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

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).

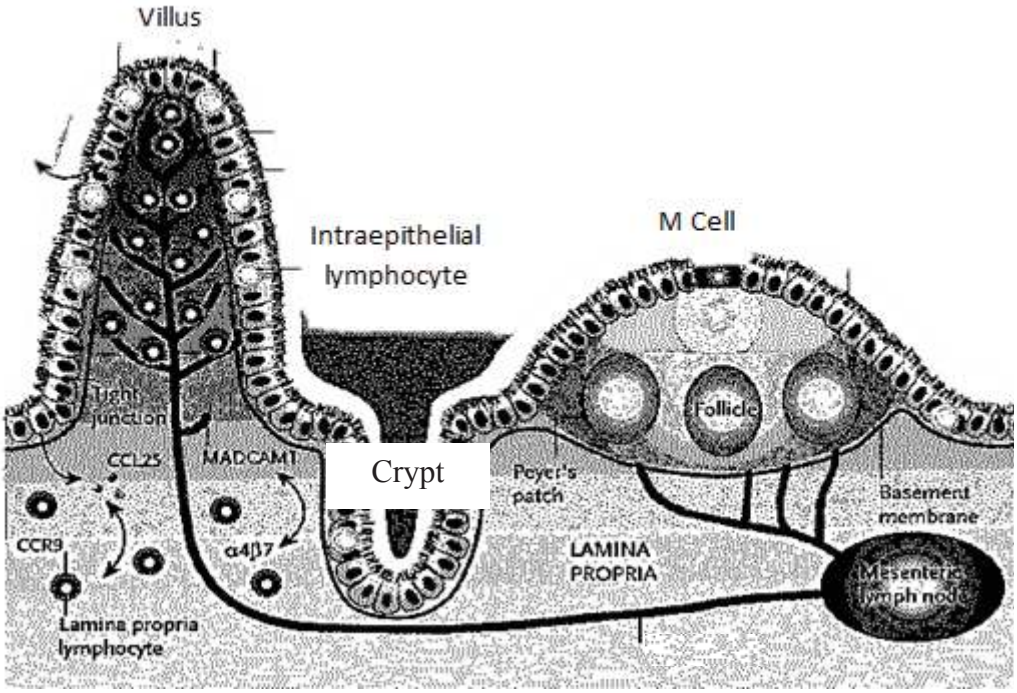

Figure 1: The Gut Associated Lymphoid Tissue (Modified from Mehandru (2007)).

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).

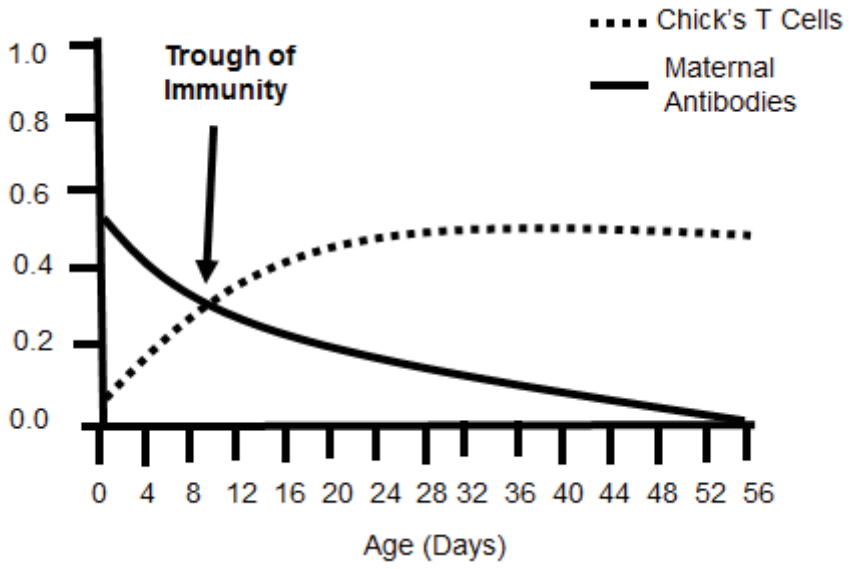

Figure 2: The Trough of Immunity Modified from Ask et al. (2007)

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