10)
‡Vitamin/mineral premix contains the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D_3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B_{12}; 29.7 mg niacin; 1.0 mg folic acid,801 mg choline;0.3 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc;25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543mg wheat middlings; 500 mg ground limestone
§The methionine premix contained 0.5kg/kg DL- Methionine and 0.5kg/kg wheat middlings

Table 21: Trial 2 Diet Formulations for the Finisher Period (Day 25-35) with Tasco® at 0.5%, 1.75%, and 3.0% Compared to Inulin at 2.5%

	Control	0.5% Tasco®	1.75% Tasco®	3.0% Tasco®	2.5% Inulin
Ingredient (% as fed)					
Corn	54.52	53.56	51.07	48.63	49.61
Soybean Meal	27.65	27.81	28.23	28.63	28.47
Wheat	10.00	10.00	10.00	10.00	10.00
Poultry Fat	4.03	4.33	5.14	5.92	5.61
Ground Limestone	1.73	1.73	1.72	1.71	1.71
Mono-Dicalcium Phosphorus	0.55	0.56	0.57	0.58	0.58
Pel-Stick	0.50	0.50	0.50	0.50	0.50
Tasco® *	-	0.50	1.75	3.0	-
Inulin†	-	-	-	-	2.5
Vitamin/mineral Premix‡	0.50	0.50	0.50	0.50	0.50
Iodized Salt	0.42	0.42	0.42	0.42	0.42
Methionine Premix§	0.10	0.10	0.10	0.11	0.11
Total	100	100	100	100	100
Calculated Analysis					
Metabolizable Energy (kcal/kg)	3200	3200	3200	3200	3200
Protein	18.0	18.0	18.0	18.0	18.0

*Tasco® was provided by Acadian Seaplants Ltd.(Dartmouth, NS)
†Inulin was provided from Cargill Inc. (Wayzata MN) as Oliggo-Fiber™ DS2 inulin (average DP≤10)
‡Vitamin/mineral premix contains the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D_3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B_{12}; 29.7 mg niacin; 1.0 mg folic acid,801 mg choline;0.3 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc;25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543mg wheat middlings; 500 mg ground limestone
§The methionine premix contained 0.5kg/kg DL- Methionine and 0.5kg/kg wheat middlings

4.4.3 Analysis of Growth Performance

Growth variables measured and analyses were the same as used in Trial 1 (section 3.4.3).

4.4.4 Sample Collection

Sample collection and analysis of physiological variables followed the same procedures used in Trial 1 (section 3.4.4)

4.4.5 Litter Salmonella Population Analysis

For litter analysis, a modified version of the procedure established by Rybolt et al. (2005) was used as follows. Litter samples were collected from each pen on day 0 and on day 35. Samples were collected aseptically randomly from several areas within the pen. A composite sample of litter from all pens in a room was also collected. Samples were stored at -20°C prior to being moved to -80°C several weeks before analysis. For most probable number (MPN) determination of *Salmonella* presence, 25±0.2g of litter was measured out from each sample, mixed with 225mL of buffered peptone water made up to the manufacturer's instructions (Oxoid Ltd. 2011) in sterile filter stomacher bags, and then stomached for 1 minute. A spiked litter control was also prepared by adding 1100 cells of *Salmonella Heidelburg* in 25 mL buffered peptone water to 25±0.2 g of autoclaved litter in a stomacher bag which was then stomached for 1 minute. The concentration of *Salmonella Heidelburg* in the spiked control was determined by

comparing the optical density to a McFarland standard and was verified by plating on Xylose Lactose Tergitol® 4 (XLT4) agar and incubating at 35±2°C for 24 hours.

Ten mL from each stomacher bag was added to each of three autoclaved tubes as a 10^{-1} dilution. One mL was then added from the first dilution to three replicates of 9 mL of buffered peptone water for the second dilution and so on for a total of three replicates each of 4 serial dilutions; 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Tubes were then capped and incubated at 35±2°C for 24 hours. After 24 hours of incubation samples were transferred from the buffered peptone water to Rapport- Vassiliadis Soya (RVS) and Tetrathionate (TT) broths made up to the manufacturer's instructions (Oxoid Ltd. 2011). 0.1mL aliquots were taken from each tube of the serial dilutions and added to 10 mL of RVS broth. The RVS broth was then incubated at 42±1°C for 24 hours. Likewise, 1 mL aliquots were taken from each tube of the serial dilution and added to 9 mL of TT broth. Tubes with TT broth were incubated at 35±2°C for 24 hours.

Following the 24 hours incubation, an isolation loop from each tube of each broth was streaked onto both Brilliant Green (BG) and XLT4 agar plates made up to the manufacturer's instructions (Oxoid Ltd. 2011). The plates were then incubated at 35±2° C for 24 hours. After 24 hours incubation all plates were read to determine MPN. All positive plates were recorded for each dilution. Positive colonies on BG agar were determined as red colonies surrounded by a bright red medium. Positive colonies on the XLT4 agar were determined as black or red colonies with a black center. A *Salmonella* colony was picked from each positive plate and streaked and stabbed on pre –prepared

Lysine Iron Agar (LIA) slants made up according to the manufacturer's instructions (Oxoid Ltd. 2011). The slants were then incubated at 35±2° C for 24 hours.

After 24 hours LIA slants were read to confirm positive *Salmonella* colonies. Positive colonies were determined as slants with a red slant, purple butt, and black precipitate. Positive LIA slants were confirmed using an Oxoid *Salmonella* latex kit (Oxoid Ltd. 2011). Positive samples were determined as those forming a precipitate within 2 minutes of mixing. Only samples confirmed positive on the plates, slants, and with the agglutination test were given a final positive result for determination of MPN. For determination of *Salmonella* concentrations in the original samples an MPN chart for a 4 serial dilution analysis was referred to.

4.4.6 Analysis of Intestinal Histomorphology

Intestinal histomorphology analysis followed the same procedure used in Trial 1 (section 3.4.5).

4.4.7 Statistical Analysis

Birds were randomly allocated to one of the five supplement treatments and one of the two litter treatments with 6 pens per supplement by litter combination. The trial was a 5 by 2 randomized blocked factorial design with supplement and litter treatment as the main factors and room as the block. Pen was used as the experimental unit. Data were analyzed using ANOVA in SAS 9.2 (SAS Institute Inc., Cary, NC). Growth data and percent mortality were analyzed as repeated measures with day as a factor. Where interactions with day were significant (α=0.05) data was sliced by day and analyzed

separately. Data on physiological variables were analyzed as single measurement. Any significant main or interaction effects (α=0.05) were analyzed using Tukeys (Littell et al. 1996) to differentiate the means.

Statistical Model for Trial 2 Repeated Measures Analysis:

$$\gamma_{ijklm}=\mu+Supplement_i+Litter_j+Supplement_i*Litter_{ij}+Day_k+Supplement*Day_{ik}+Litter*Day_{jk}+Supplement*Litter*Day_{ijk}+Room_l+Є_{ijklm}$$

Statistical Model of Trial 2 Single Measures Analysis:

$$\gamma_{ijkl=}Supplement_i+Litter_j+Supplement*Litter_{ij}+Room_k+Є_{ijkl}$$

Statistical models for experimental design are shown above where γ is the response of the parameter being measured, μ is the overall mean response of that parameter, $Supplement_i$ is the effect of supplement for the i^{th} level of supplement (i=1-5), $Litter_j$ is the effect of litter for the j^{th} level of litter treatment (j=1-2), Day_k is the effect of the k^{th} level of day(k=1-3), $Room_l$ is the effect of the l^{th} level of the block (room) (l=1-4) , and Є is the effects of the uncontrollable factors for the the i^{th} level of supplement, j^{th} level of litter, k^{th} level of day, l^{th} level of the block (room), and m^{th} repetition. The statistical model for a single measurement analysis is likewise displayed above where all terms are the same, with the exception that day is no longer a factor.

4.5 Results

The block used in this trial, room, was not significantly different for any parameter ($p \geq 0.10$). Data was therefore reanalyzed with the block removed.

4.5.1 Growth Performance

Results of ANOVA analysis for each statistical model component are shown in Table 22.

Table 22: ANOVA p-values for Trial 2 Growth Variable Analysis

Growth Variable	Body Weight	Body Weight Gain	Feed Intake	Feed to Gain
ANOVA				
Supplement	<0.001	<0.001	0.008	0.03
Litter	0.88	0.60	<0.001	<0.001
Supplement*Litter	0.97	0.59	0.70	0.45
Day	<0.001	<0.001	<0.001	<0.001
Supplement* Day	<0.001	0.17	0.11	<0.001
Litter*Day	0.11	0.01	0.02	0.02
Supplement*Litter*Day	0.56	0.26	0.14	0.004

Dietary supplementation had an effect on BW (Table 22). During the starter period feeding 1.75% Tasco® increased BW over all other supplements except the control. Feeding the control increased BW over 3.0% Tasco®, and all dietary supplements increased BW over 2.5% Inulin (Figure 14). During the grower and finisher periods

feeding 2.5% inulin resulted in lower BW than any other dietary treatments. In addition, 1.75% Tasco® increased BW over 3.0% Tasco® and the control (Figure 14).

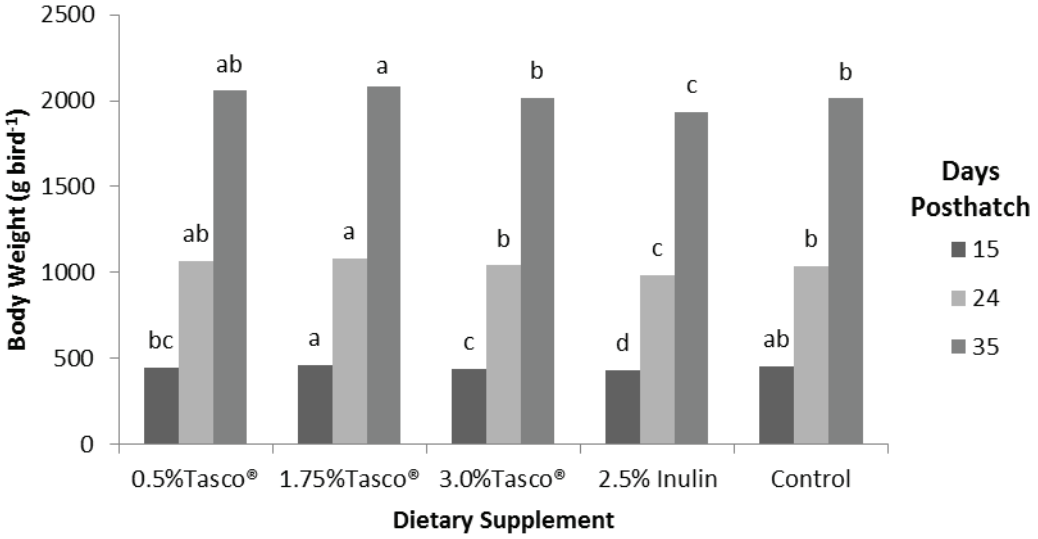


Figure 14: Trial 2 Body Weights (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-15), Grower (Day 16-24), and Finisher (Day 25-35) Experimental Periods with Dietary Supplementation of Tasco® or Inulin

$^{a-c}$ means with different letters within an experimental period are significantly different ($p \leq 0.05$)

Dietary supplementation had an effect on BWG and FI when all three growth periods were analyzed together ($p \leq 0.05$)(Table 22). Feeding Tasco® at 1.75% resulted in higher BWG than 3.0% Tasco®. All dietary treatments resulted in higher BWG than 2.5% inulin (Table 23). There were no interactive effects among litter treatment and supplement response for BW (Appendix B Table B-7), BWG (Appendix B Table B-8), or FI (Appendix B Table B-9).

Table 23: Trial 2 Body Weight Gain (g bird^{-1}) and Feed Intake (g bird^{-1}) of Broiler Chickens Averaged over the Starter (Day 0-15), Grower (Day 16-24), and Finisher (Day 25-35) Experimental Periods with Dietary Supplementation of Tasco® or Inulin

	Body Weight Gain (g bird^{-1})	Feed Intake (g bird^{-1})
Dietary Supplement		
0.5% Tasco®	671.0±5.5^{ab}	1152.5±13^{ab}
1.75% Tasco®	680.4±5.5^{a}	1199.0±13^{a}
3.0% Tasco®	657.0±5.5^{b}	1169.4±13^{ab}
2.5% Inulin	629.5±5.5^{c}	1131.3±13^{b}
Control	659.5±5.5^{ab}	1171.0±13^{ab}

$^{a-c}$ means±SEM with different superscripts within a column are significantly different (p≤0.05)

There was an effect of litter treatment on BWG (Table 22). In the starter period, birds on new litter had lower BWG than those on previously used litter while in the grower period the opposite occurred. There were not differences in BWG in the finisher period (Figure 15).

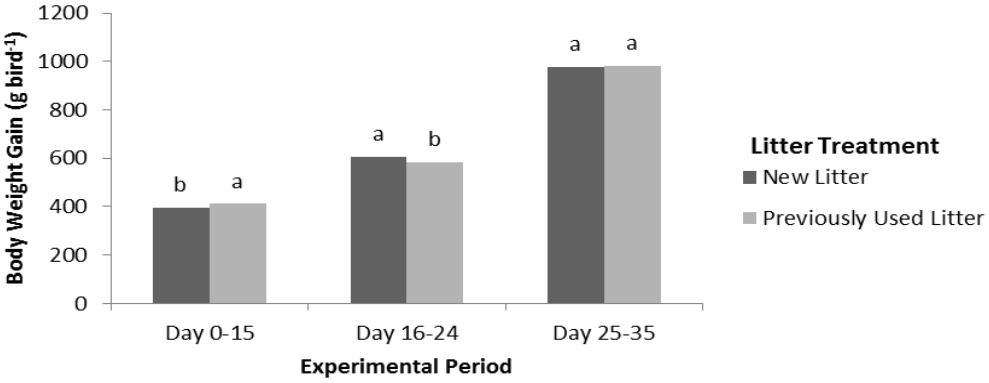


Figure 15: Trial 2 Body Weight Gain (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-15), Grower (Day 16-24), and Finisher (Day 25-35) Experimental Periods with Placement on New or Previously Used Litter

$^{a-b}$ means with different letters within an experimental period are significantly different (p≤0.05)

Supplement had an effect on FI ($p \leq 0.05$) (Table 22). Feeding the 1.75% Tasco® resulted in higher FI measured over all experimental periods than 2.5% inulin (Table 23). Litter treatment also had an effect on the FI of the birds (Table 22). During all experimental periods birds on the new litter had significantly higher FI than those on previously used litter (Figure 16).

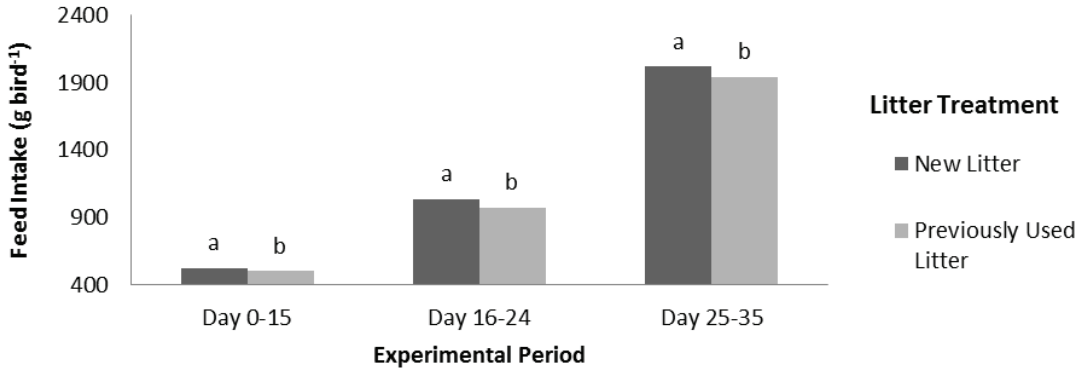


Figure 16: Trial 2 Feed Intakes (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-15), Grower (Day 16-24), and Finisher (Day 25-35) Experimental Periods with Placement on New or Previously Used Litter

$^{a-b}$ means with different letters within an experimental period are significantly different ($p \leq 0.05$)

Effects of both supplement and litter treatments were observed for feed to gain ($p \leq 0.05$) (Table 22). No differences were observed among birds on previously used litter while, within the birds housed on new litter, there were variations in feed to gain according to supplement fed. During the finisher period birds on new litter fed 2.5% inulin resulted in significantly poorer feed to gain than those given 0.5% Tasco® (Table 24).

Table 24: Trial 2 Feed to Gain (g:g) of Broiler Chickens for the Starter (Day 0-15), Grower (Day 16-24), and Finisher (Day 25-35) Experimental Periods with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

		Days Posthatch		
		0-15	16-24	25-35
Litter Treatment	Dietary Supplement			
New Litter	0.5% Tasco®	1.35±0.02	1.55±0.04	2.01±0.04[b]
	1.75% Tasco®	1.31±0.02	1.73±0.04	2.04±0.04 [ab]
	3.0% Tasco®	1.32±0.02	1.68±0.04	2.11±0.04 [ab]
	2.5% Inulin	1.30±0.02	1.85±0.04	2.21±0.04 [a]
	Control	1.29±0.02	1.77±0.04	2.08±0.04[ab]
Previously Used Litter	0.5% Tasco®	1.23±0.02	1.61±0.04	1.99±0.04 [b]
	1.75% Tasco®	1.24±0.02	1.66±0.04	1.98±0.04 [b]
	3.0% Tasco®	1.23±0.02	1.61±0.04	2.04±0.04 [ab]
	2.5% Inulin	1.24±0.02	1.77±0.04	1.93±0.04 [b]
	Control	1.24±0.02	1.71±0.04	1.98±0.04 [b]

[a-b] means±SEM with different superscripts within a column are significantly different (p≤0.05)

4.5.2 Bird Health

There was a significant effect of supplement*day on mortalities (Table 25).

Table 25: ANOVA p-values for Trial 2 Mortality (%) Analysis

	Mortality (%)
ANOVA	
Supplement	0.03
Inclusion Level	0.89
Supplement*Inclusion Level	0.84
Day	<0.001
Supplement* Day	0.01
Inclusion Level*Day	0.27
Supplement*Inclusion Level*Day	0.95

No treatment differences were observed in mortalities during the starter or grower periods ($p>0.05$). However, in mortalities which occurred during the finisher period feeding 2.5% inulin resulted in significantly higher mortalities than 0.5% Tasco®. Tasco® fed at 0.5% and 3.0% resulted in significantly lower overall mortalities than 2.5% inulin (Figure 17). The most common causes of mortality were septicemia, leg deformities, peritonitis, acute death syndrome, and ascites, as well as omphalitis in the early growth period, but were not treatment related.

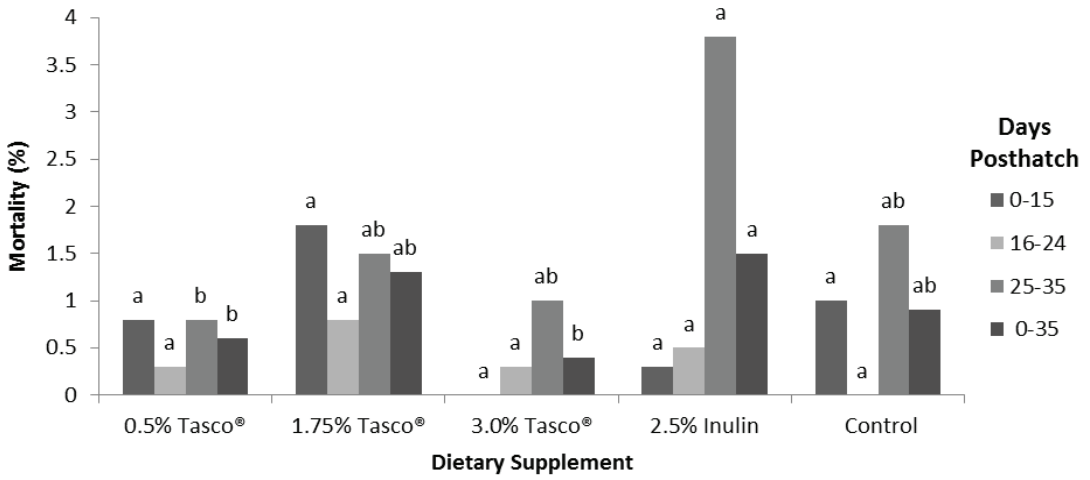


Figure 17: Trial 2 Mortality (%) of Broiler Chickens During the Starter (Day 0-15), Grower (Day 16-24), and Finisher (Day 25-35) Experimental Periods and over Whole Experimental Period with Dietary Supplementation of Tasco® or Inulin

[a-b] means with different letters within an experimental period are significantly different ($p \leq 0.05$)

4.5.3 Intestinal pH

Birds on new litter had significantly higher pH of cecal contents than those on previously used litter on day 21 (6.6±0.1 and 6.2±0.1 respectively, $p=0.001$) and higher pH of jejunal contents than those on previously used litter on day 35 (6.2±0.1 and 5.8±0.1 respectively, $p<0.001$). Supplement also had an effect on cecal content pH (Table 26). Feeding 3.0% Tasco® resulted in lower cecal content pH than 2.5% inulin or the control on day 21. The volume of cecal and jejunal contents from day 7 birds was too low to obtain accurate pH measurements. No effects were observed on pH of jejunal contents on day 21 or 35 or cecal content pH on day 35 ($p>0.05$) (Table 26). Placement on new or used litter did not affect dietary supplement effects on pH of either the jejunum or cecum on any sample day (Appendix B Table B-10).

Table 26: Trial 2 pH of the Cecal and Jejunal Contents of Broiler Chickens on Days 21 and 35 Posthatch with Dietary Supplementation of Tasco® or Inulin

	Cecal Content pH		Jejunal Content pH	
Days Posthatch	**21**	**35**	**21**	**35**
Dietary Supplement				
0.5% Tasco®	6.4±0.2[ab]	6.3±0.1	6.0±0.1	5.9±0.1
1.75% Tasco®	6.4±0.2 [ab]	6.3±0.1	6.0±0.1	6.0±0.1
3.0% Tasco®	5.9±0.1 [b]	6.4±0.1	6.1±0.1	6.0±0.1
2.5% Inulin	6.7±0.1 [a]	6.4±0.1	6.1±0.1	5.9±0.1
Control	6.6±0.2 [a]	6.6±0.1	6.0±0.1	6.1±0.1
ANOVA (p-value)				
Supplement	0.002	0.39	0.56	0.70
Litter	0.001	<0.001	0.15	<0.001
Supplement*Litter	0.86	0.23	0.89	0.31

[a-b] means±SEM with different superscripts within the same column are significantly different (p≤0.05)

4.5.4 Organ Weights

Relative organ weights of both immunological and intestinal organs were found to be affected by litter treatments (Table 27 and 28). Day 35 relative bursa weights were higher for birds on new litter (2.3±0.1 and 1.9±0.1 mg/g respectively, p=0.01). Day 21 relative cecal and ileal weights were found to be higher in birds on previously used litter (6.4±0.3 and 5.1±0.3 mg/g respectively, p=0.001) and (12.2±0.4 and 10.9 ±0.4 mg/g respectively, p=0.04).

Table 27: ANOVA p- values for Trial 2 Relative Bursa and Spleen Weight Analysis

	Relative Bursa Weight			Relative Spleen Weight		
Days Posthatch	**7**	**21**	**35**	**7**	**21**	**35**
ANOVA						
Supplement	0.29	0.72	0.96	0.88	0.66	0.71
Litter	0.35	0.87	0.009	0.99	0.54	0.06
Supplement*Litter	0.89	0.11	0.43	0.12	0.53	0.85

Table 28: ANOVA p-values for Trial 2 Relative Cecal and Ileal Weight Analysis

	Relative Cecal Weight			Relative Ileal Weight		
Days Posthatch	**7**	**21**	**35**	**7**	**21**	**35**
ANOVA						
Supplement	0.25	0.31	0.004	0.48	0.69	0.14
Litter	0.50	0.001	0.28	0.16	0.04	0.54
Supplement*Litter	0.98	0.91	0.14	0.25	0.64	0.14

Supplementation also had an effect on relative cecal weights on day 35 (Table 29). The 3.0% Tasco® increased relative cecal weights over 0.5% and 1.75% Tasco®. There were no effects on relative cecal weights on day 7 (Table 30), ileal weights on day 7 or 35 (Table 30), bursa weight on day 7 or 21 (Table 31) or spleen weights on any of the sampling days (Table 31).

Table 29: Trial 2 Relative Cecal Weights (mg cecal weight: g body weight) of Broiler Chickens Day 7, 21, and 35 Posthatch with Dietary Supplementation of Tasco® or Inulin

	Relative Cecal Weight (mg:g)		
Days Posthatch	**7**	**21**	**35**
Dietary Supplement			
0.5% Tasco®	7.2±0.5	5.4±0.4	3.4±0.3[b]
1.75% Tasco®	8.0±0.4	5.9±0.4	3.8±0.2[b]
3.0% Tasco®	8.6±0.5	6.5±0.4	4.8±0.2[a]
2.5% Inulin	7.7±0.5	5.3±0.4	3.8±0.2[ab]
Control	7.4±0.4	5.8±0.4	4.2±0.2[ab]

[a-b] means±SEM with different superscripts within the same column are significantly different (p≤0.05)

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Table 30: Trial 2 Relative Cecal and Ileal Weights (mg organ: g body weight) of Broiler Chickens Day 7, 21, and 35 Posthatch with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

		Relative Cecal Weight (mg:g)			Relative Ileal Weight (mg:g)		
Days Posthatch		7	21	35	7	21	35
Litter Treatment	**Dietary Supplement**						
New Litter	0.5% Tasco®	7.0±0.6	5.1±0.6	2.8±0.4	21.7±2.2	11.2±0.9	10.4±0.6
	1.75% Tasco®	7.9±0.6	5.3±0.6	4.0±0.3	21.7±2.0	11.9±0.9	10.1±0.6
	3.0% Tasco®	8.7±0.9	5.8±0.6	4.8±0.4	26.4±2.0	11.5±0.9	10.3±0.6
	2.5% Inulin	7.5±0.7	4.6±0.6	4.4±0.3	25.8±2.0	9.6±0.9	11.1±0.6
	Control	7.1±0.8	4.9±0.6	3.6±0.3	21.0±2.0	10.3±0.9	10.2±0.6
Previously Used Litter	0.5% Tasco®	7.4±0.7	5.7±0.6	4.0±0.3	20.4±2.0	11.9±0.9	11.1±0.6
	1.75% Tasco®	8.1±0.6	6.5±0.6	3.5±0.4	22.3±2.0	11.9±0.9	8.6±0.6
	3.0% Tasco®	8.5±0.8	7.2±0.6	4.9±0.3	22.9±2.0	12.5±0.9	11.3±0.6
	2.5% Inulin	7.9±0.6	6.0±0.6	4.1±0.3	19.2±2.0	11.9±0.9	9.6±0.6
	Control	7.7±0.7	6.7±0.6	4.2±0.3	22.8±2.0	12.6±0.9	10.3±0.6

Table 31: Trial 2 Relative Bursa and Spleen Weights (mg organ: g body weight) of Broiler Chickens Day 7, 21, and 35 Posthatch with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

		Relative Bursa Weight (mg:g)			Relative Spleen Weight (mg:g)		
Days Posthatch		7	21	35	7	21	35
Litter Treatment	**Dietary Supplement**						
New Litter	0.5% Tasco®	1.5±0.2	2.9±0.2	2.0±0.2	0.7±0.1	0.7±0.1	0.7±0.1
	1.75% Tasco®	1.9±0.2	2.8±0.2	2.2±0.2	0.6±0.1	0.8±0.1	0.7±0.1
	3.0% Tasco®	1.8±0.2	2.6±0.2	2.4±0.2	0.7±0.1	0.8±0.1	0.7±0.1
	2.5% Inulin	2.1±0.2	2.9±0.2	2.4±0.2	0.8±0.1	0.8±0.1	0.9±0.1
	Control	1.9±0.2	2.3±0.2	2.4±0.2	0.8±0.1	0.9±0.1	0.8±0.1
Previously Used Litter	0.5% Tasco®	1.5±0.2	2.4±0.2	2.0±0.2	0.8±0.1	0.8±0.1	0.8±0.1
	1.75% Tasco®	1.4±0.2	2.6±0.2	2.1±0.2	0.9±0.1	0.8±0.1	0.9±0.1
	3.0% Tasco®	1.9±0.2	2.4±0.2	1.8±0.2	0.7±0.1	0.8±0.1	1.0±0.1
	2.5% Inulin	2.0±0.2	2.8±0.2	1.8±0.2	0.6±0.1	0.7±0.1	0.9±0.1
	Control	1.8±0.2	3.0±0.2	1.8±0.2	0.7±0.1	0.8±0.1	0.8±0.1

4.5.5 Intestinal Histomorphology

ANOVA analysis for intestinal histomorphology measurements are shown in Table 32 and 33.

Table 32: ANOVA p- values for Trial 2 Mucosal Depth and Breakage Score Analysis

	Mucosal Depth			Breakage Score		
Days Posthatch	**7**	**21**	**35**	**7**	**21**	**35**
ANOVA						
Supplement	0.44	0.81	0.44	0.40	0.39	0.11
Inclusion Level	0.11	0.60	0.62	0.09	0.14	0.40
Supplement*Inclusion Level	0.64	0.10	0.69	0.73	0.09	0.28

There were no effects on any of the day 7, 21, or 35 intestinal histomorphology measurements taken ($p>0.05$) (Table 34-36). After histological processing and sectioning of ileal tissue, day 21 villi were found to be extremely damaged and so sufficient readings to perform statistical analysis were only possible for breakage scores and mucosal depth

Table 33: ANOVA p- values for Trial 2 Intestinal Histomorphology Measurement Analysis

	Villi Height:Crypt Depth Ratio		Villi Width		Crypt Depth		Villi Height		Villi Apparent Area	
Days Posthatch	**7**	**35**	**7**	**35**	**7**	**35**	**7**	**35**	**7**	**35**
ANOVA										
Supplement	0.34	0.51	0.07	0.58	0.28	0.48	0.62	0.47	0.47	0.83
Inclusion Level	0.98	0.29	0.58	0.43	0.74	0.21	0.80	0.99	0.74	0.73
Supplement* Inclusion Level	0.39	0.52	0.66	0.19	0.38	0.42	0.21	0.86	0.33	0.34

Table 34: Trial 2 Day 7 Ileal Intestinal Histomorphology Measurements of Broiler Chickens with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

Litter Treatment	Dietary Supplement	Breakage Score	Villi Height (µm)	Villi Width (µm)	Crypt Depth (µm)	Villi Height:Crypt Depth (µm:µm)	Mucosal Depth(µm)	Villi Apparent Area (mm^2)
New Litter	0.5% Tasco®	4.0±0.3	529.2±27.6	122.8±9.0	105.7±8.5	5.2±0.3	171.8±11.5	0.07±0.005
	1.75% Tasco®	3.0±0.3	511.5±21.4	141.0±6.9	108.5±6.6	4.9±0.2	181.3±11.5	0.07±0.004
	3.0% Tasco®	3.0±0.4	505.4±27.6	109.5±9.0	110.7±8.5	4.7±0.3	195.7±14.1	0.06±0.005
	2.5% Inulin	4.0±0.3	438.7±47.8	116.6±15.5	107.7±14.7	4.1±0.5	178.5±11.5	0.05±0.009
	Control	3.0±0.3	554.7±27.6	108.9±9.0	134.3±8.5	4.2±0.3	166.6±11.5	0.06±0.005
Previously Used Litter	0.5% Tasco®	3.0±0.3	506.0±21.4	127.2±6.9	112.5±6.6	4.7±0.2	161.8±11.5	0.07±0.004
	1.75% Tasco®	3.0±0.3	484.4±23.9	129.8±7.8	112.9±7.4	4.4±0.3	166.2±11.5	0.06±0.005
	3.0% Tasco®	3.0±0.3	530.8±33.8	117.9±11.0	119.3±10.4	4.7±0.4	177.9±11.5	0.06±0.006
	2.5% Inulin	3.0±0.3	537.8±27.6	121.6±9.0	116.5±8.5	4.7±0.3	151.8±11.5	0.07±0.005
	Control	3.0±0.3	503.5±21.4	119.3±6.9	115.3±6.6	4.5±0.2	174.9±11.5	0.06±0.004

Table 35: Trial 2 Day 21 Ileal Intestinal Histomorphology Measurements of Broiler Chickens with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

Litter Treatment	Dietary Supplement	Breakage Score	Mucosal Depth(µm)
New Litter	0.5% Tasco®	4.0±0.2	272.8±21.3
	1.75% Tasco®	4.0±0.2	236.6±23.3
	3.0% Tasco®	4.0±0.2	238.5±21.3
	2.5% Inulin	4.0±0.2	273.3±21.3
	Control	4.0±0.2	287.9±21.3
Previously Used Litter	0.5% Tasco®	3.0±0.2	258.7±21.3
	1.75% Tasco®	4.0±0.2	311.5±21.3
	3.0% Tasco®	4.0±0.2	268.7±21.3
	2.5% Inulin	4.0±0.2	243.1±21.3
	Control	4.0±0.2	262.2±21.3

Table 36: Trial 2 Day 35 Ileal Intestinal Histomorphology Measurements of Broiler Chickens with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

Litter Treatment	Dietary Supplement	Breakage Score	Villi Height (µm)	Villi Width (µm)	Crypt Depth (µm)	Villi Height:Crypt Depth (µm:µm)	Mucosal Depth(µm)	Villi Apparent Area (mm^2)
New Litter	0.5% Tasco®	3.0±0.3	939.9±80.3	149.3±14.0	188.8±13.9	5.0±0.5	313.2±23.6	0.16±0.02
	1.75% Tasco®	3.0±0.3	681.9±139.1	147.8±24.2	163.3±24.1	4.2±0.9	313.2±23.6	0.11±0.03
	3.0% Tasco®	3.0±0.3	841.4±80.3	134.0±14.0	153.6±13.9	5.5±0.5	318.0±23.6	0.13±0.01
	2.5% Inulin	2.0±0.3	873.3±69.6	171.3±12.1	172.9±12.0	5.0±0.4	283.3±26.0	0.17±0.02
	Control	3.0±0.3	845.8±80.3	180.6±14.0	140.3±13.9	6.2±0.5	295.3±23.6	0.16±0.02
Previously Used Litter	0.5% Tasco®	3.0±0.3	899.8±69.6	140.4±12.1	151.5±12.0	6.0±0.4	344.8±23.6	0.14±0.02
	1.75% Tasco®	4.0±0.3	795.6±98.4	150.0±17.1	160.2±17.0	5.1±0.6	305.2±23.6	0.14±0.02
	3.0% Tasco®	3.0±0.3	881.3±80.3	163.1±14.0	153.8±13.9	5.7±0.5	315.1±23.6	0.16±0.02
	2.5% Inulin	3.0±0.3	799.2±62.2	156.0±10.8	143.2±10.8	5.6±0.4	321.0±23.6	0.14±0.02
	Control	3.0±0.3	801.4±98.4	134.7±17.1	148.1±17.0	5.4±0.6	275.1±23.6	0.12±0.02

4.5.6 Litter Salmonella Levels

Litter analysis of all samples was not completed. One half of the 64 Day 35 samples were analyzed with no *Salmonella* being detected. The author was satisfied that, until microbiological profiles of the birds were determined and could suggest otherwise, no *Salmonella* was present at detectable levels in the flock.

4.6 Discussion

4.6.1 Growth Performance

As in the first trial, Tasco® improved growth performance over inulin in this trial. Improvement over inulin occured in BW, feed to gain, BWG, and FI. Unlike in the first trial Tasco® only improved BW over the control. Tasco® improved BW over the control at 1.75%. Lack of improvement over the control does not necessarily indicate an inability to achieve results from Tasco® supplementation in a production setting however. The stressor used in this study may not have been strong enough to result in growth improvement, as commonly prebiotics have no effect without a stressor present (Janczyk et al. 2010; Catalá-Gregori et al. 2008). Results from this study must therefore be interpreted with the knowledge that in most variables, Tasco® was comparable to the control. When compared to the inulin treatment, the lowest level of Tasco® (0.5%) showed beneficial effects. The 0.5% Tasco® improved BW, feed to gain, and BWG over inulin. Of the three levels of Tasco® fed, 0.5% and 1.75% were found to most consistently improve growth variables. With the exception of BW, most effects on growth occured in the later growth periods rather than the starter period. In BW the treatment response from 0.5% Tasco® improved from the starter to the grower and

finisher periods while 1.75% consistently performed well. This indicates that effects at 0.5% were either compensatory growth from physiological effects enacted in the early growth stages or Tasco® was not present at high enough levels to result in a response by earlier bacterial populations. It may then have been more efficiently fermented by bacterial populations established in the grower period, perhaps due to increased cross-feeding. An increase in bacteria able to utilize primary fermentation products of Tasco® would lead to a greater proliferation in a variety of microflora species (Buddington 2009). These observations regarding Tasco®'s optimal inclusion level are made in comparison to the poorly performing inulin, not the control, and so their value is unclear. However, they do indicate that there were differences in how each inclusion level performed and give some indication of which inclusion levels might best be examined in future studies.

The 2.5% inulin did not perform as well as expected from previous studies using inulin in broilers as it did not improve growth at all over the control. Rebole et al. (2010) found inulin to increase BWG, Tianxing et al. (1999) found FOS to increase BW and improve feed conversion ratio, and Catala- Gregori et al. (2008) found FOS to increase BWG in broilers. However, other studies have been performed which did not find inulin or FOS to increase growth (Gier et al. 2009; Janardhana et al. 2009; Rehman et al. 2008). Factors such as chain length of inulin fed can affect the bacterial response and thus the response in growth variables (Patterson et al. 2010). The inulin used in this study had a low average DP of ≤10 which would likely cause it to have more of an effect in the more proximal digestive tract (Patterson et al. 2010), and be more easily fermented by various bacteria (Biekela et al. 2002). This does not explain why the same inulin was found to improve growth in the first trial but not in the current trial. Perhaps bacterial populations

present in the earlier flock were able to ferment inulin, but they were not present in this flock. Previous studies have suggested that ANOD is not fermented by typical probiotic bacteria such as *Bifidobacterium* and *Lactobacillus* where as inulin is (Dierick et al. 2009; Gardiner et al. 2008). Even if bacteria able to ferment inulin were not present in this study, it therefore would not necessarily mean that Tasco® could not be fermented. Thus, effects from Tasco® would be observed but not from inulin, as in this trial. Due to inulin's poor performance improvements of Tasco® supplementation compared to the inulin treatment are difficult to interpret.

4.6.2 Bird Health

Rather than decreasing mortalities as expected, inulin was actually found to increase percent mortality over 0.5% Tasco® in the finisher period. Reasons for this are not clear. It is an effect which was not observed in Trial 1. If bacterial populations present were not able to ferment inulin then the birds would have been at a disadvantage in pathogen resistance. However, it is still not clear why the control would then not have resulted in increased mortalities. Tasco® at 0.5% and 3.0% reduced percent mortality averaged over all growth periods compared to inulin. However, if inulin was not performing optimally in this parameter the importance of this observation becomes decreased.

4.6.3 Intestinal pH

The pH of cecal contents was found to be lowered by Tasco® supplementation in this trial, indicating a beneficial effect on fermentation. The 3.0% Tasco® decreased cecal content pH on day 21. Decreases in pH are thought to indicate SCFA production in

response to prebiotic supplementation and can be detrimental to growth of acid – sensitive pathogenic species (Van Immerseel et al. 2003; Russell and Diez-Gonzalez 1997). Alteration of the pH on day 21 but not 35 is most likely due to changes in bacterial populations as the birds aged, leading to altered fermentation patterns.

The 2.5% inulin did not decrease cecal or jejunal content pH compared to the control, which could further indicate that it was not being fermented as expected. Alternatively it could indicate that it was just not being fermented to the same degree as Tasco®, and thus was not able to increase SCFA concentrations enough to alter pH.

4.6.4 Organ Weights

On day 35, 3.0% Tasco® increased relative cecal weights comparatively to the other Tasco® levels, though not over inulin or the control. SCFA, particularly butyrate, are known to stimulate growth of colonic epithelial cells (Candela et al. 2010). The increased relative cecal weight may therefore have been due to increased production of SCFA; however it was not accompanied by a decrease in pH. This indicates that if there was an increase in SCFA, it was not great enough to alter pH. Alternatively, it may be that some other mechanism was acting in the birds on those treatments to increase relative cecal weight which was not measured in this study, such as mucin production or goblet cell number, due to influences of the bacterial population in those birds.

4.6.5 Intestinal Histomorphology

Similarly to Trial 1, there were no effects of supplement on intestinal histomorphology measurements on day 35. However, treatment differences occurred in Trial 1 day 7 and 21 ileal samples whereas in this trial no treatment differences were

observed on those days. Day 7 and 21 samples were more damaged in this trial as demonstrated by the breakage scores tending to be higher in Trial 2 than Trial 1 samples. Reasons for this are not known though it is most likely due to handling during the sampling process as there were no influences of supplement or litter treatments. This increased damage may have masked any effects of dietary or litter treatment on other histomorphology measurements by causing fewer villi to be available to take measurements from, which would explain the lack of treatment effect. It may also be that in the first trial, differences in villi height and apparent area as well as crypt depth were observed between Tasco® and inulin as a whole, rather than individual levels of the supplements. In this trial, individual levels were analyzed separately making it more difficult for differences to be observed.

4.6.6 Effect of Dietary Supplement on Trough of Immunity

No physiological variables were found to be altered in the day 7 data collected. This would indicate that the feed additives had no effect on improving the birds' nutrient absorption or pathogen resistance during this time, unlike in the first trial. Lack of effect may be due to competitive exclusion effects of the litter creating uniformity in the birds on previously used litter and a lack of pathogen presence in birds on new litter. It may also be due to increased exposure of birds to the excreta of other birds and moisture due to placement on litter rather than in cages, hindering Tasco®'s actions during this time period.

4.6.7 Effect of the Used Litter Challenge

The lack of *Salmonella* detected in the litter samples is consistent with Baurhoo et al. (2009) who also sampled litter from a facility with optimal biosecurity enforcement and sanitary conditions, as there was in this trial. Conversely, *Salmonella* presence has been detected in previously used litter in other studies. When Chinivsagam et al. (2010) used MPN analysis to determine pathogen presence in litter from 28 farms in Australia *Salmonella* was detected in 5 of the 6 farms that re- used their litter at levels ranging from 4 to 93 MPN/g of litter and in 15 of 22 farms that only used litter once at levels of 4 to $1.1*10^5$ MPN/g of litter. It is possible that *Salmonella* was not detected, in addition to the biosecurity protocols, due to the lack of chick exposure to *Salmonella* in the hatchery. Lahellec et al. (1986) found that incoming chicks, rather than the environment, were the greatest contributors to *Salmonella* serovars in broiler houses.

Environmental factors other than biosecurity can also affect *Salmonella* populations. Factors such as litter moisture content, temperature, and ammonia were all found to affect pathogen survival in litter (Santos et al. 2005; Williams 1978). The samples collected were frozen prior to analysis which may have altered ability to detect *Salmonella*. However, the pH and water activity of samples was not measured so it is unclear if these had any affect. The agars and broths used in this study for MPN analysis were also used by Rybolt et al. (2005) to successfully detect *Salmonella* in 51.6% of litter samples, thus showing that the methodology used most likely did not play a role in the lack of *Salmonella* detected. It is possible that while *Salmonella* was not found in the litter, other pathogens were present which were not tested for. Chinivasagam et al. (2010) found *E. coli* and *Campylobacter* to also be present in both farms that re- used litter and

farms that used litter only once. *E. coli* was found at the highest levels of the three pathogens measured. Had the litter samples been tested for *E. coli* or *Campylobacter* therefore positive samples may have been detected.

It was expected that the previously used litter treatment would increase the results observed with Tasco® treatment as the presence of stressors has been shown to enhance Tasco® response (Kannan et al. 2007; Allen et al. 2001; Bailey et al. 1991; Orban et al. 1997). While *Salmonella* was not detected, litter treatment was shown to have an effect, although not always what was anticipated. Treatment differences in feed to gain occured, but only within birds placed on new litter. Birds on previously used litter had no differences. Birds on new litter had increased FI during all experimental periods and decreased BWG in the starter period but improved BWG in the grower period. Once in the finisher stage no differences in BWG were observed in birds on different litter types at all. This indicates some effect of litter treatment on growth, though not one that supplementation with Tasco® or Inulin was able to overcome.

Previous studies using Tasco® in stressed animals have not produced differences in growth variables, but rather physiological variables (Kannan et al. 2007; Allen et al. 2001). In this study, relative bursa weights were found to be higher in birds on new litter, indicating an increased immune response in these birds. The lack of effect observed on spleen weight may indicate that this was solely stimulation of the local gut immune system and that systemic immunity was not affected. These observations are the opposite of what was expected. Allen et al. (2001) found immune function to be enhanced in steers fed Tasco®, but only within the steers that had grazed endophyte infected pastures, not those that had grazed uninfected pastures. Lack of effect in birds on previously used

litter may indicate that the bacteria in the previously used litter acted as a competitive exclusion treatment to the chicks, providing them with benefical bacteria from the previous flock. This would have served to benefit birds on all dietary treatments creating less of a stressor rather than more. The previous flock had very low mortality rate of only 1.3% indicating that the pathogen load in the litter was most likely very low. Competitive exclusion effects may have resulted in a more uniform growth response regardless of supplement. Further supporting this theory are the observations of lower jejunal content pH, increased relative cecal weights, and increased relative ileal weights in birds on previously used litter. The lower pH of jejunal contents would be consistent with a high degree of fermentation occurring from the beneficial microbe populations. This effect did not carry through to the ceca, where pH was higher in birds on previously used litter. The increased relative cecal and ileal weights may indicate an effect on increasing mucin production. Though competitive exclusion may have been responsible for lack of dietary treatment effect, there was still enough of a challenge present to prevent equal growth response in birds on new and previously used litter. The only way to be certain of the effect previously used litter treatment can have is to carry out the trial again using spiked litter with known amounts of particular pathogens.

4.6.8 Trial Differences

While Tasco® was shown to improve growth variables in both trials to some degree, Tasco® performed much better compared to the control in the first trial. In Trial 1 the intermediate levels performed very poorly while in Trial 2, 1.75% Tasco® was the only level to improve growth compared to the control. Variations in microflora present in each flock may explain these differences as they would alter how well each

polysaccharide present in Tasco® was fermented. Tasco®'s exact mode of action is yet to be discovered and may allow for a clearer interpretation of these findings.

Another possible explanation for treatment differences between Trial 1 and Trial 2 is the housing of birds in cages versus pens. Garcia et al. (2006) found birds grown in battery cages to have improved growth performance in a study comparing the growth of birds from day 21 to 38. However, the opposite was found by Santos et al. (2008) where birds raised on litter in pens were found to have improved feed conversion, lower cecal *Salmonella* levels, and increased villi height, villi SA, villi height to crypt depth ratio, and increased mucosal depth in a study looking at growth of birds from day 0 to 42. Growing birds in floor pens with litter allows them to have increased exposure to the feces of other birds. This can act to increase their pathogen exposure (Garcia et al. 2006) but can also act as a competitive exclusion treatment allowing them to show improved growth performance (Santos et al. 2008).

Between Trial 1 and 2 there were dietary differences. In Trial 1 birds were fed diets in mash form throughout the trial. In Trial 2 birds were given diets as crumbles during the starter period and then switched to pelleted diets for the remainder of the birds' growth. Dietary form has been found to have an influence on growth performance. Pelleting is thought to lower the amount of energy birds must use to break up the feed and so allow them to have more energy available for growth (Jensen et al. 1962). El-Deek and Brikaa (2009) found ducks fed pelleted diets were able to utilize their feed more efficiently than ducks given mash diets and Engberg et al. (2002) and Bennet et al. (2002) found birds fed pellets to have increased FI and BWG. Similarly, when feeding

seaweed to ducks, Jensen et al. (1962) found that ducks receiving diet in pelleted form had increased BW and BWG.

As a potential prebiotic, the effects able to be produced by Tasco® supplementation may be dependant on the presence of microbial populations in the gut that are able to ferment it (Gudiel – Urbano and Goni 2002). Therefore, any environmental factors which would alter the nature of the microbial populations present in the chicks' immediate surroundings in the hatchery, in transport, and in the production facility may alter what effects are observed from Tasco® supplementation. Changes of these types in the flocks of chicks from each trial would influence results.

Increased performance with birds in pens and fed pelleted diets would help explain why BW, BWG, and FI tended to be higher in the second trial and feed efficiency seemed to be improved. Tasco® improved more growth variables over inulin in Trial 2 than 1 such as BW, FI, BWG, and feed to gain and improved % mortality over inulin, however inulin had poor performance in Trial 2. Conversely, improvement over the control fed birds occured to a much greater degree in Trial 1, possibly indicating that the caged birds had more stressors present than those in the floor pens, such as higher stocking density, allowing a greater feed additive response.

4.7 Conclusions

Tasco® did not result in as great of an improvement in growth as in Trial 1. The 1.75% Tasco® improved BW over the control but was equal to the control in all other growth variables. The 0.5% Tasco® improved BW, feed to gain, and BWG over inulin, which performed poorly in this study. Tasco® did display prebiotic like effects in altering

relative cecal weights and decreasing cecal content pH. Results of this trial were inconclusive regarding the value of Tasco® in a commercial production setting due to lack of a strong stressor.

Chapter 5. Effects Of Tasco®, Inulin, And An Antibiotic On Growth Of Broiler Chickens Raised In Pens

5.1 Abstract

Performance of candidate prebiotic Tasco®, made of sun dried *Ascophyllum nodosum*, was compared to the known prebiotic inulin and the antibiotic virgniamycin in an extended growing period. Based on previous trials, seven dietary treatments of 0.25%, 0.5%, 1.0%, 2.0%, and 2.0% Pulse (fed day 0-14 only) Tasco®, a negative control, a positive control with antibiotic, and 2.5% inulin were fed to male broiler chickens. Diets were formulated for the starter (day 0-14), grower (day 15-24), finisher 1 (day 25-35), and finisher 2 (day 36-45) experimental periods. Tasco® at 0.25% and 0.5% increased finisher 1 body weight over the negative control ($p \leq 0.05$) and 0.25%, 1.0%, and 2.0% Pulse Tasco® increased overall body weight gain over the negative control ($p \leq 0.05$). Tasco® at 1.0% and 2.0% increased grower and finisher 1 feed intake over both controls ($p \leq 0.05$). Tasco® at 0.25%, 1.0% and 2.0% improved finisher 2 feed intake over the negative control, and 1.0% and 2.0% Tasco® improved feed intake over 2.5% inulin in the finisher 2 period ($p \leq 0.05$). There was no effect on feed to gain. Tasco® at 0.25% increased finisher 2 body weight over 2.0% Tasco® with opposite results for day 7 relative bursa weight and day 21 cecal content pH and villi breakage score ($p \leq 0.05$) indicating varying modes of action for different levels. Overall, Tasco® was comparable to the antibiotic in improving growth. Tasco® at 0.25% and 0.5% particularly were shown to improve growth of broilers, showing further promise of low levels and affordability of Tasco® for producers.

Keywords: *Tasco®, seaweed, inulin, antibiotic, poultry, prebiotics*

5.2 Introduction

There is some indication that Tasco® has effects as a pulse treatment fed at low levels for short periods of time (F. Evans personal communication; Allen et al. 2001; Bach et al. 2008); however, this use of Tasco® has not been tested in monogastrics previously. There is some indication that prebiotics may have extended effects when growing periods are lengthened. This allows physiological changes occurring in earlier growth periods to result in improved growth in later periods (Baurhoo et al. 2009). Some American poultry production facilities grow out their birds to 45 days of age and so compensatory growth effects would be of interest to this industry.

The two previous trials found low levels of Tasco®, such as 0.5%, to improve growth performance comparatively, and often better than, the higher levels. No evaluation of lower levels has been conducted with broilers. Decreasing Tasco®'s inclusion level further was therefore of interest in this trial to determine if the improved effect on growth could be observed when Tasco® was fed as low as 0.25% of the diet. The previous two trials did not include an antibiotic to compare Tasco® and a commercial inulin product against, making a final assessment of Tasco®'s potential as an antibiotic alternative impossible. This trial therefore included an antibiotic treatment for comparison.

5.3 Objectives

An objective of this trial was to determine the effect of Tasco® supplementation when fed to birds grown to 45 days of age. A second objective was to evaluate Tasco® at levels as low as 0.25%, determine the effectiveness of Tasco® as a pulse treatment, and compare Tasco® directly to a commonly used antibiotic.

5.4 Materials and Methods

5.4.1 Animals and Husbandry

Fourteen hundred and twenty four male Ross 508 broilers from Clark's Hatchery (Burtts Corner, NB) were used in this trial which took place from December 2010 through January 2011. Prior to shipping, chicks were vaccinated with 0.05 mL Mareck's vaccine (Intervet/Schering –Plough, Kirkland, QC). Chicks were received at day of hatch and upon arrival were randomly placed in forty two, 2.13m x 1.40m pens with soft wood shavings as litter at a stocking rate of 30 birds per pen in two of the climate controlled rooms and 29 birds per pen in one of the climate controlled rooms at the Atlantic Poultry Research Centre in Truro, NS. Stocking densities were 0.44 kg/m^2 on day 0 and 26.02 kg/m^2 on day 45. Treatments were equally repeated with 2 repetitions per room. Birds were provided with feed via tube feeders and water via nipple drinkers immediately and feed and water continued to be provided *ad libitum* throughout the trial. Upon placement birds were provided feed scattered on 51cm x 43cm cardboard boxes for the first week as well as feed provided in the tube feeders. Lighting and temperature schedules followed those reported in Trial 1 (Appendix A Table A-1). All procedures were carried out in accordance with the Canadian Council on Animal Care guidelines (CCAC 2009).

5.4.2 Diets

Diets were formulated to be isonitrogenous and isocaloric within period. The diets were formulated for each of the four grower periods; starter (day 0-14) (Table 37), grower (day 15-24) (Table 38), and finisher which included the finisher 1 (day 25-35) and finisher 2 phases (day 36-45) (Table 39).

Table 37: Trial 3 Diet Formulations for the Starter Period (Day 0-14) with Tasco® Fed at 0.25%, 0.5%, 1.0%, 2.0%, and 2.0% Pulse Treatment Compared to 2.5% Inulin and an Antibiotic

	Negative Control	Positive Control	0.25% Tasco®	0.5% Tasco®	1.0% Tasco®	2.0% Tasco®	2.0% Pulse Tasco®*	2.5% Inulin
Ingredient (% as fed)								
Soybean Meal	40.77	40.78	40.88	40.96	41.12	41.47	41.47	41.66
Corn	40.86	40.84	40.34	39.81	38.80	36.69	36.69	35.61
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Poultry Fat	4.04	4.05	4.23	4.41	4.77	5.51	5.51	5.9
Ground Limestone	1.79	1.79	1.79	1.79	1.78	1.78	1.78	1.77
Mono-Dicalcium Phosphorus	0.72	0.72	0.72	0.72	0.73	0.74	0.74	0.75
Pel- Stik	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Tasco® †	-	-	0.25	0.50	1.0	2.0	2.0	-
Inulin‡	-	-	-	-	-	-	-	2.50
Virginiamycin§	-	0.25	-	-	-	-	-	-
Vitamin/mineral Premix¶	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Iodized Salt	0.44	0.44	0.44	0.44	0.44	0.44	0.44	0.45
Methionine Premix**	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Total	100	100	100	100	100	100	100	100
Calculated Analysis								
Metabolizable Energy (kcal/kg)	3050	3050	3050	3050	3050	3050	3050	3050
Protien (%)	23.0	23.0	23.0	23.0	23.0	23.0	23.0	23.0

*2.0% Pulse Tasco® was composed of Tasco® fed at 2.0% for the Starter Period Only (Day 0-14). Birds were then switched to a diet with no additives.

†Tasco® was provided by Acadian Seaplants Ltd (Dartmouth, NS)

‡Inulin was provided from Cargill Inc. (Wayzata MN) as Oliggo-Fiber™ DS2 inulin (average DP≤10)

§Virginiamycin was added as the product Stafac 44 provided by Elanco Animal Health (Guelph,ON)

¶Vitamin/mineral premix contains the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D_3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B_{12}; 29.7 mg niacin; 1.0 mg folic acid,801 mg choline;0.3 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc;25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543mg wheat middlings; 500 mg ground limestone

**The methionine premix contained 0.5kg/kg DL- Methionine and 0.5kg/kg wheat middlings

Table 38: Trial 3 Diet Formulations for the Grower Period (Day 15-24) with Tasco® Fed at 0.25%, 0.5%, 1.0%, 2.0%, and 2.0% Pulse Treatment Compared to 2.5% Inulin and an Antibiotic

	Negative Control	Positive Control	0.25% Tasco®	0.5% Tasco®	1.0% Tasco®	2.0% Tasco®	2.0% Pulse Tasco®*	2.5% Inulin
Ingredient (% as fed)								
Corn	48.54	48.49	48.02	47.47	46.44	44.33	48.54	43.28
Soybean Meal	32.99	32.99	33.07	33.17	33.34	33.69	32.99	33.86
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Poultry Fat	4.57	4.59	4.76	4.95	5.31	6.06	4.57	6.43
Ground Limestone	1.72	1.72	1.72	1.71	1.71	1.70	1.72	1.70
Mono-Dicalcium Phosphorus	0.58	0.58	0.59	0.59	0.59	0.60	0.58	0.61
Pel- Stik	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Tasco® †	-	-	0.25	0.50	1.0	2.0	-	-
Inulin‡	-	-	-	-	-	-	-	2.50
Virginiamycin§	-	0.25	-	-	-	-	-	-
Vitamin/mineral Premix¶	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.20
Iodized Salt	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Methionine Premix**	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.20
Total	100	100	100	100	100	100	100	100
Calculated Analysis								
Metabolizable Energy (kcal/kg)	3150	3150	3150	3150	3150	3150	3150	3150
Protien (%)	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0

*2.0% Pulse Tasco® was composed of Tasco® fed at 2.0% for the Starter Period Only (Day 0-14). Birds were then switched to a diet with no additives.

†Tasco® was provided by Acadian Seaplants Ltd (Dartmouth, NS)

‡Inulin was provided from Cargill Inc. (Wayzata MN) as Oliggo-Fiber™ DS2 inulin (average DP≤10)

§Virginiamycin was added as the product Stafac 44 provided by Elanco Animal Health (Guelph,ON)

¶Vitamin/mineral premix contains the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D_3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B_{12}; 29.7 mg niacin; 1.0 mg folic acid,801 mg choline;0.3 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc;25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543mg wheat middlings; 500 mg ground limestone

**The methionine premix contained 0.5kg/kg DL- Methionine and 0.5kg/kg wheat middling

Table 39: Trial 3 Diet Formulations for the Finisher 1 (Day 25-35) and Finisher 2 (Day 36-45) Periods with Tasco® Fed at 0.25%, 0.5%, 1.0%, 2.0%, and 2.0% Pulse Treatment Compared to 2.5% Inulin and an Antibiotic

	Negative Control	Positive Control	0.25% Tasco®	0.5% Tasco®	1.0% Tasco®	2.0% Tasco®	2.0% Pulse Tasco®*	2.5% Inulin
Ingredient (% as fed)								
Corn	54.52	54.50	54.05	53.56	52.57	50.60	27.65	49.61
Soybean Meal	27.65	27.65	27.72	27.81	27.97	28.30	54.52	28.47
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Poultry Fat	4.03	4.03	4.18	4.33	4.65	5.28	4.03	5.61
Ground Limestone	1.73	1.73	1.73	1.73	1.72	1.71	1.73	1.71
Mono-Dicalcium Phosphorus	0.55	0.55	0.55	0.56	0.56	0.57	0.55	0.58
Pel- Stik	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Tasco® †	-	-	0.25	0.50	1.0	2.0	-	-
Inulin‡	-	-	-	-	-	-	-	2.50
Virginiamycin§	-	0.25	-	-	-	-	-	-
Vitamin/mineral Premix¶	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Iodized Salt	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Methionine Premix**	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11
Total	100	100	100	100	100	100	100	100
Calculated Analysis								
Metabolizable Energy (kcal/kg)	3200	3200	3200	3200	3200	3200	3200	3200
Protien (%)	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0

*2.0% Pulse Tasco® was composed of Tasco® fed at 2.0% for the Starter Period Only (Day 0-14). Birds were then switched to a diet with no additives.

†Tasco® was provided by Acadian Seaplants Ltd (Dartmouth, NS)

‡Inulin was provided from Cargill Inc. (Wayzata MN) as Oliggo-Fiber™ DS2 Inulin (average DP≤10)

§Virginiamycin was added as the product Stafac 44 provided by Elanco Animal Health (Guelph,ON)

¶Vitamin/mineral premix contains the following per kg of diet: 9750 IU vitamin A; 2000 IU vitamin D_3; 25 IU vitamin E; 2.97 mg vitamin K; 7.6 mg riboflavin; 13.5 mg Dl Ca-pantothenate; 0.012 mg vitamin B_{12}; 29.7 mg niacin; 1.0 mg folic acid,801 mg choline;0.3 mg biotin; 4.9 mg pyridoxine; 2.9 mg thiamine; 70.2 mg manganese; 80.0 mg zinc;25 mg copper; 0.15 mg selenium; 50 mg ethoxyquin; 1543mg wheat middlings; 500 mg ground limestone

**The methionine premix contained 0.5kg/kg DL- Methionine and 0.5kg/kg wheat middlings

Diets met or exceeded the NRC (1994) nutrient requirements for birds at each stage of growth. Seven dietary treatments were fed. Tasco®, provided by Acadian Seaplants Ltd. (Yarmouth, NS), was fed at 0.25%, 0.5%, 1.0%, and 2.0% as well as a 2.0% pulse treatment, which was fed to the chicks during the starter period only after which the birds were switched to a diet with no feed additive. The commercial inulin, provided by Cargill Inc. (Wayzata, MN) as Oliggo- Fiber™ DS2 inulin with average DP≤10, was fed at 2.5%. A positive control containing the antibiotic virginiamycin and a negative control with no feed additive were also fed. Diets were fed in crumble form during the starter period and pelleted form during the grower and both finisher periods. Dietary treatments were randomly distributed among the pens in each room with 6 pens per treatment.

5.4.3 Analysis of Growth Performance

Collection of growth performance data followed the same procedure as in Trial 1 (section 3.4.3) with the exception of a day 45 data collection where all birds in a pen were weighed and their feed was weighed back and recorded.

5.4.4 Sample Collection

Collection of data on physiological variables followed the same procedure as Trial 1 (section 3.4.4) with the addition of a day 45 sample collection where one bird per pen was euthanized by cervical dislocation. The ileum and ceca were emptied by gentle squeezing and weighed. Each bird was weighed and the ileum, jejunum, ceca, bursa, and spleen were removed. A 0.5- 1.0 cm section was removed from the middle of the ileum and rinsed in deionized water and then placed in 10% buffered formalin for storage. The

cecal and jejunal contents were collected and the pH was measured with an Accumet AP62 portable pH/mV meter. The bursa and spleen were weighed. Relative organ weights were calculated as a ratio of organ weight to BW.

5.4.5 Analysis of Intestinal Histomorphology

Intestinal histomorphology analysis followed the same procedures as described in Trial 1 (section 3.4.5).

5.4.6 Statistical Analysis

Birds were randomly allocated to one of the 7 dietary treatments with 6 pens per treatment. The trial was a single factor randomized block design with dietary supplement as the main factor and room as the block. Pen was used as the experimental unit. Data was analyzed using ANOVA in SAS 9.2 (SAS Institute Inc, Cary, NC). Growth data and % mortality were analyzed as repeated measures with day as a factor. Where interactions with day were significant (α=0.05) data was sliced by day and analyzed separately. Data on physiological variables was analyzed as single measurement. Any significant main or interaction effects (α=0.05) were analyzed using Tukeys (Littell et al. 1996) to differentiate the means.

Statistical Model for Trial 3 Repeated Meausures Analysis:

$$\gamma_{ijkl} = \text{Supplement}_i + \text{Day}_j + \text{Supplement*Day}_{ij} + \text{Room}_k + \epsilon_{ijkl}$$

Statistical Model for Trial 3 Single Measures Analysis:

$$\gamma_{ijk} = \text{Supplement}_i + \text{Room}_j + \epsilon_{ijk}$$

Statistical models for experimental design are shown above where γ is the response of the parameter being measured, μ is the overall mean response of that parameter, $Supplement_i$ is the effect of supplement for the i^{th} level of supplement (i=1-7), Day_j is the effect of the j^{th} level of day(j=1-4), $Room_k$ is the effect of the k^{th} level of the block (room) (k=1-3), and Є is the effects of the uncontrollable factors for the the i^{th} level of supplement, j^{th} level of day, k^{th} level of the block (room), and l^{th} repetition. The statistical model for a single measurement analysis is likewise displayed above where all terms are the same, with the exception that day is no longer a factor.

5.5 Results

The block used in this trial, room, was not found to be significant for any parameter ($p \geq 0.10$). Data was therefore reanalyzed with the block removed.

5.5.1 Growth Performance

Results of ANOVA analysis for each statistical model component are shown in Table 40.

Table 40: ANOVA p-values for Trial 3 Growth Variable Analysis

Growth Variable	Body Weight	Body Weight Gain	Feed Intake	Feed to Gain
ANOVA				
Supplement	<0.001	0.001	<0.001	0.70
Day	<0.001	<0.001	<0.001	<0.001
Supplement* Day	<0.001	0.16	0.01	0.41

There was a supplement*day interaction for BW per bird (Table 40). Dietary supplementation was found to affect BW per bird during all growth periods (Figure 18).

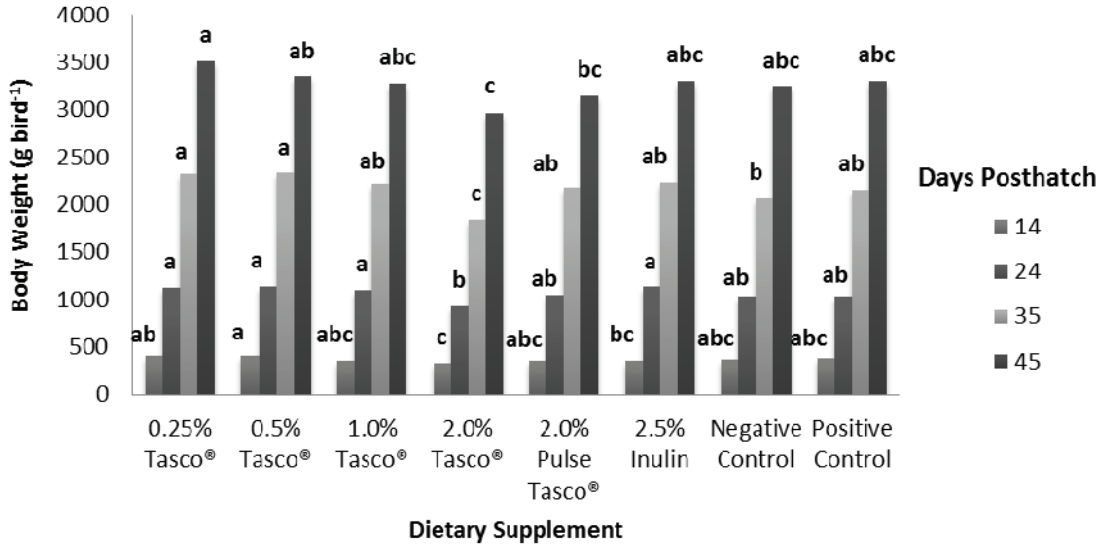

Figure 18: Trial 3 Body Weight (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), Finisher 1 (Day 25-35), and Finisher 2 (Day 36-45) Experimental Periods with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

$^{a-c}$ means with different letters within an experimental period are significantly different ($p \leq 0.05$)

During all experimental periods feeding 0.25% and 0.5% Tasco® improved BW over 2.0% Tasco®. In addition, feeding Tasco® at 0.5% increased BW over 2.5% inulin in the starter period, 0.25% and 0.5% Tasco® increased BW over the negative control in the finisher 1 period, and 0.25% Tasco® increased BW over 2.0% Pulse Tasco® in the finisher 2 period. 2.0% Tasco® had lower BW than 1.0% Tasco® and 2.5% inulin in the grower period and the negative control in the finisher 1 period.

Supplement had an effect on BWG averaged over all growth periods (Table 40). 0.25%, 1.0%, and 2.0% Pulse Tasco® as well as the positive control resulted in increased average BWG over the negative control (Table 41).

Table 41: Trial 3 Body Weight Gain (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), Finisher 1 (Day 25-35), and Finisher 2 (Day 36-45) Experimental Periods and Whole Experimental Period with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

	Days Posthatch				
	0-14	**15-24**	**25-35**	**36-45**	**Overall Average 0-45**
Dietary Supplement					
0.25% Tasco®	360.2±45.8	706.2±45.8	1160.7±45.8	1170.2±45.8	849.3±19.0[a]
0.5% Tasco®	314.1±45.8	679.9±45.8	1103.7±45.8	1061.6±45.8	789.8±19.0[ab]
1.0% Tasco®	372.4±45.8	745.5±45.8	1219.5±45.8	1002.5±45.8	835.0±19.0[a]
2.0% Tasco®	334.4±45.8	763.1±45.8	1125.2±45.8	1010.8±50.1	808.4±19.0[ab]
2.0% Pulse Tasco®	342.0±45.8	715.0±45.8	1115.3±45.8	1121.0±45.8	823.3±19.0[a]
2.5% Inulin	291.0±45.8	697.2±45.8	1034.1±45.8	1097.5±45.8	779.9±19.0[ab]
Negative Control	272.4±45.8	617.0±45.8	911.1±45.8	1087.6±45.8	722.0±19.0[b]
Positive Control	325.4±45.8	664.8±45.8	1126.4±45.8	1171.9±45.8	822.1±19.0[a]

[a-b] means±SEM with different superscripts within a column are significantly different (p≤0.05)

There was a supplement*day interacton for FI (p≤0.05) (Table 40). Supplementation resulted in differences in FI during the grower and finisher 1 and 2 periods (p≤0.05). During the grower and finisher 1 periods feeding 1.0% Tasco® increased FI over 0.5% Tasco® and the negative control. The negative control resulted in lower FI than 2.0% Pulse Tasco® during the grower period and 2.0% Tasco® during the finisher 1 period. Feeding 1.0% and 2.0% Tasco® resulted in increased FI over the positive control in the finisher 1 period. During the finisher 2 period feeding 1.0% Tasco® increased FI over 2.0% Pulse Tasco®, 2.5% Inulin, and the negative control. In addition,

2.0% Tasco® increased finisher 2 FI over 2.5% Inulin and, along with 0.25% Tasco®, increased finisher 2 FI over the negative control (Figure 19).

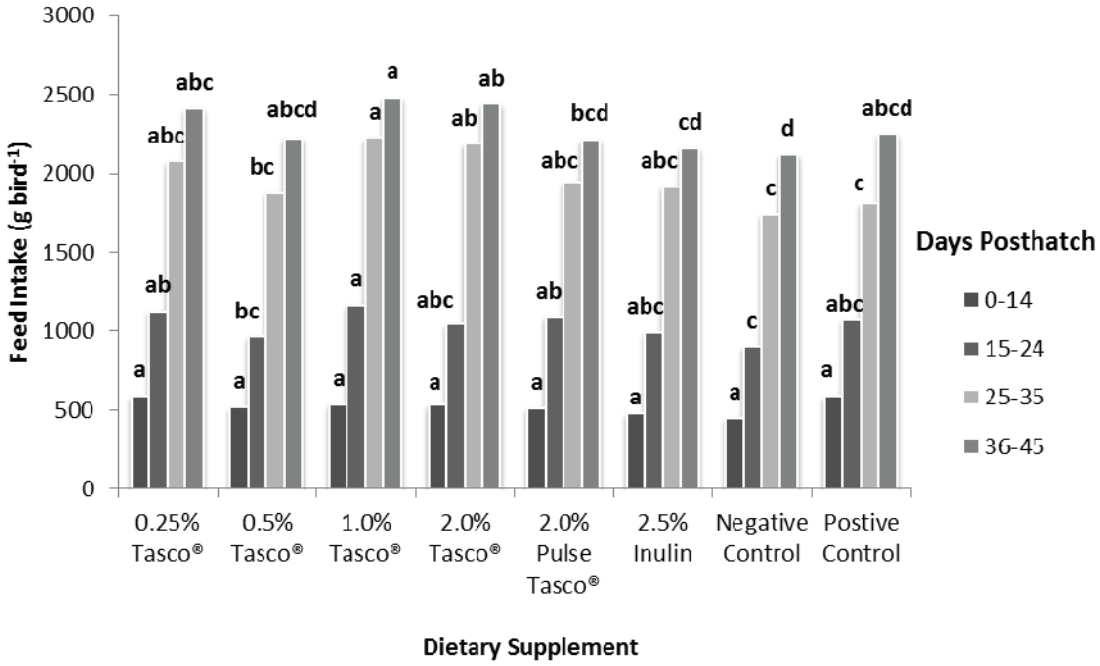

Figure 19: Trial 3 Feed Intake (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), Finisher 1 (Day 25-35), and Finisher 2 (Day 36-45) Experimental Periods with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

$^{a-d}$ means with different letters within an experimental period are significantly different ($p \leq 0.05$)

There were no effects on feed to gain ($p > 0.05$) (Table 40 and 42)

Table 42: Trial 3 Feed to Gain (g:g) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), Finisher 1 (Day 25-35), and Finisher 2 (Day 36-45) Experimental Periods with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

	Days Posthatch			
	0-14	**15-24**	**25-35**	**36-45**
Dietary Supplement				
0.25% Tasco®	1.63±0.10	1.60±0.10	1.79±0.10	2.07±0.10
0.5% Tasco®	1.65±0.10	1.43±0.10	1.71±0.10	2.14±0.10
1.0% Tasco®	1.44±0.10	1.57±0.10	1.83±0.10	2.19±0.11
2.0% Tasco®	1.59±0.10	1.37±0.10	1.95±0.10	1.89±0.12
2.0% Pulse Tasco®	1.50±0.10	1.52±0.10	1.75±0.10	1.97±0.10
2.5% Inulin	1.70±0.10	1.43±0.10	1.85±0.10	2.01±0.10
Negative Control	1.66±0.10	1.54±0.10	1.92±0.10	2.03±0.10
Positive Control	1.53±0.11	1.65±0.10	1.61±0.10	1.93±0.10

5.5.2 Bird Health

There were no effects of supplement on mortalities ($p>0.05$) (Table 43)

Table 43: ANOVA p- values for Trial 3 Mortality (%) Analysis

	Mortality (%)
ANOVA	
Supplement	0.07
Day	<0.001
Supplement* Day	0.12

Main causes of mortalities were septicemia, prolapsed intestines, pericarditis, soft bones, ascites, flip over disease and culling for bad legs, as well as omphalitis in the early growing period, but were not treatment related. Overall mortalities (%) for 0.25% Tasco®, 0.5% Tasco®, 1.0% Tasco®, 2.0% Tasco®, 2.0% Pulse Tasco®, 2.5% inulin, the negative control, and the positive control were 1.3, 3.2, 1.5, 2.2, 3.2, 4.4, 4.3, and 2.9 ± 0.8 respectively.

5.5.3 Intestinal pH

On day 21, supplemention was found to have an effect on cecal content pH ($p \leq 0.05$). Feeding 2.5% inulin resulted in lower day 21 cecal content pH than 0.25% and 2.0% Pulse Tasco®. Feeding Tasco® at 2.0% resulted in lower pH of cecal contents than 0.25% Tasco® (Table 44). The volume of cecal contents from day 7 birds was too low for accurate pH measurements. No effects were observed on day 35 or 45 cecal content pH (Table 44) or day 7, 21, 35, or 45 jejunal content pH ($p > 0.05$) (Table 45).

Table 44: Trial 3 pH of Cecal Contents of Broiler Chickens Day 21, 35, and 45 Posthatch with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

	Days Posthatch		
	21	**35**	**45**
Dietary Supplement			
0.25% Tasco®	7.8±0.2[a]	7.0±0.2	6.4±0.2
0.5% Tasco®	7.3±0.3 [abc]	6.0±0.2	6.3±0.2
1.0% Tasco®	7.3±0.2 [abc]	6.7±0.3	6.5±0.2
2.0% Tasco®	6.7±0.2 [bc]	6.7±0.2	6.8±0.2
2.0% Pulse Tasco®	7.4±0.2 [ab]	6.7±0.2	6.3±0.2
2.5% Inulin	6.6±0.2 [c]	6.1±0.2	6.3±0.2
Negative Control	6.9±0.6 [abc]	6.5±0.3	6.2±0.2
Positive Control	6.8±0.2 [abc]	6.4±0.2	6.3±0.2
ANOVA (p-value)			
Supplement	0.02	0.07	0.59

[a-c] means±SEM with different superscripts within a column are significantly different ($p \leq 0.05$)

Table 45: Trial 3 pH of Jejunal Contents of Broiler Chickens Day 7, 21, 35, and 45 Posthatch with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

	Days Posthatch			
	7	**21**	**35**	**45**
Dietary Supplement				
0.25% Tasco®	6.1±0.2	6.1±0.1	5.7±0.2	5.4±0.3
0.5% Tasco®	6.1±0.2	6.1±0.1	5.8±0.2	6.0±0.3
1.0% Tasco®	6.5±0.2	6.1±0.1	5.8±0.2	6.1±0.3
2.0% Tasco®	5.8±0.2	6.1±0.1	5.7±0.2	5.9±0.3
2.0% Pulse Tasco®	6.1±0.2	6.1±0.1	5.7±0.2	5.7±0.3
2.5% Inulin	6.1±0.2	6.0±0.1	5.6±0.2	5.7±0.3
Negative Control	6.0±0.2	6.0±0.1	6.2±0.2	5.7±0.3
Positive Control	6.1±0.2	6.2±0.1	5.9±0.2	5.9±0.3
ANOVA (p-value)				
Supplement	0.31	0.33	0.52	0.73

5.5.4 Organ Weights

Supplement had effects on both immunological and intestinal weights ($p \leq 0.05$). There was an effect of supplement on cecal weight on day 21 ($p \leq 0.05$) where feeding 2.5% inulin resulted in higher relative cecal weights than 1.0% and 2.0% Pulse Tasco® (Table 46). There were no effects on relative cecal weights on day 7, 35, and 45 ($p > 0.05$). There was no effect on relative ileal weight during any growth period ($p > 0.05$)(Table 46). On day 7 feeding 2.0% and 2.0% Pulse Tasco® as well as 2.5% inulin resulted in increased relative bursa weights over 0.25% Tasco® (Table 47).

Table 46: Trial 3 Relative Cecal and Ileal Weights (mg organ weight:g body weight) of Broiler Chickens on Day 7, 21, 35, and 45 Posthatch with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

	Relative Cecal Weight				Relative Ileal Weight			
Days Posthatch	**7**	**21**	**35**	**45**	**7**	**21**	**35**	**45**
Dietary Supplement								
0.25% Tasco®	6.3±0.6	4.8±0.3[ab]	3.1±0.4	3.1±0.3	20.7±2.0	11.6±0.6	9.6±0.7	8.3±0.7
0.5% Tasco®	7.5±0.6	4.4±0.3 [ab]	3.6±0.4	2.9±0.3	19.7±2.2	11.9±0.6	8.8±0.7	9.2±0.7
1.0% Tasco®	6.4±0.6	3.9±0.3 [b]	3.4±0.4	3.3±0.4	23.6±2.0	12.7±0.6	10.0±0.7	8.8±0.7
2.0% Tasco®	7.0±0.6	5.1±0.3 [ab]	3.5±0.4	3.2±0.4	19.0±2.0	11.8±0.6	9.9±0.7	7.9±0.7
2.0% Pulse Tasco®	8.0±0.6	4.2±0.3 [b]	4.3±0.4	3.4±0.3	27.8±2.0	11.4±0.6	8.4±0.7	7.9±0.7
2.5% Inulin	9.1±0.6	5.7±0.3 [a]	3.7±0.4	3.6±0.3	23.9±2.0	11.9±0.6	9.5±0.7	8.8±0.7
Negative Control	7.1±0.6	4.8±0.3 [ab]	3.9±0.4	3.8±0.3	19.0±2.0	11.5±0.6	9.8±0.7	8.6±0.7
Positive Control	7.0±0.6	4.6±0.3 [ab]	4.1±0.4	3.4±0.3	20.9±2.0	11.4±0.6	9.2±0.7	7.6±0.7
ANOVA (p-value)								
Supplement	0.09	0.01	0.49	0.42	0.04	0.80	0.70	0.70

[a-b] means±SEM with different superscripts within the same column are significantly different (p≤0.05)

Table 47: Trial 3 Relative Spleen and Bursa Weights (mg organ weight:g body weight) of Broiler Chickens on Day 7, 21, 35, and 45 Posthatch with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

	Relative Spleen Weight				Relative Bursa Weight			
Days Posthatch	**7**	**21**	**35**	**45**	**7**	**21**	**35**	**45**
Dietary Supplement								
0.25% Tasco®	0.9±0.1	0.9±0.1	1.0±0.1	1.2±0.1	1.5±0.2[b]	2.3±0.2	2.3±0.2	1.8±0.2
0.5% Tasco®	0.7±0.1	1.0±0.1	1.0±0.1	1.2±0.1	2.1±0.2[ab]	2.5±0.2	1.9±0.2	1.9±0.2
1.0% Tasco®	0.8±0.1	0.9±0.1	1.0±0.1	1.0±0.1	1.7±0.2[ab]	2.7±0.2	1.8±0.2	1.9±0.2
2.0% Tasco®	0.6±0.1	0.8±0.1	1.3±0.1	1.1±0.1	2.4±0.2[a]	2.4±0.2	1.5±0.2	2.0±0.2
2.0% Pulse Tasco®	0.1±0.2	0.9±0.1	1.0±0.1	1.3±0.1	2.3±0.2[a]	2.7±0.2	2.0±0.2	2.0±0.2
2.5% Inulin	0.8±0.1	0.9±0.1	0.7±0.1	1.2±0.1	2.4±0.2[a]	2.2±0.2	1.7±0.2	1.8±0.2
Negative Control	0.7±0.1	1.0±0.1	1.0±0.1	1.1±0.1	1.9±0.2[ab]	2.4±0.2	2.1±0.2	1.5±0.2
Positive Control	0.7±0.1	0.9±0.1	1.2±0.1	1.1±0.1	1.8±0.2[ab]	2.5±0.2	2.2±0.2	2.1±0.2
ANOVA (p-value)								
Supplement	0.03	0.62	0.20	0.75	<0.001	0.83	0.27	0.42

[a-b] means±SEM with different superscripts within the same column are significantly different (p≤0.05)

There were no other effects on relative bursa weight on day 21, 35, or 45 (Table 47) or on relative spleen weight during any growth period ($p>0.05$)(Table 47).

5.5.5 Intestinal Histomorphology

No treatment differences were observed in measurements taken on intestinal samples for any sample day (Table 48-50) ($p>0.05$) except for day 21 where feeding 0.25% Tasco® resulted in higher breakage scores than 2.0% Tasco® (Table 50). On day 7 there was a significant effect on villi apparent area ($p=0.04$), however Tukeys did not reveal any differences between treatments. After histological processing and sectioning of ileal tissue, day 35 and 45 villi were found to be extremely damaged and so sufficent readings to perform statistical analysis were only able to be taken of breakage scores and mucosal depth (Table 50).

Table 48: Trial 3 Ileal Villi Width (µm) and Apparent Area (mm^2) of Broiler Chickens on Day 7 and 21 Posthatch with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

	Villi Width (µm)		Villi Apparent Area (mm^2)	
Days Posthatch	**7**	**21**	**7**	**21**
Dietary Supplement				
0.25% Tasco®	116.7±10.8	123.9±37.2	0.06±0.01	0.11±0.03
0.5% Tasco®	120.8±15.3	133.0±16.7	0.10±0.02	0.10±0.01
1.0% Tasco®	147.6±8.8	137.3±18.6	0.07±0.01	0.10±0.02
2.0% Tasco®	123.1±7.6	131.6±15.2	0.05±0.01	0.10±0.01
2.0% Pulse Tasco®	140.3±8.8	131.2±16.7	0.09±0.01	0.11±0.01
2.5% Inulin	125.8±8.8	164.2±16.7	0.06±0.01	0.12±0.01
Negative Control	96.1±15.3	163.3±18.6	0.03±0.02	0.12±0.02
Positive Control	142.2±10.8	173.7±16.7	0.07±0.01	013±0.01
ANOVA (p-value)				
Supplement	0.14	0.39	0.04	0.87

Table 49: Trial 3 Ileal Intestinal Histomorphology Measurements of Broiler Chickens on Day 7 and 21 Posthatch with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

	Villi Height (µm)		Crypt Depth (µm)		Villi Height:Crypt Depth Ratio (µm:µm)	
Days Posthatch	**7**	**21**	**7**	**21**	**7**	**21**
Dietary Supplement						
0.25% Tasco®	482.9±69.7	874.7±118.0	122.4±15.7	129.4±27.4	4.1±0.6	6.8±0.9
0.5% Tasco®	808.1±98.6	714.6±52.8	142.7±22.3	132.6±12.2	5.7±0.8	5.5±0.4
1.0% Tasco®	511.5±56.9	727.7±59.0	125.8±12.9	129.8±13.7	4.2±0.5	5.8±0.5
2.0% Tasco®	437.4±49.3	727.7±48.2	130.0±11.1	145.6±11.2	3.6±0.4	5.3±0.4
2.0% Pulse Tasco®	659.7±56.9	780.9±52.8	140.7±12.9	150.4±12.2	4.7±0.5	5.5±0.4
2.5% Inulin	462.8±56.9	675.5±52.8	134.4±12.9	148.1±12.2	3.6±0.5	4.7±0.4
Negative Control	356.2±98.6	724.3±59.0	121.9±22.3	142.4±13.7	3.0±0.8	5.3±0.5
Positive Control	521.7±69.7	697.1±52.8	114.7±15.7	151.0±12.2	4.7±0.6	4.7±0.4
ANOVA (p-value)						
Supplement	0.05	0.78	0.91	0.88	0.24	0.32

Table 50: Trial 3 Ileal Breakage Score and Mucosal Depth (µm) of Broiler Chickens on Day 7, 21, 35, and 45 Posthatch with Dietary Supplemention of Tasco®, Inulin, or an Antibiotic

	Breakage Score				Mucosal Depth (µm)			
Days Posthatch	**7**	**21**	**35**	**45**	**7**	**21**	**35**	**45**
Dietary Supplement								
0.25% Tasco®	4.0±0.3	4.0±0.3[a]	3.0±0.3	4.0±0.2	180.1±16.3	225.1±19.0	271.4±21.4	333.2±25.9
0.5% Tasco®	4.0±0.3	3.0±0.3 [ab]	3.0±0.3	4.0±0.2	182.1±16.3	241.3±19.0	273.3±21.4	323.0±25.9
1.0% Tasco®	3.0±0.3	3.0±0.3 [ab]	3.0±0.3	4.0±0.2	173.0±16.3	197.2±19.0	280.0±21.4	363.0±25.9
2.0% Tasco®	3.0±0.3	2.0±0.3 [b]	4.0±0.3	4.0±0.2	198.3±16.3	264.0±19.0	292.5±21.4	340.3±25.9
2.0% Pulse Tasco®	3.0±0.3	3.0±0.3 [ab]	4.0±0.3	4.0±0.2	162.6±16.3	236.9±19.0	271.9±21.4	342.5±25.9
2.5% Inulin	4.0±0.3	3.0±0.3 [ab]	3.0±0.3	4.0±0.2	199.7±16.3	224.1±19.0	250.0±21.4	378.7±25.9
Negative Control	4.0±0.3	3.0±0.3 [ab]	3.0±0.3	4.0±0.2	199.9±16.3	213.2±19.0	242.1±21.4	383.1±25.9
Positive Control	4.0±0.3	3.0±0.3 [ab]	4.0±0.3	4.0±0.2	207.6±16.3	268.7±19.0	206.7±21.4	334.1±25.9
ANOVA (p-value)								
Supplement	0.46	0.04	0.96	0.39	0.49	0.15	0.16	0.63

[a-b] means±SEM with different superscripts within the same column are significantly different (p≤0.05)

5.6 Discussion

5.6.1 Growth Performance

As with the previous trial, Tasco® increased growth performance in this trial. Tasco® improved growth performance over the negative control in all growth variables measured except for feed to gain. The 0.25% and 0.5% Tasco® showed improved BW and 0.25%, 1.0%, and 2.0% Pulse Tasco® improved BWG and FI over the negative control. Feeding the 0.5% Tasco® improved BW and feeding the 1.0% and 2.0% Tasco® improved FI over inulin. Unlike in Trial 2, 2.5% inulin performed comparatively to Tasco® in this trial. It would seem that the poor performance of birds given inulin in Trial 2 may have been a matter of the growing conditions in that trial.

5.6.2 Bird Health

No effects on mortalities occured in this trial ($p>0.05$), unlike in Trial 2. This may have been due to a lack of stressors present.

5.6.3 Intestinal pH

On day 21, feeding 2.5% inulin was found to decrease pH of cecal contents compared to the Tasco® Pulse treatment and 0.25% Tasco®. Feeding 2.0% Tasco® also resulted in decreased cecal content pH compared to 0.25% Tasco®. This decrease in pH would indicate a beneficial increase in fermentation of the prebiotic. However, the decreased pH of cecal contents with 2.0% Tasco® did not correspond to a positive increase in BW, as birds on that treatment performed poorly in that growth parameter compared to 0.25% Tasco®. The lower pH of cecal contents from 2.5% inulin did not correspond with improved growth in any parameter over 0.25% Tasco® or 2.0% Pulse

Tasco® treatment. Perhaps the increase in bacterial fermentation caused by the supplements was not enough to increase growth of the birds. It is interesting to note that effects of dietary treatment on cecal content pH were seen on day 21 only in this trial, as they were in Trial 2. The appearance of these differences on day 21 and no other day may be a reflection of the transitional bacterial communities on day 12 through 17 altering the fermentation patterns of the feed additives (Torok et al. 2009).

5.6.4 Organ Weights

The 2.5% inulin decreased cecal content pH over 2.0% Pulse Tasco®. This was accompanied by increased relative cecal weight on day 21 compared to 1.0% and 2.0% Pulse Tasco®. This may indicate that the decreased pH was due to increased SCFA. SCFA such as butyrate cause proliferation of epithelial cells (Bosscher 2009) which would result in increased relative cecal weights. Possibly, the lack of resulting body growth was due to increased energy used in cell proliferation. This supports the hypothesis that bacterial populations present in the younger birds may be better able to ferment the additives, while transitional bacterial communities in older birds may have lessened ability to do so.

Differences in relative weights of immunological organs were recorded. On day 7, feeding 2.5% inulin, and 2.0% and 2.0% Pulse Tasco® resulted in increased bursa weights over 0.25% Tasco®. Lymph follicles of the bursa are a major site of lymphocyte production (Li et al. 2009) and so an increase in the weight of this organ may be due to increased production of lymphocytes. The spleen is also a major site of lymphocyte production (Li et al. 2009) and has been shown to increase in relative weight in birds fed

probiotics (Awad et al. 2009); however, it is more of a reflection of systemic immunity. As in Trial 2, the lack of effect on this organ implies stimulation of local gut immunity rather than systemic immunity. The increased immune function was not reflected in decreased % mortalities, perhaps due to lack of a challenge.

5.6.5 Intestinal Histomorphology

While the same response in intestinal histomorphology measurements that was observed in Trial 1 day 7 and 21 samples was not observed in this trial, as in Trial 2, there was a response in breakage score on day 21. On that day, feeding 0.25% Tasco® resulted in higher breakage scores than 2.0% Tasco®. This echoes the effect on day 21 pH of cecal contents in which 2.0% Tasco® decreased cecal content pH compared to 0.25% Tasco®. In addition, on day 7 feeding 0.25% Tasco® lowered relative bursa weights compared to 2.0% Tasco® and 2.0% Pulse Tasco® which were at that time the same diet. There therefore seems to be a recurring effect in which 2.0% Tasco® is able to improve physiological variables compared to 0.25% Tasco®, yet performs more poorly in growth variables such as BW. This may indicate differences in Tasco®'s mode of action when altering physiological variables versus growth variables. Lack of effect of treatment on any other intestinal histomorphology measurement, unlike in trial 1, is unexplained. The effect on villi height, villi apparent area, and crypt depth was only observed in the cage trial and so it may be that the competitive exclusion effect of exposure to flockmates' excreta decreases the effects of the prebiotics on intestinal histomorphology. It may also be the analysis of dietary treatment levels separately in this trial, as in Trial 2, making it more difficult for individual treatment differences to be observed. The lack of effect of treatment on intestinal histomorphology on day 35 and 45 is consistent with that in the

previous two trials, indicating a consistent lack of effect of dietary supplementation with Tasco® and inulin on intestinal histomorphology in later growing periods.

5.6.6 Effect of the Longer Growing Period

When Baurhoo et al. (2009) fed MOS to broiler chickens up until day 34, intestinal histomorphology and gut environment variables were found to be altered by the supplement in the later growth period. These changes were not reflected in growth performance, and it was suggested that if the birds were grown for a longer period of time than the alterations in the other variables may have resulted in improved growth. A longer growing period results in increased stocking densities as the birds grow in size, thus possibly increasing stress on the birds. Increased stocking densities were found to decrease BW of birds when birds were raised in sanitary conditions (Feddes et al. 2002). Due to these studies it was thought that extending the growing period for broilers to 45 days would result in increased performance from the birds on Tasco® and inulin treatments. The extended growth period was also intended to reflect the extended growth used for broiler chickens in the United States.

The finisher 2 period resulted in differing results from the treatments. Improvement of BW over the negative control by 0.25% and 0.5% Tasco® in the finisher 1 period was no longer detected in the finisher 2 period. Likewise, 1.0% and 2.0% Tasco® no longer showed improved FI over the positive control in the finisher 2 period, though 0.25% Tasco® did show improvement over the negative control in the finisher 2 but not the finisher 1 period. There were no treatment differences in the physiological variables during the finisher 1 and 2 periods. These differences appeared only on day 7

and 21 sampling days and were reflected in growth differences during the finisher 1 and 2 periods. In general, less improvement over the controls was observed in the finisher 2 period and so Tasco® may not require an extended growing period in which to have a full effect at certain levels. The stressor of increased stocking densities was less severe than that used by Feddes et al. (2002). Densities reached 9.06 birds/m^2 in this trial while that shown to affect growth by Feddes et al. (2002) was 23.8 birds/m^2 when birds were raised to 42 days of age. This may indicate that densities were not great enough to play a role in the treatment differences seen in the longer growing period. In addition, Tasco®'s physiological effects occurred earlier than those observed by Baurhoo et al. (2009) and so compensatory effects on growth are able to be measured as early as the normal finisher period in the Canadian industry of day 25-35.

5.6.7 Comparison with an Antibiotic Growth Promoter

Previous trials in this study did not include an AGP to compare Tasco® against. It was therefore of interest in this trial to determine if Tasco® provided comparable results to antibiotics, which is the only way in which Tasco®'s feasibility as an antibiotic replacement could be determined. Patterson and Burkholder (2003) stated that antibiotics should be included in all studies with prebiotics due to the large role that stress plays in response to these treatments. If there is no response to the antibiotic then it indicates that not enough of a challenge was present to require growth promotants and therefore a lack of response from the prebiotic should not be taken as an indication of ineffectiveness.

A response to the AGP was observed in this trial in improved BWG over the negative control. Compared to the positive control, Tasco® resulted in comparable BW,

BWG, and feed to gain as well as improved FI in the finisher 1 period. This indicates that Tasco® acts as well as, and at times better than, the AGP.

5.6.8 Pulse Treatment Performance

Previous studies in beef cattle by Bach et al. (2008) and Allen et al. (2001) have indicated that when Tasco® is fed as a pulse treatment, fed at a low dose for a short period of time, results seem to improve and in fact extend past the time in which Tasco® is present in the diet. Bach et al. (2008) fed Tasco® to steers at 10g/kg for 14 days, 20 g/kg for 7 days, and 20 g/kg for 14 days and found improved resistance to *E. coli* inoculation from Tasco® at 10g/kg for 14 days and 20g /kg for 7 days. No *E. coli* was detected in the steers fed 20g/kg for 7 days up to 65 days post challenge. Allen et al. (2001) fed Tasco® to steers on endophyte infected pastures during pasturing only, with effects on immunity carrying through into the feedlot period. In the present study the pulse treatment, where Tasco® was fed at 2.0% during the starter period only, did not perform as well as was expected. This treatment did result in improved FI over both controls during the grower period and improved overall BWG over the negative control. In addition, the treatment increased BW over 2.0% Tasco® in the finisher 1 period. There were no other improvements compared to other treatments after the birds on the pulse treatments were switched to a diet with no feed additives. Bach et al. (2008) also saw no improvement in BW of animals given pulse treatments compared to the control, only reductions in *E. coli* and Allen et al. (2001) saw only effects on immune function, not on growth. Thus, the pulse treatment's lingering effects may affect pathogen resistance only and not extend to increases in growth. However, in this study the pulse treatment had no effect on immune organ weights. It may be that, if acting as a prebiotic, when Tasco® is

present it is increasing beneficial bacterial populations and being fermented into SCFA to increase growth. The large beneficial bacterial populations Tasco® supported may remain in place even when Tasco® is removed from the diet, thus competitively excluding pathogens. However, the increased substrate for fermentation would no longer be present which would result in decreased production of SCFA and so a lack of growth effect would be observed. This theory cannot be further examined until measurement of microbial population changes currently underway are completed. It is also possible that more of a response to the pulse treatment was not observed as no challenges were presented to the animals as there were in the studies by Bach et al. (2008) and Allen et al. (2001). This treatment should be re-examined at various levels of Tasco® to determine the optimal level for the pulse treatment and with a defined stressor present such as a pathogen challenge.

5.6.9 Tasco® Levels of Interest

The low levels of 0.25% and 0.5% Tasco® fed in this study showed promising results. The 0.25% and 0.5% both improved BW over the negative control and 0.25% Tasco® improved BWG and FI over the negative control. Both of these levels showed comparable results to the positive control in all growth variables measured. While both improved growth performance it is interesting to note that the same response was not observed on physiological variables. At 0.25%, Tasco® resulted in higher pH of cecal contents than inulin and 2.0% Tasco® on day 21, increased ileal villi breakage score on day 21 compared to 2.0% Tasco®, and decreased relative bursa weight compared to 2.0% Tasco®, 2.0% Tasco® Pulse, and 2.5% inulin on day 7. It could be that the alterations in physiological variables by 2.0% Tasco®, and in bursa weight by 2.5% inulin, served to

prevent pathogen infection; however, the changes in bacterial fermentation they resulted from were not great enough to increase growth. In prebiotic studies it is often found that gut environment and bacterial populations are altered while no response in growth occurs (Baurhoo et al. 2007; Janardhana et al. 2009). This scenario occurs most often when there is a lack of stressor present (Catala – Gregori et al. 2008). Another explanation would be that Tasco®'s mode of action in increasing growth is somewhat distinct from its mode of action in preventing bacterial infection and so each are affected differently with differing inclusion levels. It could be for example, that Tasco® increases growth by alteration of bacterial populations slightly, causing increased bacterial fermentation but perhaps not enough to sustain large populations of bacteria. Alternatively, Tasco®'s mode of action in alteration of physiological variables may require high levels of particular bioactive polysaccharide(s). If this causes too high of a bacterial proliferation competition for nutrients would be increased (Yang et al. 2009), causing decreased growth. The cause of this difference may become clearer once microbial population differences amongst treatments have been examined.

5.6.10 Effect of Dietary Supplement on the Trough of Immunity

On day 7 interlevel effects of Tasco® only were observed. The improvement of various physiological variables over inulin observed during this time in the first trial was not repeated either in this trial or in Trial 2. This further indicates that the change of birds to pens on litter may be responsible for the lack of treatment effects, due to influence of increased exposure to excrement. Analysis of microbial profiles currently underway should further indicate factors responsible for lack of influence on physiological variables.

5.7 Conclusions

Tasco® fed at 0.25% had beneficial effects on growth performance, though not on physiological variables measured, while the opposite was true for Tasco® fed at 2.0%. The 0.5% Tasco® improved growth as it had in both previous trials. Tasco® was comparable to the antibiotic in all variables measured and improved FI over the antibiotic in the finisher 1 period. Results from the trial further confirmed improvement of growth due to Tasco® and showed it to be comparable, and in some variables better than, an antibiotic.

Chapter 6. Conclusion

6.1 Cross Trial Comparison

Tasco® improved growth at very low levels in all trials in the feed such as 0.25% and 0.5%, increasing its cost effectiveness. Tasco® at 0.5% was shown to increase BW over the control in both Trial 1 and 3. In Trial 1 and 3, 0.5% Tasco® was comparable to the optimal level of inulin found, 2.5%. In Trial 2, it improved BW, BWG, and feed to gain over 2.5% inulin, though 2.5% inulin performed very poorly in that study. A summary of Tasco®'s effects compared to the negative control at different inclusion levels for each trial can be seen in Table 51. Performance of high inclusion levels varied in each trial with inconsistent results.

6.2 Meeting the Prebiotic Criteria

To be verified as a prebiotic the fiber component of Tasco® must be shown to reach the lower GIT intact, to be fermentable by specific bacterial populations and increase their activity and/or populations, and to improve the health of the host. The three trials performed showed Tasco® to enact effects in the lower GIT such as alteration of intestinal pH, intestinal histomorphology measurements, and bursa and cecal relative weights. These effects are indicative of Tasco®'s presence in the lower GIT and its fermentation by microflora. Tasco® may enact its effects in the gut via its fermentation products or by altering microbial populations directly. Shifts in physiological variables were generally beneficial, thus showing Tasco® to improve the health of the host.

Table 51: Overview of Tasco® Performance at Various Inclusion Levels in 3 Growth Trials

	Tasco® Inclusion Level (%)								
	0.25	0.5	1.0	1.5	1.75	2.0	2.0 Pulse	2.5	3.0
Trial									
1§	N/A†	+*	C‡	C	N/A	+	N/A	+	+
2¶	N/A	C	N/A	N/A	+	N/A	N/A	N/A	C
3**	+	+	+	N/A	N/A	C	+	N/A	N/A

† N/A indicates that the respective inclusion level was not fed as a treatment in that trial
* + indicates a positive response in growth compared to the negative control
‡ C indicates a comparable response to the negative control
§ In Trial 1 Broilers were housed in cages from Day 0-35 posthatch and fed Tasco® or inulin at levels from 0%-3.0% increasing in levels of 0.5%.
¶ In Trial 2 Broilers were housed in pens from Day 0-35 posthatch and fed Tasco® at 0.5%, 1.75%, and 3.0%, inulin at 2.5%, or a negative control
** In Trial 3 Broilers were housed in pens from Day 0-45 posthatch and fed Tasco® at 0.25%, 0.5%, 1.0%, and 2.0%, 2.0% Tasco® for the first two weeks only as a pulse, a positive control with antibiotic, or a negative control

Improvements in health were reflected in improved growth, particularly in the later growing periods, further supporting Tasco®'s fulfillment of the third prebiotic criteria. Though the alterations in the gut environment present strong evidence toward fulfillment of the first criteria, Tasco® fibers must be measured in the digesta of the lower GIT for any definitive statements to be made.Changes in pH in particular support microbial fermentation of Tasco®; however, microbial population changes must be measured for this requirement to be established. This analysis is currently underway using next generation sequencing.

6.3 Tasco® as a Feed Additive

Regardless of verification of Tasco®'s qualifications as a prebiotic, Tasco® in this study improved growth of broilers, thus validating its use as a feed additive. Tasco® was shown to improve BW, BWG, FI, and feed to gain compared to the commonly used additive and prebiotic, inulin. As an AGP alternative it was comparable to, and at times better than, the antibiotic virginiamycin in increasing growth of broiler chickens. Improvement of growth was found to extend into the 35-45 day growth period which would be used in the poultry industry in the United States.

6.4 Research Conclusions

In order to optimally utilize Tasco® as a feed additive, future studies should examine even lower levels of Tasco®, determine the effects of Tasco® in the presence of a well defined stressor, and look into combining Tasco® with a probiotic species able to ferment it in order to ensure its full utilization in the gut.

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Appendix A. Lighting and Temperature Schedules for Trials 1, 2 , and 3

Table A-1: Lighting and Temperature Schedules for Broiler Chickens Housed at the Atlantic Poultry Institute During Trial 1, 2, and 3

Days Post Hatch	Temperature (°C)	Light Hours	Light Intensity (lux)
Trial 1*, 2, and 3*** Schedule**			
0-1	32	24	20
2-3	31	23	20
4	30	23	20
5	30	16	15
6	29	16	15
7-8	29	16	10
9-10	28	16	5
11-12	27	16	5
13-15	26	16	5
16-17	25	16	5
18-19	24	16	5
20-22	23	16	5
23-26	22	16	5
27	21	16	5
28-31	21	17	5
32-35	21	18	5
Trial 3 Lengthened Growing Period Schedule			
36-45	21	19	5

*In Trial 1 Broilers were housed in cages from Day 0-35 posthatch and fed Tasco® or inulin at levels from 0%-3.0% increasing in levels of 0.5%.

**In Trial 2 Broilers were housed in pens from Day 0-35 posthatch and fed Tasco® at 0.5%, 1.75%, and 3.0%, inulin at 2.5%, or a negative control

*** In Trial 3 Broilers were housed in pens from Day 0-45 posthatch and fed Tasco® at 0.25%, 0.5%, 1.0%, and 2.0%, 2.0% Tasco® for the first two weeks only as a pulse, a positive control with antibiotic, or a negative control

Appendix B. Data Tables From Trials 1, 2, and 3

Table B-1: Trial 1 Body Weight (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), and Finisher (Day 25-35) Experimental Periods with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Days Posthatch		
		14	24	35
Dietary Supplement	**Supplement Inclusion level (%)**			
Tasco®	0.0	322.7±35.1	833.7±35.1	1590.0±37.8
	0.5	387.8±35.1	1001.6±35.1	1900.6±35.1
	1.0	385.9±35.1	999.2±35.1	1801.3±35.1
	1.5	379.9±35.1	935.1±35.1	1785.8±35.1
	2.0	401.3±35.1	1034.4±35.1	1892.1±37.8
	2.5	378.3±35.1	973.5±35.1	1845.3±35.1
	3.0	416.4±35.1	1086.5±35.1	1973.9±35.1
Inulin	0.0	326.4±35.1	869.6±35.1	1639.5±35.1
	0.5	352.2±35.1	915.2±35.1	1756.5±37.8
	1.0	351.7±35.1	984.4±35.1	1774.9±37.8
	1.5	373.0±35.1	991.3±35.1	1873.9±35.1
	2.0	356.1±35.1	968.6±35.1	1830.0±35.1
	2.5	377.7±35.1	982.7±35.1	1915.6±35.1
	3.0	363.2±35.1	967.4±35.1	1737.0±35.1

Table B-2: Trial 1 Feed Intake (g bird^{-1}) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), and Finisher (Day 25-35) Experimental Periods with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Days Posthatch		
		0-14	15-24	25-35
Dietary Supplement	**Supplement Inclusion level (%)**			
Tasco®	0.0	480.6±33.8	876.7±33.8	1596.4±33.8
	0.5	499.5±33.8	992.2±33.8	1784.5±33.8
	1.0	458.2±33.8	988.7±33.8	1661.4±33.8
	1.5	466.0±33.8	978.8±33.8	1773.7±33.8
	2.0	479.7±33.8	1037.6±33.8	1762.2±37.0
	2.5	507.4±33.8	1034.3±33.8	1964.4±33.8
	3.0	496.1±33.8	1034.7±33.8	1841.1±33.8
Inulin	0.0	455.8±33.8	916.8±33.8	1653.1±37.0
	0.5	446.0±33.8	942.0±33.8	1678.4±37.0
	1.0	473.2±33.8	941.8±33.8	1637.6±37.0
	1.5	480.5±33.8	973.9±33.8	1810.2±33.8
	2.0	504.4±33.8	977.4±33.8	1775.9±37.0
	2.5	484.0±33.8	989.6±33.8	1743.3±33.8
	3.0	484.2±37.0	960.2±33.8	1751.3±33.8

Table B-3: Trial 1 Feed to Gain (g:g) of Broiler Chickens During the Starter (Day 0-14), Grower (Day 15-24), and Finisher (Day 25-35) Experimental Period and Averaged over Whole Experimental Period with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Days Posthatch			
		0-14	15-24	25-35	Overall Mean 0-35
Dietary Supplement	Supplement Inclusion level (%)				
Tasco®	0.0	1.58±0.07	1.66±0.06	2.13±0.1	1.79±0.05
	0.5	1.44±0.06	1.62±0.05	2.00±0.1	1.69±0.05
	1.0	1.34±0.06	1.62±0.05	2.11±0.1	1.69±0.05
	1.5	1.38±0.06	1.77±0.05	2.09±0.1	1.75±0.05
	2.0	1.34±0.06	1.56±0.06	2.17±0.1	1.69±0.05
	2.5	1.52±0.06	1.74±0.05	2.26±0.1	1.84±0.05
	3.0	1.33±0.06	1.55±0.05	2.09±0.1	1.66±0.05
Inulin	0.0	1.61±0.06	1.63±0.06	2.26±0.1	1.83±0.05
	0.5	1.44±0.06	1.69±0.05	2.08±0.1	1.74±0.05
	1.0	1.53±0.06	1.49±0.05	1.85±0.1	1.63±0.05
	1.5	1.46±0.06	1.58±0.05	2.07±0.1	1.70±0.05
	2.0	1.53±0.06	1.60±0.05	1.99±0.1	1.71±0.05
	2.5	1.46±0.06	1.64±0.05	1.88±0.1	1.66±0.05
	3.0	1.47±0.06	1.60±0.05	2.16±0.1	1.74±0.05

Table B-4: Trial 1 Ileal Intestinal Histomorphology Measurements of Broiler Chickens on Day 7 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Ileal Histomorphology Measurement			
		Crypt Depth(µm)	Villi Height (µm)	Villi Height:Crypt Depth(µm:µm)	Villi Apparent Area (mm^2)
Dietary Supplement	**Supplement Inclusion level (%)**				
Tasco®	0.0	118.8±14.0	583.5±46.5	5.0±0.5	0.07±0.01
	0.5	139.6±9.9	473.7±32.9	3.6±0.3	0.07±0.01
	1.0	125.6±9.9	527.6±32.9	4.3±0.3	0.07±0.01
	1.5	132.9±11.4	500.8±37.9	3.9±0.4	0.06±0.01
	2.0	122.8±8.9	530.1±29.4	4.4±0.3	0.07±0.01
	2.5	143.9±9.9	522.2±32.9	3.8±0.3	0.07±0.01
	3.0	136.3±8.9	500.6±29.4	3.8±0.3	0.06±0.01
Inulin	0.0	111.8±11.4	477.9±37.9	4.3±0.4	0.05±0.01
	0.5	134.7±9.9	473.0±32.9	3.6±0.3	0.06±0.01
	1.0	113.1±8.1	455.1±26.8	4.1±0.3	0.06±0.01
	1.5	110.2±8.1	437.8±26.8	4.2±0.3	0.06±0.01
	2.0	112.8±11.4	474.7±37.9	4.3±0.4	0.06±0.01
	2.5	110.9±8.1	409.8±26.8	4.1±0.3	0.05±0.01
	3.0	110.7±14.0	430.6±46.5	4.0±0.5	0.05±0.01

Table B-5: Trial 1 Ileal Intestinal Histomorphology Measurements of Broiler Chickens on Day 21 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Ileal Histomorphology Measurement			
		Crypt Depth(µm)	Villi Height (µm)	Villi Height:Crypt Depth(µm:µm)	Villi Apparent Area (mm^2)
Dietary Supplement	**Supplement Inclusion level (%)**				
Tasco®	0.0	148.7±17.1	912.9±85.5	5.2±0.7	0.10±0.01
	0.5	146.2±9.9	760.9±49.4	5.2±0.4	0.10±0.01
	1.0	119.0±10.8	777.1±54.1	6.2±0.4	0.10±0.01
	1.5	165.9±12.1	988.2±60.5	5.7±0.5	0.13±0.01
	2.0	139.6±12.1	729.7±60.5	5.1±0.5	0.08±0.01
	2.5	149.9±14.0	763.4±69.8	5.0±0.6	0.10±0.01
	3.0	137.5±9.9	740.3±49.4	5.6±0.4	0.10±0.01
Inulin	0.0	144.4±12.1	720.3±60.5	5.2±0.5	0.09±0.01
	0.5	154.4±14.0	773.4±69.9	5.2±0.6	0.10±0.01
	1.0	123.8±10.8	719.9±54.1	6.2±0.4	0.09±0.01
	1.5	151.7±12.1	812.3±60.5	5.7±0.5	0.10±0.01
	2.0	137.5±10.8	661.4±54.1	5.1±0.4	0.09±0.01
	2.5	142.0±12.1	703.6±60.5	5.0±0.5	0.08±0.01
	3.0	149.1±10.8	809.5±54.1	5.6±0.4	0.11±0.01

Table B-6: Trial 1 Ileal Intestinal Histomorphology Measurements of Broiler Chickens on Day 35 Posthatch with Tasco® or Inulin Fed at 0%-3.0% in Increments of 0.5%

		Ileal Histomorphology Measurement			
		Crypt Depth(µm)	Villi Height (µm)	Villi Height:Crypt Depth(µm:µm)	Villi Apparent Area (mm^2)
Dietary Supplement	**Supplement Inclusion level (%)**				
Tasco®	0.0	195.9±20.1	682.3±68.2	5.3±0.8	0.13±0.02
	0.5	152.3±28.5	965.0±96.5	4.5±1.1	0.09±0.02
	1.0	165.2±16.4	557.1±55.7	5.7±0.6	0.15±0.01
	1.5	172.4±12.7	431.6±43.2	4.4±0.5	0.11±0.01
	2.0	190.6±14.2	482.5±48.3	4.3±0.6	0.12±0.01
	2.5	169.8±14.2	482.5±48.3	5.1±0.6	0.11±0.01
	3.0	150.4±16.4	557.1±55.7	5.4±0.6	0.11±0.01
Inulin	0.0	164.5±16.4	861.6±55.7	5.8±0.6	0.14±0.01
	0.5	178.1±14.2	818.5±48.3	5.0±0.6	0.12±0.01
	1.0	167.7±12.7	796.9±43.2	5.2±0.5	0.12±0.01
	1.5	179.9±20.1	846.6±68.2	4.8±0.8	0.12±0.02
	2.0	158.6±12.7	841.9±43.2	5.5±0.5	0.14±0.01
	2.5	155.0±20.1	877.3±68.2	5.9±0.8	0.15±0.02
	3.0	195.2±28.5	791.6±96.5	4.1±1.1	0.12±0.02

Table B-7: Trial 2 Body Weight (g bird^{-1}) of Broiler Chickens for the Starter (Day 15), Grower (Day 24), and Finisher (Day 35) Experimental Periods with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

		Days Posthatch		
		15	24	35
Litter Treatment	**Dietary Supplement**			
New Litter	0.5% Tasco®	438.6±14.2	1073.1±14.2	2062.1±14.2
	1.75% Tasco®	461.3±14.2	1087.0±14.2	2079.0±14.2
	3.0% Tasco®	429.5±14.2	1041.6±14.2	2031.5±14.2
	2.5% Inulin	425.6±14.2	999.3±14.2	1918.3±14.2
	Control	451.8±14.2	1043.9±14.2	2020.3±15.5
Previously Used Litter	0.5% Tasco®	455.4±14.2	1061.8±14.2	2052.1±14.2
	1.75% Tasco®	465.4±14.2	1078.9±14.2	2090.7±14.2
	3.0% Tasco®	449.3±14.2	1041.9±14.2	1998.8±14.2
	2.5% Inulin	441.6±14.2	973.9±14.2	1944.7±14.2
	Control	460.1±14.2	1030.6±14.2	2003.8±14.2

Table B-8: Trial 2 Body Weight Gain (g bird^{-1}) of Broiler Chickens for the Starter (Day 0-15), Grower (Day 16-24), and Finisher (Day 25-35) Experimental Periods with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

		Days Post hatch		
		0-15	**16-24**	**25-35**
Litter Treatment	**Dietary Supplement**			
New Litter	0.5% Tasco®	394.7±14.2	634.5±14.2	989.0±14.2
	1.75% Tasco®	417.6±14.2	625.7±14.2	992.1±14.2
	3.0% Tasco®	385.2±14.2	612.2±14.2	989.9±14.2
	2.5% Inulin	381.7±14.2	573.7±14.2	919.1±14.2
	Control	407.9±14.2	592.1±14.2	997.1±14.2
Previously Used Litter	0.5% Tasco®	412.5±14.2	606.4±14.2	990.4±14.2
	1.75% Tasco®	421.8±14.2	613.5±14.2	1011.8±14.2
	3.0% Tasco®	405.5±14.2	592.6±14.2	956.9±14.2
	2.5% Inulin	399.2±14.2	532.4±14.2	970.8±14.2
	Control	416.3±14.2	570.5±14.2	973.3±14.2

Table B-9: Trial 2 Feed Intake (g bird^{-1}) of Broiler Chickens for the Starter (Day 0-15), Grower (Day 16-24), and Finisher (Day 25-35) Experimental Periods with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

		Days Post hatch		
		0-15	16-24	25-35
Litter Treatment	Dietary Supplement			
New Litter	0.5% Tasco®	533.3±68.9	978.7±68.9	1987.4±68.9
	1.75% Tasco®	546.4±68.9	1080.4±68.9	2025.9±68.9
	3.0% Tasco®	507.6±68.9	1029.4±68.9	2085.2±68.9
	2.5% Inulin	496.0±68.9	1022.7±68.9	2106.8±68.9
	Control	524.3±68.9	1049.9±68.9	2073.0±68.9
Previously Used Litter	0.5% Tasco®	814.4±68.9	976.8±68.9	1963.7±68.9
	1.75% Tasco®	523.8±68.9	1018.9±68.9	1998.8±68.9
	3.0% Tasco®	496.7±68.9	995.7±68.9	1949.8±68.9
	2.5% Inulin	493.1±68.9	941.4±68.9	1930.3±68.9
	Control	517.2±68.9	977.9±68.9	1926.2±68.9

Table B-10: Trial 2 Cecal and Jejunal Content pH of Broiler Chickens Day 21 and 35 Post hatch with Dietary Supplementation of Tasco® or Inulin and Placement on New or Previously Used Litter

		Cecal Content pH		Jejunal Content pH	
Days Post hatch		**21**	**35**	**21**	**35**
Litter Treatment	**Dietary Supplement**				
New Litter	0.5% Tasco®	6.7±0.2	6.6±0.1	6.1±0.1	6.0±0.1
	1.75% Tasco®	6.6±0.3	6.6±0.1	6.0±0.1	6.3±0.1
	3.0% Tasco®	6.2±0.2	6.5±0.1	6.1±0.1	6.1±0.1
	2.5% Inulin	6.8±0.2	6.5±0.1	6.2±0.1	6.3±0.1
	Control	6.8±0.2	6.8±0.1	6.0±0.1	6.4±0.1
Previously Used Litter	0.5% Tasco®	6.1±0.2	6.2±0.1	6.0±0.1	5.8±0.1
	1.75% Tasco®	6.1±0.2	5.9±0.1	5.9±0.1	5.8±0.1
	3.0% Tasco®	5.6±0.2	6.3±0.1	6.1±0.1	5.7±0.1
	2.5% Inulin	6.5±0.2	6.4±0.1	6.0±0.1	5.6±0.1
	Control	6.4±0.2	6.3±0.1	5.9±0.1	5.8±0.1

Appendix C: Product Description of Oliggo-fiber™ DS2 Inulin

Table C-1: Oliggo - fiber™ DS2 Inulin Product Description adapted from Cargill Inc. Product Sheet

technical data

Oliggo-Fiber™ DS2 Inulin

Product Description

Oliggo-Fiber™ DS2 inulin is a soluble dietary fiberextracted from chicory roots by a natural process. It's a natural food ingredient. Belonging to the fructan group,inulin is a mixture of non-digestible oligo- and polysaccharides built up of fructose units with $\beta_{2\text{-}1}$ bonds, mostly ending with a glucose unit.

Application / Functionality

Oliggo-Fiber™ DS2 inulin is an easy to disperse fine granulated white powder, suitable for applications with higher incorporation levels without increasing the sugar level. It provides both nutritional and functional benefits, such as enabling fiber fortification, calorie and sugar reduction in product formulations, as well as promotingoverall digestive health and enhancing calcium absorption.

Specifications (Analytical methods available on request)

Chemical and Physical

Dry Matter (%)	≥95
Total carbohydrates (%D.M.) _	≥99.7
Inulin, Dietary Fiber (%D.M.) _	≥98
Free fructose, glucose & saccharose (%D.M.) _	≤2.0
Ash (%D.M.) _	≤0.3

Microbiological

Total Aerobic Mesophilic Count (cfu/g)	≤1000
Yeasts (cfu/g)	≤20
Molds (cfu/g)	≤20
Coliforms	neg/1g
Enterobacteriaceae	neg/1g
Escherichia coli	neg/1g
Salmonella	neg/100g
Staphylococcus aureus	neg/1g
Bacillus cereus (cfu/g)	≤100
Total aerobic thermophilic count (cfu/g):	≤2000

Typical Characteristics

Particle Size (µm)	< 500
Density (after packing) (kg/l)	~ 0.6
Dispersibility	high
Solubility	≥200 g/l
Appearance	granulated white powder
Taste	neutral to slightly sweet
pH	~ 6
Average Degree of Polymerization	< 10
Lead (ppm)	≤0.1

Nutritional Information

Nutrient Content ("as is" basis)

Calories (based on 1 Cal/g for pure inulin)	102 Cal/100g
Total Fat	0 g/100g
Saturated Fat	0 g/100g
Trans Fat	0 g/100g
Sodium	50 mg/100g
Total Carbohydrates	96 g/100g
Dietary Fiber	94 g/100g
Sugars	2 g/100g
Protein	0 g/100g
Calcium	5 mg/100g
Iron	< 1 mg/100g
Potassium	< 1 mg/100g
Moisture	4 g/100g

Table C-1: Oliggo - fiber™ DS2 Inulin adapted from Cargill Inc. Product Sheet (con.)

technical data

Oliggo-Fiber™ DS2 Inulin

Allergen Status
Does not contain common allergens: milk, eggs, fish,crustacean shellfish, tree nuts, wheat, peanuts,soybeans
.Contains no preservatives. Contains less than 10 ppm of sulphur dioxide and sulfites.

Storage / Shelf-life
4 years in dry conditions, in original sealed bag. Max. 30LC (86LF), max. 60% R.H.

Packaging: Multi-layer paper bags 20 kg net, poly-lined.

Applicable certifications
KOSHER (Passover on demand)
HALAL

Not a product of biotechnology or bioengineering.

Produced by
Cosucra Groupe Warcoing SA Belgium
(Mfg. site Warcoing Industrie, Belgium)
ISO 22000 certified by SGS Int. Certification Services

Regulatory Status
Generally Recognized as Safe (GRAS) (additional information available upon request).

Labeling: May be labeled on ingredient list as one of the following: (Chicory) inulin, (Chicory) root fiber, (Chicory) dietary fiber, (Chicory) vegetable fiber,(Chicory) fructo-oligosaccharide, (Chicory)oligofructose.

Item Number: IN-DS2

Tariff code: 11082000

Marketed by
Cargill, Incorporated

Oliggo-Fiber™ Inulin

www.oliggofiber.com

This information reflects US requirements for ingredients and allergens declaration. For countries other than US, please consult with local Cargill regulatory group.

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