Combination of brown seaweed (*Ascophyllum nodosum*) and butyric acid as in-feed growth and health promoters in broiler chickens

by

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Submitted in partial fulfillment of the requirements

for the degree of Master of Science

at

Dalhousie University

Halifax, Nova Scotia

July 2021

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ABSTRACT

Canada's broiler industry is transitioning away from the reliance of in-feed antibiotics, and investigations of alternative feed ingredients to in-feed antibiotics is required. The purpose of these studies was to determine if Tasco (brown seaweed) and Proformix (65% butyric acid) can substitute the antibiotic Bacitracin methylene disalicylate (BMD) either in combination or individually by examining broiler production and intestinal health parameters. Results including Tasco in broiler feed can improve growth and maintain intestinal health compared to broilers fed non Tasco diets. Tasco inclusion at 1.00% was the most consistent at improving growth performance. Mixing Tasco and Proformix did not produce larger broilers or improve intestinal health compared to broilers fed non Tasco + Proformix diets. In conclusion, Tasco can potentially replace in-feed antibiotics as a growth promoter. Mixing Tasco and Proformix requires further investigation, as the mixed diets did not alter broiler production and health traits compared to standard diets.

LIST OF ABBREVIATIONS USED

BMD	Bacitracin methylene disalicylate
BW	Body weight
BWG	Body weight gain
C4	η-butyric acid
cAMP	Cyclic adenosine monophosphate
CB	Calcium butyrate
CD	Crypt depth
CFIA	Canadian food inspection agency
FCR	Feed conversion ratio
FI	Feed intake
GCC	Goblet cell count per area
GI	Gastrointestinal tract
HOCl	Hypochlorous acid
IWT	Intestinal wall thickness
LAB	Lactic acid bacteria
ME	Metabolizable energy
NO	Nitric oxide
Pro	Proformix
ROS	Reactive oxygen species
SCFA	Short-chined fatty acid
SEM	Standard error of the mean

VL Villus length

VSA Villus surface area

VW Villus width

ACKNOWLEDGEMENTS

I would like to express gratitude to my supervisors Dr. Bruce Rathgeber and Dr. Stephanie Collins for their continual advice and guidance through out my years attending Dalhousie Agricultural Campus. I would also like to thank members of my committee Dr. Stefanie Colombo and Dr. Deborah Adewole for their continued support and advice based on their experience in planning and conducting research. The constant willingness of my supervisors and committee members to discuss my project was a constant source of encouragement and enlightenment throughout my studies.

I am grateful to the staff at the Atlantic Poultry Research Centre, specifically Sarah MacPherson and Krista Budgell as their aid was invaluable to the success of the project as their technical knowledge of broiler production and aid in growth data and sample collection cannot be understated. I would like to express my gratitude to Janice MacIsaac as her willingness to educate me on utilizing SAS for conducting statistics was invaluable. I would also like to thank Janice for agreeing to finish completing my statistics on my behalf when access to the software was no longer available to me to complete the remaining statistics.

I would like to thank my fellow poultry group graduate students for their aid in data collection and methodology development for data collection and sample preparation. I would like to give specific thanks to Vicki Mackintosh for agreeing to take on a few morning shifts for barn chores as this allowed for completion of the courses I was completing at the time.

I would like to give huge thanks to my friends and family for their support and advice during to process of my studies. I would like to express my gratitude to Holly Fisher for her constant source of support, friendship, and critical eye that has pushed me to aim for high quality research efforts. Lastly, I would like to thank my mother Margie Cox as she has always been a constant source of support and encouragement ever since I was born. Without her I would not be where I am today as she has helped me determine the path I wish to take in life and what the means are to reach my goals in my own way.

CHAPTER 1 INTRODUCTION

In-feed antibiotics have been used in broiler production since the 1940's, due to their ability to maintain good health, prevent disease development, and enhance the growth performance of broiler chickens (Graham et al., 2007). With increased health concerns related to antibiotic use in food animal production, such as antibiotic-resistant bacteria in poultry, the European Union banned the use of in-feed antibiotics in 2006 for the production of broiler chickens. There were concerns that this could negatively impact poultry health as well as human health from consuming these poultry products (Salim et al., 2018). Since the European ban, there has been a worldwide effort to phase out antibiotic use in broiler production, which has led to an increased occurrence of disease in broiler chickens (Salim et al., 2018). Since that time, there has been a need for alternatives that provide a similar function (Singer and Hofacre, 2006).

Alternatives to in-feed antibiotics are needed by the Canadian poultry industry in order to improve poultry performance at a level that is equally or more efficient than the use of preventative antibiotics (Diarra and Malouin, 2014). Canadian chicken farmers have been making efforts to eliminate the use of in-feed antibiotic growth promoters by consecutive categories. Canada uses a 4-category system to group antibiotics, which is based on the impact these antibiotics have on human health (Diarra and Malouin, 2014). Category 1 antibiotics are classified as having very high importance, category 2 is considered high importance, category 3 is considered medium importance, and category 4 are considered low importance (Diarra and Malouin, 2014). Category 1 antibiotics have been banned by the Canadian national agency Chicken Farmers of Canada for preventative use in the Canadian poultry industry since 2014, and category 2 antibiotics were banned for preventative use at the end of 2018 (Chicken Farmers of Canada, 2021). The industry initially projected to stop the use of category 3 antibiotics by 2020, but the timeline has been moved to an unspecified time in the future, when alternatives to these antibiotics will be available. It is only a matter of time before all classes of antibiotics will not be allowed for preventative measures in the Canadian poultry industry (Chicken Farmers of Canada, 2021).

Some of the alternatives to in-feed antibiotics that have been investigated include probiotics, prebiotics, natural marine oils and products, herbal products such as plant oils and material, and organic acids, including short-chained fatty acids (Sergeant et al., 2014) (Suresh et al., 2018). These ingredients enhance beneficial microbial populations within the gastrointestinal tract of poultry species and increase the number of bacterial metabolites that have beneficial effects on the gastrointestinal tract health and production performance of chickens (Suresh et al., 2018; Swiatkiewicz et al., 2015). They also have the potential to increase overall immunity as well as stimulate digestive health and function (Sergeant et al., 2014) (Swiatkiewicz et al., 2015). The improved digestive capabilities of chickens with their feed will lead to more efficient utilization of the feed nutrients for health and growth (Sabour et al., 2019).

Necrotic enteritis is one of the most prominent management-related diseases known to occur in commercial broiler production as a result of decreased use of in-feed antibiotics. Necrotic enteritis is a bacterial disease caused by the Gram-positive bacteria *Clostridium perfringens*, a naturally occurring species of microbe found within the digestive tract of chickens that can cause damage to the gastrointestinal tract via production of toxins and potentially competing with beneficial bacteria for resources (Si et al., 2007). Necrotic enteritis leads to increased mortality in broiler chickens and can cost the industry between two to six billion dollars per year worldwide (Wade et al., 2015).

Two in-feed antibiotic alternatives that could potentially reduce the occurrence and impact of necrotic enteritis in the broiler industry are brown seaweed and butyric acid. Brown seaweed is an applicate for controlling necrotic enteritis in broilers, as it contains non-starch polysaccharides that stimulate the activity of beneficial bacteria within the intestinal tract of broilers (Charoensiddhi et al., 2016). This can lead to increased uptake of nutrients by these bacteria and limit nutrient uptake of *C. perfringens*, reducing its activity, thus lowering toxin production (Prescott et al., 2016). *Ascophyllum nodosum* is a species of brown seaweed studied for its impact on broiler production, as it is one of the most readily available brown seaweed species (Fan et al., 2011). Implementation of butyric acid in poultry production is a potential control measure for necrotic enteritis, as butyric acid conveys antibiotic properties without the drawbacks associated with the preventative application of antibiotics in food animal production. Including butyric acid in broiler feed can reduce the presence of *C. perfringens* in the intestinal tracts of broilers (Namkung et al., 2011). This leads to reduced toxin production and limits intestinal tract epithelial damage (Namkung et al., 2011).

The purpose of this study is to determine if a combination of brown seaweed *(Ascophyllum nodosum*) and butyric acid that will have a beneficial impact on the intestinal health and production performance of broiler chickens.

CHAPTER 2 LITERATURE REVIEW

2.1 Antibiotic use in poultry

Antibiotics have been used in poultry production at both the therapeutic and subtherapeutic level since the mid 1900's (Salim et al., 2018). Antibiotics have been generally used in poultry as growth and health promoters, due to their ability to improve feed efficiency and maintain gut health (Kumar et al., 2018). Some of the most common antibiotics that have been used in poultry production in North America are enramycin, monensin, penicillin, virginamycin, and bacitracin methylene disalicylate (BMD). These antibiotics (especially BMD) are used in broiler production to prevent the occurrence of necrotic enteritis as well as improve weight gain and feed efficiency (Kumar et al., 2018). Bacitracin methylene disalicylate (BMD) is popular in the industry, as it has a narrow spectrum of antibiotic effects on the overall population of microbiota within the intestinal tracts of broilers (Proctor and Phillips, 2019).

Antibiotics work by manipulating the microbiota within the intestinal tract of the host by discouraging the growth of pathogenic microbes. This results in the stimulation of beneficial microbial growth due to lower competition (Sapkota et al., 2018). Unfortunately, if a species of bacteria is exposed to an antibiotic over a period of time, they can develop resistance to this antibiotic, and will no longer have the same effects on the species of bacteria it once affected (Wongsuvan et al., 2018). This can cause harm to the host, as the bacteria is free to infect the host without antibiotics impairing their infection rate. Genes from these antibiotic-resistant bacteria can also be vertically transmitted to the next generation, as well as horizontally transmitted to other bacteria within the host (Salim et al., 2018). This can alter the microbial composition of the host, leading to a less diverse microbial population and resulting in a deficiency of resources that the more diverse population of microbes were able to produce (Salim et al., 2018). There is also the risk that if humans eat poultry products that contain these antibiotic-resistant bacteria, then natural microbes within humans will be affected by these microbes and impair human health (Singer and Hofacre, 2006).

The European Union voted in 2006 to ban the use of antibiotics in broiler production at any level, and since then, there has been a worldwide trend of not using antibiotics in food production, due to pressure from government organizations and businesses (Salim et al., 2018). Due to this trend however, there has been an increased occurrence of certain diseases in broiler production, such as necrotic enteritis. The risk associated with antibiotic usage in food production is real, as there has been an increase in *Salmonella* infection in different regions of the world due to the overuse of antibiotics (Sapkota et al., 2018). This is an example of why there will be increased pressure against antibiotic usage in broiler production worldwide (Sapkota et al., 2018).

2.2 Bacitracin methylene disalicylate (BMD)

Bacitracin methylene disalicylate (BMD) is an antibiotic widely used in poultry production for its growth promoting properties (Koltes et al., 2017). This antibiotic is supplemented in chicken feed to promote growth, gastrointestinal performance such as improve intestinal villus lengths and beneficial alterations of intestinal microbial populations, and improve production quality of broiler chickens (Proctor and Phillips, 2019). Bacitracin is a mixture of non-ribosomal polypeptides that interact with the cell wall formation of bacteria (Neumann and Suen, 2015). This interaction prevents the dephosphorylation of the carrier for N-acetylmuramyl pentapeptide intermediates. This leads to a more permeable cell wall, making the bacteria more vulnerable to other bacteria or activity of the host immune system (Neumann and Suen, 2015). In-feed antibiotics act as growth promoters, as they encourage the growth of beneficial bacteria by inhibiting the growth of pathogenic bacteria, which increases the availability of resources for probiotics within the host (Samanta, et al., 2010). The performance and growth of broiler chickens is closely related to the quality and quantity of microbes within the digestive tract of the chicken (Samanta et al., 2010). These microbes can improve digestive activity, feed utilization, and intestinal tract immunity; which in turn can improve growth and production. A high quantity of pathogenic microbes can lead to impaired digestive activity, depressed growth, and increased occurrence of disease (Beitawi et al., 2009). While BMD is widely used to promote health and growth of chickens, increasing pressure from consumers and government officials to limit the use of antibiotics in poultry production may lead to the inability to use BMD in poultry feed in the future (Beitawi, et al., 2009). Consequences of this action are already being felt across the industry, as limiting the use of BMD and antibiotics in general has led to increased occurrences of severe diseases in broiler production (Neumann and Suen, 2015).

2.3 Necrotic enteritis

Necrotic enteritis is an enteric bacterial disease that is mainly caused by the bacterium *Clostridium perfringens* (Si et al., 2007). *Clostridium perfringens* is a Gram-positive, anaerobic, spore-forming bacterium (Alnassan et al., 2013). The strain that is the main cause for necrotic enteritis is Type A, and to a lesser extent, Type C. The mechanism that these bacteria use to inflict this disease is toxin production. Type A can produce several toxins including alpha, beta 2, necrotic enteritis B-like toxin (NetB) and TypeL; while Type

C bacterium only produces beta toxins. The toxins mainly responsible for the development of necrotic enteritis are toxins alpha and NetB (Alnassan et al., 2013). These toxins break down the epithelial wall of the intestinal tract, causing lesions. The result is a divergence of energy from maintaining general health to addressing the lesions caused by the bacterial toxins (Keyburn et al., 2006). Necrotic enteritis is a problematic disease facing the poultry industry as the intensity of its effects correlates with the overabundance of *Clostridium perfringens* within the gastrointestinal tract in chickens. Larger populations of this bacteria lead to increased production of toxins and increased damage to the intestinal tract (Paiva and McElroy, 2014). As a result, the chicken utilizes excessive energy to control necrotic enteritis and cannot maintain sufficient health and may eventually die (Keyburn et al., 2006). These lesions also create openings within the intestinal tract that can lead to the leakage of nutrients (Antonissen et a., 2014). The result is the chicken cannot properly digest and utilize those nutrients in order to maintain general health and growth (Antonissen et a., 2014).

A predisposing disease often associated with necrotic enteritis is coccidiosis. This is a disease caused by the protozoan coccidia that causes depressed weight gain and reduced feed intake. Coccidiosis also damages the epithelia of the intestine, causing protein leakage and increased mucus secretion (Alnassan et al., 2013). *Clostridium perfringens* then uses the energy from these free proteins caused by mucus breakdown by coccidiosis for replication and production of toxins. Necrotic enteritis can emerge in two to five-week-old broilers, but clinical signs of the disease do not appear until the chicken is around 17-18 days old (Si et al., 2007). It can be difficult to identify the disease early enough to treat it properly. Since the antibiotic ban in the European Union in 2006, worldwide use of in-feed antibiotic growth promoters has diminished, and it has become more challenging to control this disease (Stanley et al., 2014).

2.4 Potential Antibiotic alternatives

2.4.1 Seaweed

Seaweeds are an abundantly available natural source of biomass for many species of organisms and can be easily cultivated (Cabrita et al., 2016). Many seaweeds are rapid growers and contain a rich source of nutrients, such as non-starch polysaccharides, minerals, and vitamins (Cabrita et al., 2016). These nutrients and their natural abundance are key factors for considering seaweed as a feed additive in poultry production. Many of the nutrients found in seaweed enhance immune system activity in chickens, as well as nurture beneficial gut microbes and improve overall digestive system function (Hansen et al., 2003). A study conducted by Kulshreshtha et al. (2017) observed the effects of red seaweeds (*Chondrus crispus* and *Sarcodiotheca gaudichaudii*) on bird growth and egg production for laying hens challenged with *Salmonella enterica*. Both seaweeds were introduced to the feed at dietary inclusion levels of 2% and 4% and were compared to a basal non-medicated feed as well as a feed containing chlortetracycline (Kulshreshthta et al., 2017). Both seaweed species significantly improved feed intake compared to the antibiotic and significantly improved body weight and egg production compared to the basal diet in weeks 3 and 4 of the trial. The highest level of seaweed inclusion led to a significant reduction of *S. enterica* colonization in the ceca, as well as a significant increase in propionic acid in the digesta of the chicken (Kulshreshtha et al., 2017). This could be due to the bioactive compounds found within red seaweed acting as a prebiotic as well as improving immunity (Kulshreshtha et al., 2017). Some of these bioactive components include polyphenols, peptides, and polysaccharides which convey health benefits. These benefits include anticoagulant action, anti-inflammatory, antiviral, and antitumoral activities (Choi et al., 2014).

Seaweed can affect the microbial composition of the intestinal tract of the chicken, as microbes require nutritional components such as amino acids and carbohydrates. These nutrients could potentially enhance the population and activity of beneficial microbes within the chicken for improved health and performance (Kulshreshtha et al., 2017; Sharma et al., 2018). Seaweeds also contain high amounts of compounds known as polysaccharides. Polysaccharides are carbohydrates that consist of a number of sugar molecules bonded together (Charoensiddhi et al., 2017). These compounds are not digestible by normal digestive action and require the aid of microorganisms to break down the structure of polysaccharides as well as aid in the release and formation of beneficial molecules that can be utilized in the health and growth of the host organism (Charoensiddhi et al., 2017). The presence of polysaccharides leads to the promotion of beneficial microbes within many organisms, as these contain compounds that beneficial microbes such *Lactobacillus* and *Bifidobacterium* species utilize in fermentation for self-preservation and synthesis of beneficial microbes to the host. Because of this, many polysaccharides are considered prebiotics when utilized as a functional feed ingredient (Zhou et al., 2018). As seaweeds, such as brown seaweed contain high amounts of polysaccharides, they are also considered effective prebiotics (Charoensiddhi et al., 2016).

Including prebiotics in the feed can lead to increased growth and health performance in chickens (Charoensiddhi et al., 2017). An experiment conducted by Sweeney et al. (2016) observed the effects of brown seaweed (*Ascophylum nodosum*) on growth, gut health, immune parameters, and *Campylobacter jejuni* counts in 10-day old chicks. The treatments were control, 500ppm brown seaweed, and 1000ppm brown seaweed. They found that brown seaweed significantly decreased the amount of *C. jejuni* within the ceca, and also significantly decreased average daily gain, average daily feed intake, and slaughter weight. This contradicted the findings of Kulshreshtha et al (2017), who fed red seaweed. Dietary inclusion of brown seaweed did increase villus height within the ileum but significantly decreased villus width at 1000ppm. This trial expresses the potential for brown seaweed as a health-promoting feed ingredient, but refinement for its application to be fully realized is required (Sweeney et al., 2016).

2.4.2 Brown seaweed pigment fucoxanthin

Seaweeds are taxonomically classified by the dominant carotenoid profile that makes up the seaweed. Carotenoids are tetraterpenoids that have a 40-carbon backbone containing 11 conjugated double bonds (Mikami and Hosokawa, 2013). These compounds are responsible for the expression of color in many plants and photosynthetic organisms. In the case of seaweed, the most dominate colors are brown, red, and green (Mikami and Hosokawa, 2013). The carotenoid within brown seaweed that is responsible for its brown coloration is fucoxanthin. Fucoxanthin is a xanthophyll carotenoid with a unique chemical structure containing an allenic bond, epoxy, hydroxyl, carbonyl, and carboxyl groups (Bae et al., 2020). This xanthophyll is important for the process of photosynthesis in brown seaweed, as it acts as an antenna pigment carotenoid by coupling with the thyroid membrane. Through this action, it transfers energy from the light-harvesting complexes in brown seaweed and transfers it to the photosynthetic electron transport chain (Zarekarizi et al., 2019). Fucoxanthin has been studied in relation to human health over the years, and studies have found that that fucoxanthin has anti-oxidant, anti-inflammatory, anti-cancer, anti-obesity, and anti-diabetic properties when consumed. A study conducted by Morandi et al. (2014) evaluated fucoxanthin antioxidant and anti-inflammatory ability on human neutrophil functional properties *in vitro*. They also evaluated if fucoxanthin exerts a more significant impact when combined with vitamin C (Morandi et al., 2011). They found that fucoxanthin significantly increased phagocytic capacity when compared to the control. Phagocytes are cells that engulf and consume foreign material such as pathogenic bacteria (Morandi et al., 2014). This shows that fucoxanthin significantly increases the number of phagocytes, which in turn improves immune system functionality. Morandi et al. (2014) also found that fucoxanthin significantly decreased the presence of reactive oxygen species (ROS) superoxide anions, hydrogen peroxide, and hypochlorous acid (HOCl) (Morandi et al., 2014). This leads to less oxidation of neutrophils, less degradation of cells, and improved body health and function. They also found that the addition of fucoxanthin leads to a significant decrease in cytokine release (Morandi et al., 2014).

Cytokines are small proteins that signal the immune system to activate the inflammation response, and can lead to damaging inflammatory events if they are too abundant in number. Morandi et al. (2014) demonstrated that fucoxanthin significantly decreases the release of cytokines and therefore, decreases inflammation in neutrophils (Morandi et al., 2014). This makes it ideal as a bioprotective molecule used in humans for improved health (Zarekarizi et al., 2019). In relation to necrotic enteritis control in chickens, fucoxanthin may be one of the main components that make brown seaweed applicable as an alternative to in-feed antibiotics for improved growth and health of broiler chickens. This is mainly due to its anti-oxidant and anti-inflammatory properties. Fucoxanthin's antioxidant properties are due to its ROS scavenging activity due to its carbon 7 double allenic bonds (Kim and Pangestuti, 2011). This leads to degreased degradation of internal tissues and improved functionality. While documentation on fucoxanthin's anti-inflammatory properties is scarce, the most promising theory is fucoxanthin inhabits the activity of nitric oxide (NO) synthase and inhibits NO production (Kim and Pangestuti, 2011). Nitric oxide (NO) is a major signaling molecule responsible for inducing pathogenic inflammation; therefore, inhibiting NO synthase, leads to decreased NO activity and decreased inflammation (Heo et al., 2010). Both of these properties are important for necrotic enteritis inhibition, as fucoxanthin can improve the structural integrity of the intestinal tract, as well as potentially inhibit toxin intensity on the intestinal tract (Kim and Pangestuti, 2011). This will lead to improved functional activity and therefore better growth due to improved efficiency of nutrients in the feed being extracted within the intestinal tract (Saker et al., 2004). Unfortunately, evidence of the specific benefits of fucoxanthin on broiler health and growth is limited.

2.4.3 Butyric acid

One of the strategies to replace in-feed antibiotics has been the integration of short-chain fatty acids as feed additives (Ocejo et al., 2017). These are fatty acids with one to six carbons produced within the intestinal tract via bacterial fermentation of undigested carbohydrates. Commercially available short-chain fatty acid feed products are more commonly synthetically produced, but there are currently efforts to produce available short-chain fatty acid feed products derived from biological sources, via microbial fermentation (Wang et al., 2019). The production of short-chain fatty acids with in chickens allows for the salvaging of energy from mainly carbon sources that are not digested within the small intestine (Bedford and Gong, 2018). One example is butyric acid, which conveys health benefits across species. These effects include anti-inflammatory properties, enhanced intestinal epithelial integrity, and beneficial shifts in the composition of intestinal microbes (Ocejo et al., 2017). In chickens, butyric acid stimulates small intestinal tract development. Other actions include an improved balance of microbiota within the gut, helping to stimulate growth and improve nutrient absorption and utilization (Kulcsar et al., 2017). Butyric acid is a product of bacterial fermentation of carbohydrates within the ceca of the chicken digestive tract (Kulcsar et al., 2017). This derivative of butyrate is a popular feed additive not only for its growth-promoting qualities, but also for its antimicrobial capabilities (Donovan et al., 2016). The form of butyric acid is important for how efficient butyric acid is used within the chicken. Butyric acid in its base form is limited when exerting beneficial effects on the intestinal tract of chickens as a majority of it is destroyed by acid within the stomach when ingested (Donovan et al., 2016). Often when the effectiveness of butyric acid on intestinal health in livestock is examined, tributyrin is the most common form observed. Tributyrin is a more structurally dense molecule compared to butyric acid and is more difficult to destroy in the stomach (Donovan et al., 2016). Often microencapsulation is used to further increase the efficiency of butyric acid within chickens. Microencapsulation is a physical or chemical process used to create a barrier around a core material (Donovan et al., 2016). This further ensures the butyric acid component of tributyrin is intact by the time it reaches the intestinal tract and able to exert beneficial effects on intestinal tract health and function in chickens (Donovan et al., 2016).

Ocejo et al. (2017) conducted an experiment that reported the response of chickens to whey and calcium butyrate (CB) supplementation with regards to growth performance, histological integrity, and *Campylobacter* colonization in broiler chickens. They found 6% CB improved body weight, weight gain, and feed intake compared to the control, and for the first 21 days of the trial, significantly improved the feed conversion ratio (FCR) (Ocejo et al., 2017). Unfortunately, CB did not significantly decrease *Campylobacter* colonization in the ceca of the broiler chickens in this study (Ocejo et al., 2017). It is suggested however, that early introduction of butyrate and butyrate derivatives such as butyric acid in the diet of chickens can lead to reduced *Salmonella* colonization within the ceca (Bedford and Gong, 2018). This has also been shown in the case of *Clostridium perfringens* as well (Bedford and Gong, 2018). Namkung et al. (2011) observed the effects of different forms of butyric acid products in relation to the inhibition of bacterial species *Salmonella typhimurium* and *Clostridium perfringens* via in vitro trials. The butyric acid treatments were η-butyric acid (C4), 50% monobutyrin / 50% C4 mix, 100% monobutyrin, and a Baby C4 mix (30% monobutyrin / 50% dibutyrin / 20% triglycerides of C4) (Namkung et al., 2011). In relation to *Clostridium perfringens,* the products that had the best inhibitory effect of over 90% were η-butyric acid, and 50% monobutyrin 50% C4 mix at a concentration of 3000ppm (Namkung et al., 2011). While these are promising results, the concentration of butyric acid required to obtain these results is much larger than the concentration that the Canadian Food Inspection Agency (CFIA) will allow to be incorporated into chicken feed. This shows the potential that butyrate and its derivatives have on improved necrotic enteritis control and overall growth in chickens, but modifications to acquire these results at lower concentrations of butyric acid is still required (CFIA Administrative Schedule IV and V, 2018).

2.5 Synergy of feed ingredients

In the field of medicine, there is always an ambition to find the best quantities of a medical agent for the treatment of disease. This is because increasing the amount of medication administered for more severe cases of a disease does not lead to an additive effect on the efficiency and impact of the medication (Prasad et al., 2001). Often, increasing medication intake past a threshold can lead to unwanted side effects. The most common of these is toxicity of the active ingredient that gives the medication its medicative properties (Prasad et al., 2001). This is where the concept of synergy plays a significant role in disease treatment (Jacobs et al., 2011).

Synergy is the concept of two or more substances working in unison to produce an effect that is greater than what can be achieved with the substances working individually (Jacobs et al., 2011). In relation to disease control, this concept is commonly studied in the combination of medical treatment and improved nutritional intake. Nutritional intake is a more passive way of introducing beneficial compounds to an organism that can convey effects that are beneficial to the treatment of the disease (Levine et al., 2016). These effects can lead to direct impairment of the disease's infection rate and impact and improve the host's general health and body function. This allows the medication to work more effectively, as there will be less stress and strain on the host organism to resume homeostasis during this time (Klurfeld, 2001). This concept has been shown to work well.

However, the knowledge of the mode of action of combinations of different nutrients and medications is limited and requires increased inquiry (Jacobs et al., 2011). This is especially true as different organisms will react to combinations differently, and adjustments of combinations of nutrients will be required for optimal effect (Levine et al., 2016). By determining the mode of action of combinations of nutrients, we are able to accurately determine specific inclusion amounts in diets that will result in the optimal level for health and disease prevention and treatment (Jacobs et al., 2011).

This concept plays a significant role in this study, as previous research into determining derivatives of butyric acid's impact on necrotic enteritis in chickens has shown promise (Timbermont et al., 2010). The amounts required to observe a significant effect is unfortunately higher than what is allowed to be incorporated into poultry feed. The CFIA regulates that the maximum dietary inclusion level of butyric acid in broiler feed is 100ppm (Administrative Schedule IV and V, 2018). Namkung et al. (2011) showed that optimum levels needed for the most efficient necrotic enteritis control is 3000ppm, which is thirty times greater than what can be fed to broilers. Hence, the need to combine butyric acid with another active ingredient as although the impact of butyric acid activity in the chicken will be less significant, the activity of the other active ingredients will compensate for the lower concentration of butyric acid. The other active ingredient will convey an additive effect of butyric acid activity, or by impacting health functions in different ways, such as improving base immunity and digestive tract function. This will increase the efficiency of the butyric acid as a growth-promoting agent.

2.6 Taste perception in chickens

Most vertebrate animals have a well-developed taste system comprised of taste sensory organs and connecting nerves and nervous system for the taste sensation animal perceive when consuming feed. The organ that is well connected with this system are taste buds (Kudo et al., 2014). Taste buds are taste sensory organs often located on the tongue and are bud shaped (Liu et al., 2018) Taste buds transduce chemical signals within feed (tastants) and transmit these signals to the brain. This often results in a behavioral reaction that motivates and guilds feed intake (Rajapaksha et al., 2016). The most common responses are either the animal will eat more or less, depending on how they interoperate the signals (Rajapaksha et al., 2016). Recent research informs us on how chickens taste their feed (Cui et al., 2017). Chicken taste buds are not located on the tongue as other livestock species; they are located near the opening of salivary glands and are ovoid shaped. The most common areas are the epithelium of the palate and in the base of the oral cavity, anterior to the mandibular gland region (Rajapaksha et al., 2016). The number of taste buds correlates positively with taste sensitivity in chickens; the more taste buds a chicken has, the more sensitive they are to tastants. The average number of taste buds in chickens range from 240-360, which is dependent on the breed and sex of the chicken (Rajapaksha et al., 2016).

It is widely accepted that there are five basic taste qualities, which are: sweet, bitter, umami, sour, and salty. Different taste receptor molecules and ion channels are located in the cell membranes of different taste bud cells and are the mediation molecules for transducing different taste stimuli (Liu et al., 2018). This means that taste receptor molecules and ion channel gene expression in taste buds are responsible for taste qualities.

In other words, if genes of a taste receptor to a specific taste quality is expressed more, the more sensitive the animal will be to that taste quality (Liu et al., 2018). When compared to mammals, chickens have fewer genes for taste receptors and lack the taste receptor gene T1R2, which is responsible for the sweet taste stimulus. This means that chickens only detect four taste qualities (sour, umami, salt, and bitter) (Liu et al., 2018). Chickens are particularly sensitive to bitter tastants, as chickens display aversive behaviour when quinine chloride (bitter stimuli) is introduced to them. This is significant in this study, as butyric acid is considered a highly bitter feed ingredient (Liu et al., 2018). While butyric acid has potential as a feed additive, if its bitter stimuli overloads a chicken's taste receptors, the chicken will not ingest the feed and would not acquire the benefits of the feed. The chickens may even develop poorer production characteristics due to the lack of feed consumption (Liu et al., 2018). Determining appropriate concentrations of feed additives is important in order to encourage feed intake (Liu et al., 2018).

2.7 Objectives

The objectives of this project were to determine if brown seaweed alone has beneficial effects on broiler chicken growth performance and intestinal tract health, and determine if incorporating the combination of commercial brown seaweed species *Ascophyllum nodosum* with butyric acid in broiler feed will lead to synergistic effects on broiler growth performance and improved intestinal tract health.

2.8 Hypothesis

The hypothesis of this study was that *Ascophyllum nodosum* would lead to improved growth and intestinal tract health in broiler chickens due to its prebiotic and antioxidant properties. It was also hypothesized that combining brown seaweed with butyric acid would lead to a synergistic effect on growth performance and intestinal tract health such as heavier body weights, more efficient feed conversion ratios, longer villus lengths, wider villus widths, lower crypt depths, thicker intestinal walls, and greater goblet cell counts.

CHAPTER 3 EVALUATION OF BROWN SEAWEED (*ASCOPHYLLUM NODOSUM*) AS AN EFFECTIVE FEED ADDITIVE IN BROILER CHICKEN PRODUCTION

3.1 Abstract

There is a dearth of information on the efficiency of brown seaweed as an in-feed antibiotic alternative, and what research has been conducted is inconsistent regarding the effect of brown seaweed on growth performance of broiler chickens. The purpose of this research was to investigate how the dietary inclusion of brown seaweed (Tasco) affects broiler growth and ileal morphology. Two growth trials (Tasco trial 1 and Tasco trial 2) were conducted to evaluate brown seaweeds consistency on improving broiler growth and ileal morphology. The dietary treatments observed in this study were: basal diet, a bacitracin methylene disalicylate (BMD) medicated diet, and diets containing 0.25%, 0.50%, 0.75%, or 1.00% dietary inclusion levels of Tasco. In both trials, feeding 1.00% Tasco significantly increased body weight in market-age broilers ($p<0.05$), compared to the basal and medicated diet-fed broilers ($p<0.05$). In the second trial, after 35 days on feed, broilers fed 0.75% Tasco were significantly heavier than birds fed the medicated control diet, but this was not observed in Tasco trial 1. Diet did not influence ileal morphology, as all broilers observed had similar ileal morphological parameters and goblet cell counts ($p>0.05$). In conclusion, feeding brown seaweed to broilers at a 1.00% dietary inclusion level can positively influence growth performance. The influence of Tasco on duodenum and jejunum should be investigated, as well as its effect on microbial colonies along the intestinal tract, as changes in these parameters may explain the improvements in growth performance.

3.2 Introduction

Overuse of in-feed antibiotics is a factor that can led to the development of antibiotic-resistant pathogenic bacteria that can survive in food and the environment. For this reason, there is a desire to eliminate them from the diets of broiler chickens. As the broiler industry explores ways to transition away from the use of in-feed antibiotics, controlled studies are needed to evaluate the applicability of alternatives in terms of growth and animal health improvements (Subedi et al., 2018).

One alternative that is being investigated in the broiler industry is *Ascophyllum nodosum*. *A. nodosum*, known more commonly as rockweed, is a species of brown seaweed found in rocky intertidal areas in the coasts of North America and Northern Europe, and is tolerant of extreme temperatures, salinities, and other environmental stressors (Rayorath et al., 2008). *A. nodosum* contains exceptional prebiotic properties and is easy to cultivate (Agregan et al., 2017).

A. nodosum prebiotic properties are attributed to its high content of non-starch polysaccharides (49-52%). In broiler chickens, these compounds stimulate fermentative activity of beneficial bacteria within the intestinal tract (Lin et al., 2020; Simmons-Boyce et al., 2009). Fermentation of polysaccharides by these bacteria produces compounds that aid in improving intestinal tract function, including improved integrity of intestinal epithelium, increased digestive efficiency, and improved intestinal immunity (Ivarsson et al., 2014).

A. nodosum is easy to cultivate and is grown off coastal regions in many areas of the world (Rayorath et al., 2008). This tolerance is due to its production of stress-tolerant compounds and the mutualistic relationship *A. nodosum* has with the fungal endophyte *Mycophaerella ascophylli*, which protects *A. nodosum* from desiccation (Shukla et al., 2019). These natural survival mechanisms reduce the labour and production costs required to the farm and process this seaweed, compared to land-based crop production (Shukla et al., 2019).

Due to the increase popularity of *A. nodosum* inclusion in food animal diets, readily available *A. nodosum* products have been developed for producers to include in diets. One of these products is known as Tasco, which is a dried granulated *A. nodosum* feed product produced by Acadian Seaplants Ltd. It is marketed based on its prebiotic's properties, that lead to improved GI health and performance for optimal growth and performance (Tasco, 2019).

Research into *A. nodosum* as a feed additive in broiler production has shown promise, but its influence on growth performance is inconsistent as some studies observe increases in broiler growth, while others report decreases in growth (Choi et al., 2014) (Sweeney et al., 2016). The most consistent reports for studies evaluating dietary inclusion of *A. nodosum* in broiler production is the improvement of intestinal tract health in broilers, which includes improved intestinal morphology and modification of intestinal microbial colonies (Sweeny et al., 2017; Mohammadigheisar et al., 2020).

The objective of this study was to determine if *A. nodosum* (Tasco) can consistently improve the growth performance and intestinal health of broiler chickens when included in broiler feed at different dietary inclusion levels (0.25%, 0.50%, 0.75%, and 1.00%) in a broiler production setting. The health and growth of the broiler chickens fed different diets were monitored to compare the effect of seaweed feed to antibiotic feed.

The hypothesis is that Tasco will improve the growth performance and ileal health of broiler chickens compared to broilers fed a basal diet or a medicated diet containing bacitracin methylene disalicylate (BMD).

3.3 Materials and methods

3.3.1 Housing and caretaking

This research was conducted at the Atlantic Poultry Research Centre (Truro, N.S, Canada). Two rooms were used to house 1248 Ross 308 fast-feathered, mixed-sex broiler chickens, which was repeated in two trials (Tasco trial 1 and Tasco trial 2). Each room contained 24 pens with 26 birds per pen. These birds were kept for 34 days during Tasco trial 1 and 35 days for Tasco trial 2. Each pen was randomly assigned to one of six feed treatments, resulting in 8 replicates per treatment per room, totalling 16 replicates per treatment. Each of these growth trials were independent of each other.

The test ingredient was Tasco, a readily available *A. nodosum* feed ingredient product supplied by Acadian Sea Plants Ltd (30 Brown Ave, Dartmouth, NS, Canada). The dietary formulations are reported in Tables 3.1, 3.3, 3.5. The diets were in the form of mash for the start phase (days 0-14) and pellet for the grower and finisher phases. For the pellets, the mash form of the diet mixed with the steam between 80˚C-90˚C. However, due to the heat from the steam addition plus the friction heat of the mash leaving the die as a pellet, the pellets leave the die between 100˚C-120˚C

The studies were conducted under approved Animal Care and Use Committee (ACUC) of Dalhousie University, Nova Scotia, Canada protocols 2018-089 and 2019-051.

The diets evaluated were:

1) Basal diet
2) 0.05% Bacitracin methylene disalicylate (BMD) medicated diet
3) 0.25% Tasco diet
4) 0.50% Tasco diet
5) 0.75% Tasco diet
6) 1.00% Tasco diet

The dietary inclusion levels of each of the test ingredients selected in this study were based on a combination of manufacturer recommendation, CFIA regulations, and previous research efforts. Feed and water were provided *ad libitum*. All feed added was weighed and recorded. Daily health checks were conducted and mortalities were removed and weighed, as well as the feed in that pen to ensure feed consumption data calculated for that pen was accurate for that period. All mortalities were sent to the provincial veterinary pathology lab for necropsy and cause of death was recorded when determined.

3.3.2 Experimental diets

Table 3.1: Diet formulation for starter diets (day 0-14) fed in Tasco trials 1 and 2

Ingredients	Basal	Med	0.25% Tasco	0.50% Tasco	0.75% Tasco	1.00% Tasco
Corn	51.08	50.97	50.69	50.27	49.86	49.45
Soybean Meal	41.44	41.45	41.47	41.51	41.54	41.58
Tasco[a]	0	0	0.25	0.50	0.75	1.00
Ani/Veg Fat	2.93	2.97	3.08	3.23	3.39	3.55
Limestone	1.80	1.80	1.79	1.78	1.77	1.76
Dicalcium Phosphorus 21 P	1.24	1.24	1.24	1.24	1.24	1.24
DL Methionine Premix	0.59	0.59	0.59	0.59	0.59	0.59
MCBS7	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.41	0.41	0.39	0.37	0.35	0.33
BMD	0	0.05	0	0	0	0
Lysine HCl	0.01	0.01	0	0	0	0
Total	100	100	100	100	100	100
Calculated nutrient composition on an as fed basis						
ME (kcal/kg)	3000	3000	3000	3000	3000	3000
Crude Protein (%)	23.00	23.00	23.00	23.00	23.00	23.00
Calcium (%)	0.96	0.96	0.96	0.96	0.96	0.96
Available Phosphorus (%)	0.48	0.48	0.48	0.48	0.48	0.48
Sodium (%)	0.19	0.19	0.19	0.19	0.19	0.19
Digestible Tryptophan	0.25	0.25	0.25	0.25	0.25	0.25
Digestible Threonine	0.89	0.89	0.90	0.91	0.91	0.92
Digestible Methine and Cystine (%)	0.95	0.95	0.95	0.95	0.95	0.95
Digestible Lysine	1.28	1.28	1.28	1.30	1.31	1.32

a: The brown seaweed product name supplied by Acadian Sea Plants, ME=metabolizable energy, DL Methionine Premix is comprised 50% DL Methionine and 50% ground corn. MCBS7 is broiler vitamin premix comprised of: Vitamin A 0.156%, Vitamin D3 premix 32.00%, Vitamin E 2.00%, Vitamin K 0.194%, Riboflavin 0.215%, DL Ca-pantothenate 0.6%, Vitamin B12 0.46%, Niacin 0.808%, Folic acid 2.20%, Choline chloride 26.70%, Biotin 6.00%, Pyridoxine 0.109%, Thiamine 0.082%, Manganous Oxide 4.00%, Zinc oxide 3.05%, Copper sulfate 1.28%, Selenium premix 1.485%, Ethoxyquin 1.66%, Ground corn 7.001%, Ground limestone 10.00%.

Table 3.2: Nutrient analysis of starter diets fed in Tasco trials 1 and 2

Parameter	Basal	Med	0.25% Tasco	0.50% Tasco	0.75% Tasco	1.00% Tasco
Dry Matter (%)	88.41	88.09	88.83	88.24	88.25	88.58
Crude Protein (%)	23,78	23.68	23.28	22.97	23.24	23.06
Calcium (%)	1.05	1.16	1.04	0.96	1.20	1.10
Potassium (%)	1.05	1.04	1.07	1.05	1.05	1.03
Magnesium (%)	0.18	0.18	0.18	0.18	0.18	0.18
Phosphorous (%)	0.66	0.66	0.65	0.64	0.65	0.65
Sodium (%)	0.19	0.21	0.18	0.19	0.18	0.19
Copper (ppm)	21.53	22.74	21.49	20.38	20.63	20.60
Manganese (ppm)	133.36	141.44	132.77	141.89	142.05	144.40
Zinc (ppm)	135.15	141.67	135.32	144.10	134.68	142.50
Crude Fat (%)	5.28	5.12	5.24	5.41	5.39	5.70

Table 3.3: Diet formulation for grower diets (day 14-24) fed in Tasco trials 1 and 2

Ingredients	Basal	Med	0.25% Tasco	0.50% Tasco	0.75% Tasco	1.00% Tasco
Corn	44.32	44.22	43.91	43.49	43.08	42.67
Soybean Meal	36.48	36.49	36.51	36.55	36.59	36.62
Tasco[a]	0	0	0.25	0.50	0.75	1.00
Wheat	10.00	10.00	10.00	10.00	10.00	10.00
Ani/Veg Fat	4.59	4.63	4.75	4.91	5.06	5.22
Limestone	1.65	1.65	1.64	1.63	1.62	1.61
Dicalcium Phosphorus 21 P	1.06	1.06	1.06	1.06	1.06	1.07
DL Methionine Premix	0.53	0.53	0.53	0.52	0.52	0.52
MCBF8	0.50	0.50	0.50	0.50	0.50	0.50
Pelleting Binding Agent	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.37	0.37	0.35	0.33	0.31	0.29
BMD	0	0.05	0	0	0	0
Lysine HCl	0	0	0	0	0	0
Total	100	100	100	100	100	100
Calculated nutrient composition on an as fed basis						
ME (kcal/kg)	3100	3100	3100	3100	3100	3100
Crude Protein (%)	21.50	21.50	21.50	21.50	21.50	21.50
Calcium (%)	0.87	0.87	0.87	0.87	0.87	0.87
Available Phosphorus (%)	0.44	0.44	0.44	0.44	0.44	0.44
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18
Digestible Tryptophan	0.18	0.18	0.23	0.23	0.23	0.23
Digestible Threonine	0.82	0.82	0.82	0.82	0.84	0.84
Digestible Methine and Cystine (%)	0.87	0.87	0.87	0.87	0.87	0.87
Digestible Lysine	1.16	1.16	1.17	1.18	1.20	1.21

a: The brown seaweed product name supplied by Acadian Sea Plants, ME=metabolizable energy, DL Methionine Premix is comprised 50% DL Methionine and 50% ground corn. MCBS7 is broiler vitamin premix comprised of: Vitamin A 0.156%, Vitamin D3 premix 32.00%, Vitamin E 2.00%, Vitamin K 0.194%, Riboflavin 0.215%, DL Ca-pantothenate 0.6%, Vitamin B12 0.46%, Niacin 0.808%, Folic acid 2.20%, Choline chloride 26.70%, Biotin 6.00%, Pyridoxine 0.109%, Thiamine 0.082%, Manganous Oxide 4.00%, Zinc oxide 3.05%, Copper sulfate 1.28%, Selenium premix 1.485%, Ethoxyquin 1.66%, Ground corn 7.001%, Ground limestone 10.00%.

Table 3.4: Nutrient analysis of grower diets fed in Tasco trials 1 and 2

Parameter	Basal	Med	0.25% Tasco	0.50% Tasco	0.75% Tasco	1.00% Tasco
Dry Matter (%)	87.24	87.37	87.90	87.83	87.83	88.88
Crude Protein (%)	22.34	22.00	22.02	21.80	22.34	21.67
Calcium (%)	0.92	0.96	0.93	0.94	0.93	0.94
Potassium (%)	0.94	0.98	0.98	1.01	0.98	0.98
Magnesium (%)	0.16	0.17	0.17	0.17	0.17	0.17
Phosphorous (%)	0.58	0.60	0.59	0.60	0.60	0.59
Sodium (%)	0.17	0.18	0.16	0.17	0.17	0.17
Copper (ppm)	21.54	22.70	19.06	23.34	20.60	20.32
Manganese (ppm)	131.07	136.21	136.16	137.09	136.34	131.19
Zinc (ppm)	124.62	127.26	129.69	143.64	137.33	137.12
Crude Fat (%)	6.84	6.59	6.72	6.98	7.08	7.17

Table 3.5: Diet formulations of finisher diets (day 24-35) fed in Tasco trials 1 and 2

Ingredients	Basal	Med	0.25% Tasco	0.50% Tasco	0.75% Tasco	1.00% Tasco
Corn	49.12	49.02	48.72	48.32	47.92	47.52
Soybean Meal	31.42	31.44	31.46	31.49	31.53	31.56
Tasco[a]	0	0	0.25	0.50	0.75	1.00
Wheat	10.00	10.00	10.00	10.00	10.00	10.00
Ani/Veg Fat	5.19	5.22	5.33	5.48	5.62	5.76
Limestone	1.50	1.50	1.49	1.48	1.47	1.46
Dicalcium Phosphorus 21 P	0.90	0.90	0.91	0.91	0.91	0.91
DL Methionine Premix	0.49	0.49	0.49	0.49	0.48	0.48
MCBF8	0.50	0.50	0.50	0.50	0.50	0.50
Pelleting Binding Agent	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.38	0.38	0.36	0.34	0.32	0.30
BMD	0	0.05	0	0	0	0
Lysine HCl	0	0	0	0	0	0
Total	100	100	100	100	100	100
Calculated nutrient composition on an as fed basis						
ME (kcal/kg)	3200	3200	3200	3200	3200	3200
Crude Protein (%)	19.50	19.50	19.50	19.50	19.50	19.50
Calcium (%)	0.78	0.78	0.78	0.78	0.78	0.78
Available Phosphorus (%)	0.39	0.39	0.39	0.39	0.39	0.39
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18
Digestible Tryptophan	0.21	0.21	0.21	0.21	0.21	0.21
Digestible Threonine	0.74	0.74	0.74	0.74	0.74	0.74
Digestible Methine and Cystine (%)	0.80	0.80	0.80	0.80	0.80	0.80
Digestible Lysine	1.02	1.02	1.02	1.02	1.02	1.02

a: The brown seaweed product name supplied by Acadian seaplants, ME=metabolizable energy, DL Methionine Premix is comprised 50% DL Methionine and 50% ground corn. MCBS7 is broiler vitamin premix comprised of: Vitamin A 0.156%, Vitamin D3 premix 32.00%, Vitamin E 2.00%, Vitamin K 0.194%, Riboflavin 0.215%, DL Ca-pantothenate 0.6%, Vitamin B12 0.46%, Niacin 0.808%, Folic acid 2.20%, Choline chloride 26.70%, Biotin 6.00%, Pyridoxine 0.109%, Thiamine 0.082%, Manganous Oxide 4.00%, Zinc oxide 3.05%, Copper sulfate 1.28%, Selenium premix 1.485%, Ethoxyquin 1.66%, Ground corn 7.001%, Ground limestone 10.00%.

Table 3.6: Nutrient analysis of finisher diets fed in Tasco trials 1 and 2

Parameter	Basal	Med	0.25% Tasco	0.50% Tasco	0.75% Tasco	1.00% Tasco
Dry Matter (%)	86.62	87.16	87.38	87.36	87.14	87.37
Crude Protein (%)	19.58	20.15	19.78	19.95	21.18	20.52
Calcium (%)	0.84	0.85	0.86	0.85	0.85	0.84
Potassium (%)	0.84	0.83	0.85	0.86	0.86	0.87
Magnesium (%)	0.16	0.15	0.16	0.16	0.16	0.16
Phosphorous (%)	0.53	0.54	0.54	0.55	0.54	0.54
Sodium (%)	0.17	0.17	0.17	0.18	0.18	0.17
Copper (ppm)	19.06	19.61	18.58	21.51	20.34	21.27
Manganese (ppm)	123.72	130.12	128.13	131.68	130.21	128.75
Zinc (ppm)	124.22	131.07	127.08	130.47	135.05	131.25
Crude Fat (%)	8.16	7.88	7.41	7.67	7.80	7.96

3.3.3 Growth performance measurements

The birds were weighed in batches to determine the total weight of all birds in each pen on days 0, 14, 24, and 35. The weight of the feed remaining in the feeder was recorded on these days to determine feed consumption for each growth phase. On day 14, the feed was changed from a starter mash feed to a pelleted grower feed. On day 24, the diets were changed to a pelleted finisher feed.

Production parameters were calculated for each phase according to the following calculations:

Body weight gain (BWG)= Body weight of latest day-body weight of previous weigh day.

Feed intake (FI)= Feed consumed/number of birds in a pen.

Feed conversion ratio (FCR)= Feed intake/BWG.

If mortalities occurred during a growth phase, then feed intake would be calculated until the time the mortality occurred. For the remainder of the growth phase, the new number of birds would be used after the mortality took place. The total amount of feed ingested during this growth phase would be the result of combining the feed intakes before and after the mortality took place.

3.3.4 Digestive tract measurements

Examination of the digestive tract was conducted on days 24 and 35 of Tasco trial 1 and on days 23 and 34 for Tasco trial 2. Two birds from each pen (96 birds sampled total on each day) were euthanized via cervical dislocation, which was conducted by personnel of apt qualification. The gastrointestinal tract (GI) was removed from each bird and small intestine length was measured for intestinal index. The intestinal index calculation was (intestinal length (cm)/body weight of chicken (g))*(100%). Ileal tissue sample from each broiler was collected from the center of the ileum and preserved in formalin until histological procedures are conducted. Gonads were observed to determine the sex of the sampled birds as described by Cutting et al. (2012).

3.3.4.1 Ileal lesion scoring

The ileum of each GI tract was identified by specific landmarks, starting at the Meckel's diverticulum and ending where the ceca attach to the digestive tract (ileocecal junction). Each ileum was assessed for lesions according to the scoring system established by Shojadoost et al. (2012). The scoring system ranged from a scale of 0 to 6, with higher scores representing more intense damage with an increased number of hemorrhagic lesions present.

3.3.4.2 Ileal histomorphology analysis

Histological slides of the formalin-fixed ileal tissue samples were prepared by the Animal Health Laboratory, Agriculture and Food Operations Branch (Nova Scotia Department of Agriculture, Truro, NS, Canada). These formalin-fixed tissues were embedded in paraffin wax according to the procedure described by Bullerwell et al. (2016). Samples were dehydrated using a series of graded alcohol baths. The alcohol was effaced from the samples using xylene found within a Tissue-Tek VIP (Sakura Finetek USA inc., Torrance, CA, USA). The samples were then infiltrated with paraffin wax via Tissue-Tek VIP (Sakura Finetek USA inc., Torrance, CA, USA). A 5um cross-section was cut from the sample, and placed in a 35.5˚C water bath. The warmed sample was mounted to the slide and stained with hematoxylin and eosin.

Once the samples were fixed to the slides, they were placed under a Leica DM 750 microscope with an ICC50 camera connected to a laptop with LAS EZ software. A digital image of each sample was taken at 40x magnification. An image of a 0.01mm micrometer was used as a reference for setting up the scalebar for the software used. ImageJ software was used to measure the magnified structures of the fixed tissue in relation to the scale bar. Measurements acquired were villus length, villus width, crypt depth, intestinal wall thickness, and villus area. To determine villus surface area, the equation of Sakamoto et al. (2000), as referenced by Santos et al. (2005) was used. The villus surface area calculation used was (2*3.14)*(villus width/2)*(villus length), where the 3.14 in this equation represents pi as the software used to calculate villus surface area (Microsoft Excel) does not have the pi unit available in the equations function. Ten villi were measured per sample.

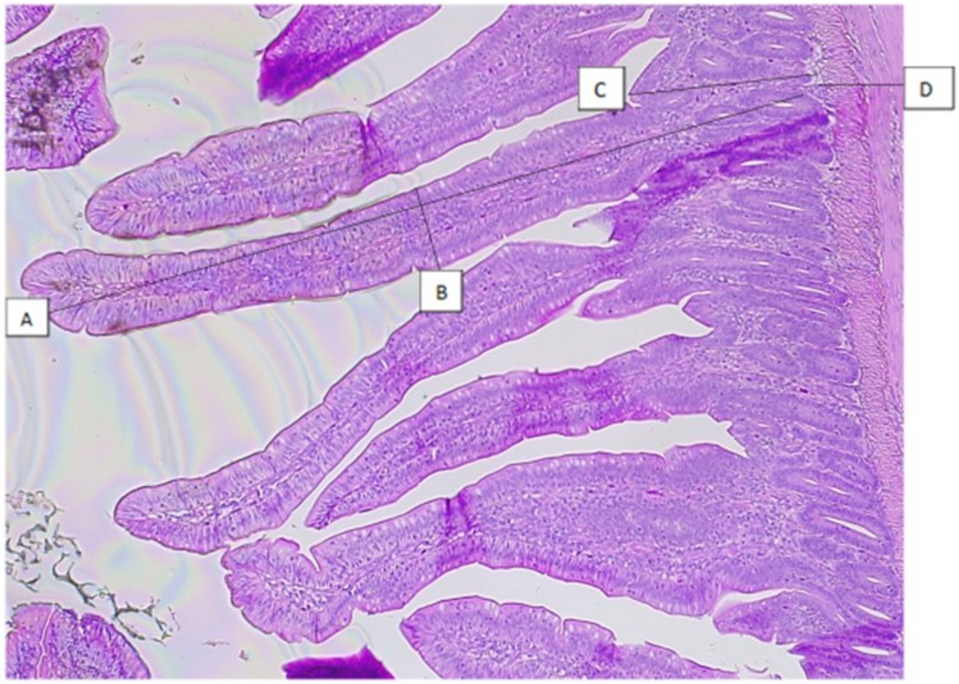

Figure 3.1: Points of measurement for histological analysis of chicken intestinal wall components: (A) villus length, (B) villus width, (C) crypt depth, (D) intestinal wall thickness.

3.3.4.3 Villus goblet cell count

The materials required for goblet cell counts were the same as the materials used in the histological analysis portion of the study, including the sample slides. An image of the tip of a villus of a histological slide was captured at 400x magnification with the use of the LAS EZ software package. Images of three villi tips were captured per sample and placed in ImageJ to perform goblet cell counts with the use of the cell counter function under the plugin command of ImageJ (Muftuoglu et al., 2011). For each new image, the cell counter was reset, initialized, and counter type was selected before placing counters on the image. Goblet cell counts per 100µm^2 per sample were calculated as: (# goblet cells/villus surface area of the image) *100. The average of the goblet cell counts per 100µm^2 was calculated rounding to the nearest whole number.

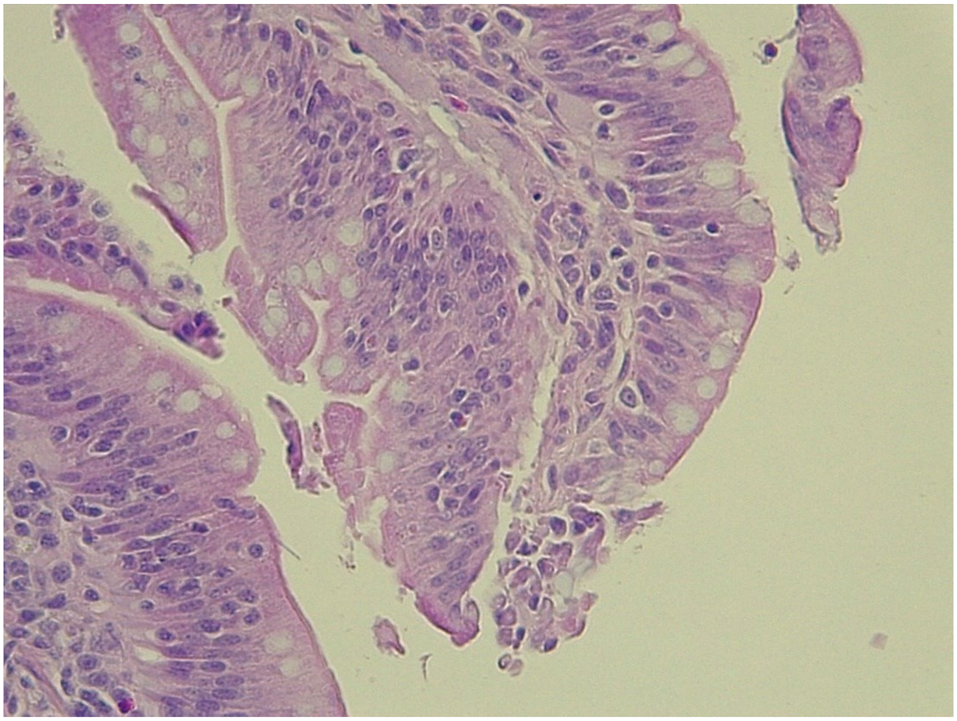

Figure 3.2: Example of image used for goblet cell counts in chicken ileal villus tips.

3.3.5 Statistical analysis

All growth data was analyzed via One-Way ANOVA with repeated measures, using mixed models through the PROC GLIMMIX package of the SAS software (version 9.4, 2012, SAS Institute Inc., Cary, NC, USA) with pens as the experimental unit. Treatment, bird age, and the interaction between treatment and bird age were fixed effects. Residuals were tested for normality as this indicated whether the assumptions from ANOVA were accurate. Least square means were determined using the LSMeans statement of SAS. If a main treatment effect was shown to be significantly different, PDMIX 800 software of SAS was utilized to determine pairwise comparisons via Tukey's pair-wise test. For the intestinal histological, intestinal index, goblet cell counts, and intestinal length data, a Two-Way ANOVA was conducted. This was done using mixed models with the PROC GLIMMIX package of the SAS software with treatment, sex, and treatment/sex interaction as the main fixed effects. The influence of the sex of the broilers was not taken into consideration for growth data analysis as it was impossible to definitively verify the sex of the birds weighed without examining internal gonadal symmetry. Least square means were determined using the LSMeans statement of SAS. The same software and codes that were used to determine pairwise comparisons for the growth data were also used for the intestinal data if a difference between treatment and/or sex was detected. For the intestinal ileum lesion score data, a Chi square test of independence was conducted, with feed treatment and lesion score as the categorical variables of interest. This was performed using the Chi square test option in SPSS statistics software (IBM SPSS Statistics for Windows, Version 26.0). The p-value of significance was 0.05.

3.4 Results

The growth data in Table 3.7 exhibit that the first 14 days showed that the feed treatment did not have a differential impact on observed growth parameters of the broiler chickens ($p>0.05$). On day 25, chickens that had consumed the 0.25% Tasco diet weighed more than chickens that had consumed the basal diet ($p<0.05$). Broilers fed all of the other dietary treatments did not experience differences in growth parameters to each other during the grower period ($p>0.05$). On day 34, broilers that had consumed the 1.00% Tasco diet were heavier than broilers that consumed the basal and medicated diets ($p<0.05$). The 1.00% Tasco diet influenced BWG, as broilers fed the 1.00% Tasco diet had a greater BWG compared to the medicated diet-fed chickens ($p<0.05$) during the finisher period of the trial. During the finisher period, the other Tasco diet-fed broilers were similar basal and medicated fed birds in terms of body weight and BWG ($p>0.05$).

The inclusion of Tasco in diets did not cause changes in feed intake or FCR during every growth phase of the trial.

The growth data in table 3.8 conveyed that during the starter period, broilers fed the 1.00% Tasco diet were more efficient with the feed they consumed when compared to the medicated and 0.25% Tasco diet broilers ($p<0.05$). All other growth parameters observed during the starter period were not affected by feed treatment ($p>0.05$). During the grower phase, the feed did not influence measured growth parameters ($p>0.05$). During the finisher phase of the growth trial, 0.75% and 1.00% Tasco-fed broilers were heavier compared to basal, 0.25%, and 0.50% Tasco diet-fed chickens ($p<0.05$). The 0.75% Tasco diet-fed broilers were also heavier than the medicated diet fed broilers at the end of the finisher phase ($p<0.05$). Feed treatment also affected BWG during the finisher phase as 0.75% and 1.00% Tasco diet-fed broilers gained more weight compared to 0.25% Tasco-fed broilers ($p<0.05$) during this phase. The 0.75% Tasco diet fed broilers gained more weight when compared to the 0.50% Tasco fed birds as well ($p<0.05$). During the finisher growth stage, 1.00% Tasco-fed broilers consumed more feed compared to the basal diet fed birds ($p<0.05$), but all other broilers ingested around the same amount of feed during this phase ($p>0.05$). The 0.25% and 0.50% Tasco diet fed broilers were not bigger than the basal and medicated diet fed broilers and did not gain significantly more weight, but they were not smaller or gain less weight than either the basal or medicated diet fed broilers ($p>0.05$).

Table 3.7: Growth performance and related parameters of broilers fed dietary inclusion of Tasco from 0.25-1.00% for a full production cycle in Tasco trial 1

Treatments	Basal	Med	0.25%	0.50%	0.75%	1.00%	SEM	p-value
Starter (D0-D14)								
BW D0(g)	39	39	39	38	39	38	0.3	0.30
BW D14(g)	374	391	406	401	365	400	12.3	0.15
BWG(g)	342	353	367	363	326	361	9.0	0.02
FI(g)	476	483	524	503	479	500	24.0	0.70
FCR	1.39	1.38	1.43	1.39	1.47	1.39	0.030	0.35
Grower (D14-D25)								
BW D25(g)	1200^{b}	1209^{ab}	1267^{a}	1242^{ab}	1203^{b}	1257^{ab}	12.3	0.0003
BWG(g)	828	818	861	841	838	857	9.0	0.009
FI(g)	1147	1172	1215	1181	1218	1219	23.98	0.18
FCR	1.39	1.43	1.41	1.40	1.45	1.42	0.030	0.78
Finisher (D25-D34)								
BW D34(g)	2165^{c}	2154^{c}	2241^{bc}	2204^{abc}	2189^{bc}	2259^{a}	12.3	<0.0001
BWG(g)	964^{ab}	945^{b}	974^{ab}	962^{ab}	987^{ab}	1003^{a}	9.0	0.0004
FI (g)	1459	1413	1514	1476	1472	1490	24.0	0.09
FCR	1.51	1.50	1.56	1.53	1.49	1.49	0.030	0.65

Body weight gain (BWG)= Body weight of latest day-body weight of previous weigh day.
Feed intake (FI)= the sum of all recorded feed addition days-feed weight back of last day of feed period/ number of birds.
Feed conversion ratio (FCR)= Feed intake/BWG.
Standard error of the mean (SEM)= standard deviation/number of birds.

Table 3.8: Growth performance and related parameters of broilers fed dietary inclusion of Tasco from 0.25-1.00% for a full production cycle in Tasco trial 2

Treatments	Basal	Med	0.25%	0.50%	0.75%	1.00%	SEM	p-value
Starter (D 0-D14)								
BW D0(g)	37	38	38	38	38	38	17.1	1.00
BW D14(g)	368	359	355	378	392	398	17.1	0.40
BWG(g)	331	321	318	340	355	360	12.0	0.07
FI(g)	454	449	454	465	481	459	18.9	0.87
FCR	1.37^{ab}	1.40^{a}	1.43^{a}	1.37^{ab}	1.36^{ab}	1.27^{b}	0.02	0.0003
Grower (D14-D25)								
BW D24(g)	1076	1080	1056	1083	1145	1128	17.1	0.002
BWG(g)	708	721	701	706	753	730	12.0	0.03
FI(g)	947	952	960	962	1020	997	18.9	0.05
FCR	1.34	1.32	1.37	1.36	1.36	1.37	0.02	0.67
Finisher (D25-D35)								
BW D35(g)	2164^{c}	2190^{bc}	2130^{c}	2160^{c}	2287^{a}	2263^{ab}	17.1	<0.0001
BWG(g)	1087^{abc}	1110^{abc}	1074^{c}	1076^{bc}	1142^{a}	1136^{ab}	12.0	<0.0001
FI(g)	1737^{b}	1774^{ab}	1755^{ab}	1763^{ab}	1832^{ab}	1846^{a}	18.9	0.0002
FCR	1.60	1.60	1.63	1.64	1.60	1.63	0.02	0.67

Body weight gain (BWG)= Body weight of latest day-body weight of previous weigh day.
Feed intake (FI)= the sum of all recorded feed addition days-feed weight back of last day of feed period/ number of birds.
Feed conversion ratio (FCR)= Feed intake/BWG.
Standard error of the mean (SEM)= standard deviation/number of birds.

Table 3.9 exhibited that the frequency of intense lesions on the ileum of broilers was not affected by diet treatment as a majority of birds examined did not show any lesions in Tasco trial 1 (p>0.05).

Table 3.10 showed that feed treatment in Tasco trial 2 had similar effects on the frequency of intense lesions on the ileum of broilers as feed treatment did in Tasco trial 1 (p>0.05).

Table 3.11 conveyed that all of the intestinal health performance and health parameter data collected in Tasco trial 1was not influenced by the inclusion of Tasco in broiler diets as the intestinal micromorphological parameters of broilers fed Tasco diets were similar to broilers fed the basal and medicated diets (p>0.05). The Tasco diet fed broilers also did not have a higher number of goblet cells per 100μm^2 of villi compared to the basal and medicated diet fed broilers ($p>0.05$). For a majority of the data collected, sex of the broilers did not impact histomorphology of the broiler's intestinal tract; except for the villus length as the 1.00% Tasco fed male birds had longer villi than the female broilers fed 0.75% Tasco diet.

Based on the intestinal index data shown in Table 3.12, it appears that feed treatment did not impact intestinal index on day 25 of Tasco trial 1 ($p>0.05$), but the sex of the broilers did. The intestinal indexes of the females fed basal, 0.75% Tasco, and 1.00% Tasco diets were larger than males fed 0.25% Tasco diets ($p<0.05$). The intestinal tract index of these females was not larger than the indexes of males fed 0.50% Tasco diets or any other bird of either sex fed any of the other diets that was not previously stated ($p>0.05$). For the intestinal index data collected on day 34, neither feed treatment or sex of the bird caused an alteration of intestinal index ($p>0.05$).

Based on the data exhibited in Table 3.13, a majority of the ileal performance data collected was not differentiated due to the inclusion of Tasco in broiler diets ($p>0.05$). The only ileal morphological data where sex had a difference was intestinal wall thickness, where the female broilers fed the 0.75% Tasco diet had thicker intestinal walls compared to the male broilers fed 0.75% Tasco ($p<0.05$). The females fed 0.75% Tasco diets had thicker intestinal walls than male broilers fed basal and medicated diets, as well as females fed basal, 0.25% Tasco, and 0.50% Tasco diets ($p<0.05$). The inclusion of Tasco in broiler diets did not cause a change in the number of goblet cells per 100um^2 of intestinal villi, compared to the broilers fed basal and medicated diets ($p>0.05$).

Based on the intestinal index data exhibited in Table 3.14 in Tasco trial 2 on day 24, feed treatment did not alter broiler intestinal index ($p>0.05$), but the sex of the broilers did. Females fed the medicated feed had larger intestinal indexes than males fed the basal, medicated, 0.25% Tasco, 0.50% Tasco, and 0.75% Tasco diets ($p<0.05$). All other females and males fed 1.00% Tasco diet did not have distinctly different intestinal indexes compared to the females fed medicated diets ($p>0.05$). Similar to day 24 results, intestinal indexes collected on day 35 did not differ due to diet treatment ($p>0.05$), but were affected by the sex of the chicken. Female broilers fed Medicated, 0.25% Tasco, and 0.75% Tasco diets had larger intestinal indexes than males fed 0.75% Tasco diets ($p<0.05$). All other males fed other diets did not have larger or smaller intestinal indexes compared to females on day 35 ($p>0.05$).

Table 3.9: Ileal lesion scores for broiler chickens fed experimental diets in Tasco trial 1

Treatment	# of birds with score 0	# of birds with score 1	# of birds with score 2	# of birds with score 3	# of birds with score 4	# of birds with score 5	# of birds with score 6	Average score	p-value
Day 25									
Basal	13	2	1	0	0	0	0	0.25	0.76
Med	10	2	3	0	1	0	0	0.75	
0.25%	11	2	2	1	0	0	0	0.56	
0.50%	10	4	2	0	0	0	0	0.50	
0.75%	11	3	2	0	0	0	0	0.44	
1.00%	13	3	0	0	0	0	0	0.19	
Day 34									
Basal	7	5	2	1	1	0	0	1	0.83
Med	8	4	2	2	0	0	0	0.88	
0.25%	7	7	2	0	0	0	0	0.69	
0.50%	9	6	0	1	0	0	0	0.56	
0.75%	9	3	2	2	0	0	0	0.81	
1.00%	8	6	0	2	0	0	0	0.75	

Table 3.10: Ileal lesion scores for broiler chickens fed experimental diets in Tasco trial 2

Treatment	# of birds with score 0	# of birds with score 1	# of birds with score 2	# of birds with score 3	# of birds with score 4	# of birds with score 5	# of birds with score 6	Average score	p-value
Day 24									
Basal	7	8	1	0	0	0	0	0.62	0.68
Med	11	4	1	0	0	0	0	0.38	
0.25%	9	6	1	0	0	0	0	0.50	
0.50%	12	3	1	0	0	0	0	0.31	
0.75%	6	9	1	0	0	0	0	0.69	
1.00%	10	5	1	0	0	0	0	0.44	
Day 35									
Basal	2	14	0	0	0	0	0	0.88	0.44
Med	4	12	0	0	0	0	0	0.75	
0.25%	3	11	2	0	0	0	0	0.94	
0.50%	1	14	1	0	0	0	0	1.00	
0.75%	5	10	1	0	0	0	0	0.75	
1.00%	2	14	0	0	0	0	0	0.88	

Table 3.11: Ileal morphological data of broiler chickens on day 34 of Tasco trial 1

Treatments	Basal		Med		0.25%		0.50%		0.75%		1.00%		SEM	p-value
Sex	M	F	M	F	M	F	M	F	M	F	M	F		
VL (µm)	922[ab]	788[ab]	777[ab]	680[ab]	910[ab]	857[ab]	912[ab]	810[ab]	818[ab]	676[b]	1036[a]	759[ab]	64.0	0.62
VW (µm)	221	282	165	206	267	228	228	222	222	216	226	256	28.0	0.55
CD (µm)	186	193	167	142	172	156	187	184	182	172	178	180	21.0	0.98
IWT (µm)	361	304	329	254	280	263	316	301	340	238	336	294	45.0	0.92
VSA (um^2)	6300	6971	3982	4338	7614	6200	6610	5709	5608	4498	7204	5997	744.0	0.63
GCC ($100um^2$)	25	18	20	24	20	16	18	16	22	23	18	16	3.0	0.61

VL=villus length, VW=villus width, CD=crypt depth, IWT=intestinal wall thickness, VSA=villus surface area, GCC=goblet cell count

Table 3.12: Intestinal indices (%body weight) of broiler chickens in Tasco trial 1

Treatments	Basal		Med		0.25%		0.50%		0.75%		1.00%		SEM	p-value
Sex	M	F	M	F	M	F	M	F	M	F	M	F		
Day 25	12.39[ab]	14.01[a]	12.52[ab]	13.36[ab]	11.87[b]	13.42[ab]	13.78[a]	13.30[ab]	13.30[ab]	13.90[a]	12.57[ab]	13.81[a]	0.39	0.13
Day 34	8.78	9.12	8.40	9.32	7.93	8.83	7.90	8.57	8.16	8.93	7.83	8.84	0.30	0.90

Table 3.13: Ileal morphological data of broiler chickens on day 35 of Tasco trial 2

Treatments	Basal		Med		0.25%		0.50%		0.75%		1.00%		SEM	p-value
Sex	M	F	M	F	M	F	M	F	M	F	M	F		
VL (µm)	824	792	743	788	908	780	804	890	804	993	929	826	79.0	0.39
VW (µm)	233	250	293	239	207	272	194	190	262	239	228	216	43.0	0.82
CD (µm)	134	164	107	152	142	156	134	158	130	150	137	128	13.0	0.45
IWT (µm)	275[b]	266[b]	266[b]	312[ab]	306[ab]	264[b]	318[ab]	272[b]	274[b]	364[a]	281[ab]	330[ab]	16.0	0.005
VSA (um^2)	6059	6112	6717	5982	6102	6676	4664	5214	6457	7101	6590	5596	1260	0.97
GCC ($100um^2$)	18	28	18	25	18	25	22	25	18	24	25	26	2.0	0.40

VL=villus length, VW=villus width, CD=crypt depth, IWT=intestinal wall thickness, VSA=villus surface area, GCC=goblet cell count

Table 3.14: Intestinal indices (%body weight) of broiler chickens in Tasco trial 2

Treatments	Basal		Med		0.25%		0.50%		0.75%		1.00%		SEM	p-value
Sex	M	F	M	F	M	F	M	F	M	F	M	F		
Day 24	13.05^{b}	15.42^{ab}	13.62^{b}	16.77^{a}	13.39^{b}	15.43^{ab}	13.64^{b}	14.71^{ab}	13.50^{b}	15.92^{ab}	14.31^{ab}	15.11^{ab}	0.62	0.43
Day 35	7.25^{ab}	7.93^{ab}	7.40^{ab}	8.17^{a}	7.43^{ab}	8.12^{a}	7.42^{ab}	7.87^{ab}	6.71^{b}	8.13^{a}	7.45^{ab}	7.42^{ab}	0.28	0.22

3.5 Discussion

The inclusion of Tasco in broiler feed improved growth performance compared to standard broiler feed and feed containing BMD, an antibiotic growth promoter ($p<0.05$). Among the Tasco diets in Tasco trial 1, 1.00% Tasco inclusion level improved BW and BWG compared to the 0.25% and 0.75% Tasco levels at the end of the finisher phase ($p<0.05$). At the end of the finisher phase of Tasco trial 2, the 1.00% Tasco diet improved BW and BWG compared to the 0.25% and 0.50% Tasco level diets ($p<0.05$).

Wiseman (2012) examined Tasco as a prebiotic candidate for improving the gut health of broiler chickens. The impact of Tasco on broiler growth performance and histomorphology of the intestinal tract was examined. Similar to this study, they found that Tasco is an effective alternative to in-feed antibiotic growth promoters as in one of their growth trials broilers fed Tasco diets had increased or similar BW and BWG as broilers fed feed containing the antibiotic growth promoter virginiamycin ($p>0.05$). They found that including Tasco in broiler feed can increase BW and BWG of broilers as early as day 14 ($p<0.05$). This trend continued throughout the remaining growth stages of the study, with only broilers fed 1.0% and 1.5% Tasco diets gaining similar weights to broilers fed a basal diet ($p>0.05$). However, their body weights were still greater than the basal fed birds ($p<0.05$).

The improved growth performance of the broilers fed 1% Tasco in this study could be attributed to the prebiotic properties of Tasco stimulating the formation of more beneficial microbial colonies as the chickens grew (Shi et al., 2019). This could have increased the production of beneficial compounds via microbial fermentation that enhanced growth performance and general immunity. Some of these bacteria could convert carbohydrates such as the glucose in Tasco into metabolites, such as lactic acid, acetic acid, butyric acid, ethanol, or carbon dioxide (Forte et al., 2018). Some of these metabolites, such as acetic acid and butyric acid, are known as short-chained fatty acids (SCFA). SCFA's are acids that are produced by microbials within the intestinal tract of monogastric animals, and can have beneficial influences on digestive tract performance (Yacoubi et al., 2016). One example being serving as an energy source to intestinal epithelial cells and encourage cell proliferation (Yacoubi et al., 2016). SCFA's are also capable of reducing intestinal pH, which creates an unfavorable environment for pathogenic microbial activity. This lowers competition for nutrients found in other components of the feed that the chicken can use for growth and maintain immune system function (Forte et al., 2018).

The results of this study, fall in line with other studies investigating brown seaweed's effect on broiler growth and intestinal health. For example, Mohammadigheisar et al (2020), investigated the impact of a seaweed blend comprised of brown, red, and green seaweeds on broiler growth when included in feed at levels of 0%, 0.5%, 1%, and 2%. They observed improved growth performance of broilers with similar weights to the current study. However, they found that seaweed improved growth performance as early as the starter phase ($p<0.05$). The trials conducted in the current study showed that brown seaweed's impact on growth was during the grower phase in Tasco trial 1. This was not repeated in Tasco trial 2. The seaweed blend in the research of Mohammadigheisar et al (2020) did lead to increase in feed intake of birds consuming feed containing the seaweed blend ($p<0.05$). Increased feed consumption could lead to increased nutrient uptake and growth performance, due to increased nutrient volume within the digestive tract (Romero et al., 2011).

In the growth trials conducted for the current study, brown seaweed did not have an impact on feed intake, except for the 1.00% Tasco feed during the finisher phase of Tasco trial 2. The combination of the brown, red, and green seaweed may have produced a chemical composition that was different from that of each of the species individually. This may have altered the tastants, and resulted in a taste that was favored over a standard feed (Collins et al., 2016). The most likely taste quality that would have been influenced by the addition of this blend would be salt, as the seaweed species used in the blend were grown in oceans surrounding Ireland and South Asia. The combination could have caused a variation of the salt tastants in the feed and produced a flavor that is unique to the combination that chickens favor and will consume more of the combination than feed containing each of the species individually (Collins et al., 2016).

Choi et al (2014) investigated brown seaweed by-product and fermented brown seaweed by-product (*Undaria pinnatifida*) in broiler feed evaluating broiler growth performance and blood profiles. While brown seaweed did not produce heavier chickens compared to the control (standard broiler feed) it did improve BWG during the grower and finisher phases ($p<0.05$). Comparatively, broilers grown in the current trial did produce bigger birds. This could indicate that the species of brown seaweed used in Tasco is more applicable to broilers than other brown seaweed species. The chemical composition of *Ascophyllum nodosum* is different from the other seaweed species tested by Choi et al (2014). Factors that can influence chemical compositions of seaweed species include: harvesting time, geographical locations, and seasons (Michalak and Mahrose, 2020).

As seaweeds are considered a rich source of phenolic compounds, minerals, and non-starch polysaccharides, it is imperative to minimize changes in their chemical composition during processing into available products. Certain processing methods can lead to changes in seaweed's properties that can impair its applicability as a feed additive. In the production of Tasco the main processing method performed on this ingredient is sun drying of *A. nodosum* as fresh brown seaweed contains 75-80% water which needs to be removed in order to stabilize it for application as a feed ingredient (Charles et al., 2020). The differences in the effect of brown seaweed on broiler growth between this study and Choi et al (2014), is the processing method used for testing their product was to apply microbial species: *Bacillus subtilis*, *Pediococcus acidilacti*, *Pediococcus pentosaceus*, *Saccharomyces cerevisiae*, and *Aspergillus oryzae* to *U. pinnatifida*. These microbes would ferment the seaweed product, and increase nutrient availability for digestion, due to seaweed's high non-starch polysaccharide content making it difficult to digest.

Different processing methods can alter the physiochemical properties of seaweed such as changing its natural microbial composition, that aid in producing compounds that give seaweed its benefits to broiler performance and health (Olmo et al., 2020).The microbes Choi et al (2014) added to the seaweed may have altered the levels of bioactive components in the brown seaweed, due to interaction with the natural microbial composition of the seaweed or utilizing compounds that are required for replication, that could have been used by the broilers for growth. (Chan et al., 1997). Whereas the sun drying method used in Tasco production may have just targeted seaweed water content, and having little impact on altering other nutritional parameters of *A. nodosum* (Charles et al., 2020).

Acadian Seaplants recommend inclusion levels of Tasco in broiler diets between 0.25% and 0.50% throughout all stages of production for optimum growth performance. However, the results of this study convey that Tasco works optimally at a 1.00% dietary inclusion level. The studies conducted by Acadian Seaplants may have used different species of broilers than the species used in this trial (Ross 308), and different broiler species may require different amounts of Tasco to optimize performance (Evans and Critchley, 2014). Housing environment can also influence nutrient amounts required for optimum performance as a more stressful environment may lead to greater levels of Tasco required to meet optimum performance (Choi et al., 2014). It is possible that the inclusion rate recommended by the company may be minimum amounts required to see an increase in performance as both growth trials conducted for the current study did show that 0.25% and 0.50% inclusion rates of Tasco in broiler feed can lead to adequate or improved growth performance ($p<0.05$). However, 1.00% was the most frequent at improving broiler growth performance ($p<0.05$). Broiler producer will prefer the steady effect of 1.00% inclusion level of Tasco on growth performance of broilers, as it is easier to predict broilers sizes and profit the producer will make (Tallentire et al., 2016). An inclusion rate that may produce bigger birds for one flock but produce smaller broilers in the next is difficult to recommend, as producers will not make a consistent profit and had a difficult time determining expenses (Tallentire et al., 2016). It is recommended that this product be tested on multiple strains during different growth stages of production in different situations based of the results of this study. Observing if the same inclusion levels are best applicable to all broiler species grown in the industry or if certain strains require specific amounts under certain conditions for optimum growth.

Inclusion of Tasco did not have a greater impact on the bulk of ileal morphological parameters and goblet cell content compared to broilers fed standard feed and feed containing BMD ($p>0.05$). Diet and sex of the broilers did seem to have an influence of intestinal wall thickness in Tasco trial 2 ($p<0.05\%$). In Tasco trial 2,0.75% Tasco female broilers had thicker intestinal wall thickness than basal diet-fed broilers, medicated females, female 0.25%Tasco-fed broilers, female 0.50% Tasco-fed broilers, and male 0.75% Tasco-fed broilers. It is possible that the inclusion of Tasco in the diet caused an increase in beneficial microbial activity. This could result in some species of bacteria in the ileum such as *Lactobacillus*, *Bifidobacteria*, and *Bacillus* spp to directly adhere enteric pathogenic microbes or produce compounds that limit pathogenic activity (Mora et al., 2020). This results in lower pathogenic adherence to intestinal epithelial and reduced influence of pathogens on intestinal epithelial integrity, immunity, and inflammation, leading to thicker intestinal walls (Tomaszewska et al., 2018). As the differences seen with Tasco are between female broilers fed lower inclusion levels of Tasco than 0.75%, this indicates that female broilers require higher inclusion levels of Tasco in their diet compared to males to have sufficient intestinal epithelial integrity. This could be due to the difference in nutrient requirements for female broilers from males as their physiology is different, mainly their more complex reproductive system (Jiang et al., 2020). As this was not seen in Tasco trial 1 further research into the influence of sex on diet utilization to determine the mode of action of Tasco included feed between males and female broilers.

When observing sex of broiler alone, in Tasco trial 2, it appears that female broilers contain more goblet cells per 100μm^2 of intestinal villi than males ($p<0.05$). This impact of sex on goblet cell counts was not seen in Tasco trial 1 ($p>0.05$), so the results found in the second growth trial could be due to the selection of birds examined rather than sex having a significant impact on goblet cell populations on the intestinal villi of broilers (Kable et al., 2020).

Goblet cells are the main cells in the intestinal tract that are responsible for mucus production. The number of goblet cells are an indication of immune function in the intestinal tract as the more goblet cells present indicates more mucus is produced (Faderl et al., 2015). Mucus provides the epithelial layer of the intestinal tract with an extra layer of protection from pathogenic microbes and pathogenic metabolites as it contains salts, lipids, and proteins with protective functions. As feeding diets containing Tasco did not increase goblet cell counts compared to the basal or medicated feeds, increased mucus would not likely be a mechanism for adaptation to necrotic enteritis infection as the level of protection would be similar (Quintana-Hayashi et al., 2018).

The sex of broilers could potentially affect intestinal morphology as the female villus length of broilers fed 0.75% Tasco diet were shorter than the villus of males fed 1.00% Tasco treatments in Tasco trial 1. However, the influence of sex on intestinal villus length was not observed again in Tasco trial 2. It is possible that male and female broilers contain different microbial populations. Through microbial activity and compound production via fermentation of feedstuff, these microbes could influence the intestinal system of broilers to modify intestinal morphology and goblet cell content in the intestinal tract (Kable et al., 2020). It is also possible that intestinal morphology could be influenced by sex of broiler, due to the difference in reproductive tracts. Since females have a more complex reproductive tract, they require more nutrient utilization on that aspect of normal function (Jiang et al., 2020). As a result, nutrients that could be used to promote improved intestinal morphology are used to maintain sufficient reproductive performance (Jiang et al., 2020)

There was no difference in ileal lesion scores in relevance to the frequency or intensity of lesions of broilers fed Tasco-included diets compared to broilers fed the basal and medicated diets ($p>0.05$). These results were likely due to the fact that the birds in this study were housed in a bio-secure research facility, which lowered the incidence of pathogens on flock health (Fike et al., 2005). The observed impact of Tasco on intestinal health was only analyzed during the last day of each of the trials. It is possible that Tasco had a more profound impact on intestinal health and activity during earlier stages of growth (Opheim et al., 2016). The early improvement of intestinal morphology may have led to improved growth performance near the end of each of the growth trials due to the boost in intestinal tract performance early on (Opheim et al., 2016).

Wiseman (2012) examined Tasco's impact on intestinal histomorphology. They examined villus height (length), villus width, crypt depth, mucosal depth (intestinal wall thickness), and intestinal villi breakage score. They observed that the addition of Tasco to broiler feed does not improve any of the examined intestinal micromorphological parameters, but did not impair these parameters either (Wiseman, 2012). Many of the observed intestinal histomorphology parameters examined in the study exhibited that neither the inclusion of Tasco or Virginiamycin altered the intestinal morphology of broilers compared to being fed a basal diet ($p>0.05$). The only intestinal histomorphology characteristic examined where Tasco had a difference compared to broilers fed feed containing virginiamycin was in the day 21 villus breakage score, where broilers fed a 2.0% Tasco diet had a lower score than broilers fed a virginiamycin containing feed diet ($p<0.05$). This trend was not seen at either day 35 or 45 of the study, so it cannot be definitively stated that Tasco improves intestinal villi integrity over virginiamycin ($p>0.05$) (Wiseman, 2012). Many of the results found in their study are similar to the results of the overlapping parameters examined in the current study as Tasco's ability to improve growth and maintain intestinal morphology health under standard broiler grower parameters was observed in this study.

Mohammadigheisar et al (2020) investigated the impact of a seaweed blend on jejunal histomorphology and found no increase villus length or crypt depth ($p>0.05$). This was similar to the villus lengths obtained at the end of the growth trials during the current study. This indicates that the inclusion of seaweed in broiler feed may lead to improvements to other characteristics in intestinal tract functionality besides intestinal morphology in relation to improved growth performance.

Future controlled bacterial challenge studies may be more likely to demonstrate potential benefits of Tasco on intestinal tract health. It is especially paramount when most research efforts are conducted on seaweed's impact on intestinal health and function, as the birds observed in these trials are often challenged either by pathogenic agents or environmental stressors (Mohammadigheisar et al., 2020). These stressors encourage the birds to utilize the additional nutrients found in seaweed in order to maintain sufficient intestinal health and function (Choi et al., 2014).

When examining the influence of the sex of the broiler on intestinal morphology and health, further research needs to be conducted in order to confirm if microbial populations differ between male and female broilers, and if these alterations impact goblet cell content and broiler intestinal villi morphology (Kable et al., 2020).

3.6 Conclusion

Results from the growth trials did not indicate that there are changes to ileal micromorphological parameters or stimulation of ileal immune function when Tasco is included in the diet at the levels tested. Tasco inclusion did improve growth performance of broiler chickens compared to broilers fed a basal and BMD diets and could be an effective antibiotic alternative to broilers grown under conditions that were used in these studies. When examining how Tasco improved broiler growth performance, future studies should examine its impact on other parts of the intestinal tract apart from the ileum as Tasco may have a more significant impact on morphological and immune parameters in earlier or later stages of the digestive process. These improvements could explain how the improvement of growth of broilers fed diets containing Tasco occurred when their ileal micromorphological and ileal immunity parameters are similar to broilers fed basal and in-feed antibiotic containing feeds.

CHAPTER 4 EVALUATION OF SYNERGY BETWEEN BROWN SEAWEED AND BUTYRIC ACID COMBINATIONS FOR IMPROVED BROILER CHICKEN PRODUCTION

4.1 Abstract

Few alternatives to in-feed antibiotics have proven to be as effective as traditional in-feed antibiotics at maintaining intestinal health and growth performance of broiler chickens. The purpose of this study was to investigate the effect of a combination of brown seaweed (*Ascophyllum nodosum*; Tasco) and butyric acid in broiler feed on growth and intestinal tract health. The diets examined were a basal diet, BMD included diet, 0.50% Tasco diet, 1.00% Tasco diet, Proformix (0.06% for starter, 0.04% for grower and finisher) included diet, 1.00% Tasco + Proformix diet, and 0.50% Tasco + Proformix diet. Mixture diet-fed birds did not perform better than broilers fed any of the other dietary treatment ($p>0.05$). The inclusion of commercially available Tasco or butyric acid (Proformix) individually or in combination in broiler diets, did not improve intestinal health indicators or morphology when compared to broilers fed the basal and medicated diets ($p>0.05$). Further research is required to confirm if combining Tasco and Proformix in broiler feed can improve broiler production traits and health as the results of this study may have been influenced by the broilers rearing environment. Investigations should observe the influence of the mixture of butyric acid and brown seaweed in *Clostridium perfringens* challenged environment. The mixture of brown seaweed and butyric acid may be effective at limiting *C. perfringens* activity and maintain adequate growth performance, but require the presence of pathogens for beneficial nutrients and compounds in these ingredients to be utilized.

4.2 Introduction

The effort to become antibiotic-free within the broiler industry, has also led to an increased frequency of diseases that traditional in-feed antibiotics once kept in check. One of these diseases is necrotic enteritis, an intestinal disease that is caused by *Clostridium perfringens*, and is most prominent in the ileum that is characterized by structural breakdown of the intestinal tract (Jang et al., 2012; Namkung et al., 2011). Destruction of key intestinal tissue results in reduced digestive tract function and higher energy and nutrient requirements for broiler chicken growth. If this disease is present at elevated levels in broiler flocks, it can lead to high mortality rates, and significant costs for the broiler industry (Timbermont et al., 2010). The increased incidence of this disease has led to research efforts directed toward evaluation of in-feed antibiotic alternatives that can eliminate or limit necrotic enteritis activity in the intestinal tract of broiler chickens.

Short chain fatty acids have been evaluated as candidates for replacement of antibiotics. One of the most prominent short chain fatty acids evaluated is butyric acid and its derivatives, butyrate and tributyrin (Bedford and Gong, 2018). Butyrate is a short chain fatty acid (SCFA) produced within the intestinal tract of animals and humans via microbial fermentation. Butyrate possesses antimicrobial capabilities, making it an attractive feed additive to utilize for the control of pathogenic bacteria in food animal production (Bedford and Gong, 2018).

Namkung et al. (2011) investigated the inhibitory effects of butyric acid glycerides in combination with other butyrate derivatives against *Salmonella typhimurium* and *Clostridium perfringens* via *in vitro* analysis. They found that a mixture of 50% η-butyric acid, and 50% monobutyrin at a concentration of 3000 ppm led to 90%

inhibition of *C. perfringens*. They also observed that η-butyric acid by itself at a concentration of 3000 ppm can lead to 100% inhibition of *S. typhimurium*. However, this level of inclusion of butyric acid derivatives cannot be included in Canadian broiler feeds as the maximum amount of butyric acid and its derivatives permitted to be included in broiler feed is 100ppm (CFIA Administrative Schedule IV and V, 2018).

Most studies investigating the synergy between in-feed antibiotic alternatives in broiler feeds combine alternatives of similar origin and activity in the hopes of achieving an additive effect on broiler growth and health, while being incorporated at lower levels. Few to no studies currently investigate mixtures that have an inter-mutual relationship in relation to the broiler performance (Ayllon et al., 2017).

The objective of this study was to investigate wither or not brown seaweed will have an inter-mutual relationship with butyric acid when incorporated into broiler feed. The hypothesis of this study is that brown seaweed (Tasco) will act as a prebiotic, encouraging the growth and activity of beneficial bacteria located in the ileum; while butyric acid (Proformix) will act as a bactericide and discourage the activity of pathogenic bacteria (Nari and Ghasemi, 2020). This will result in the combination of Tasco and Profomix to improve broiler performance compared to the effects of each individual test ingredient. Verification of the relationship between brown seaweed and butyric acid will be achieved by investigating the impact of feed treatments on broiler growth performance and intestinal health.

4.3 Materials and methods

4.3.1 Broiler housing and caretaking

Two rooms at the Atlantic Poultry Research Centre (Truro, N.S, Canada) were used to house 1248 mixed-sex Ross 308 fast-feathering broiler chickens. Each room had 21 pens holding 26 birds/pen. Each pen was randomly assigned to one of seven feed treatments, resulting in 3 replicate pens/feed treatment/ room, totaling 6 replicates/feed treatment.

Test diets were:

1) Basal diet
2) 0.05% BMD included diet
3) 0.50% Tasco diet
4) 1.00% Tasco diet
5) 0.06% Proformix (starter) and 0.04% Proformix (grower/finisher) diet
6) 1.00% Tasco + 0.06% Proformix (starter) and 0.04% Proformix (grower/finisher) diet
7) 0.5% Tasco + 0.06% Proformix (starter) and 0.04% Proformix (grower/finisher) diet

The test ingredients examined in this study were Tasco and Proformix. Tasco is a *Ascophyllum nodosum* feed ingredient product supplied by Acadian Sea Plants Ltd (30 Brown Ave, Dartmouth, NS B3B 1X8). Proformix (Pro) is a dietary feed ingredient containing 65% butyric acid marketed by Probiotech (6225 Boulevard Choquette, Saint-Hyacinthe, QC J2S 8L2). The remaining 35% of Proformix was comprised of vegetable fat, sodium bicarbonate, lime, and a flavouring agent. The dietary formulations of the dietary treatments examined are reported in Tables 4.1, 4.3, and 4.5. The following study was conducted under approved Animal Care and Use

Committee (ACUC) of Dalhousie University, Nova Scotia, Canada protocol 2019-023.

Test dietary inclusion levels of Tasco investigated in this study were selected based on the results found in Tasco trial 1, with 0.50% considered the low Tasco inclusion level, and 1.00% considered the high Tasco inclusion level. Feed and water were provided *ad libitum*. All feed added was weighed and recorded. Daily health checks were conducted and mortalities were removed, and weighed, as well as the feed in that pen to ensure feed consumption data calculated for that pen was accurate for that period. All mortalities were sent to the provincial veterinary pathology lab for necropsy and cause of death was recorded when determined.

4.3.2 Experimental diets

Table 4.1: Starter (Day 0-14) diet formulations including BMD, Tasco, and Proformix

Ingredient	Basal	Medicated	0.50% Tasco	1.00% Tasco	Proformix	0.50% Tasco + Proformix	1.00% Tasco + Proformix
Corn	51.08	50.97	50.27	49.45	50.95	50.15	49.32
Soybean Meal	41.44	41.45	41.51	41.58	41.46	41.53	41.60
Ani/Veg Fat	2.93	2.97	3.23	3.55	2.97	3.28	3.59
Limestone	1.80	1.80	1.78	1.76	1.80	1.78	1.76
Dicalcium Phosphorus	1.24	1.24	1.24	1.25	1.24	1.24	1.25
MCBF8	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL Methionine Premix	0.59	0.59	0.59	0.58	0.59	0.59	0.58
Salt	0.41	0.41	0.37	0.33	0.41	0.37	0.33
BMD	0	0.05	0	0	0	0	0
Tasco[a]	0	0	0.50	1.00	0	0.50	1.00
Profromix[b]	0	0	0	0	0.06	0.06	0.06
Lysine HCl	0.01	0.01	0	0	0.01	0	0
Total	100	100	100	100	100	100	100
Calculated nutrient composition on a as fed basis							
ME (kcal/kg)	3000	3000	3000	3000	3000	3000	3000
Crude Protein (%)	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Calcium (%)	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Available Phosphorus (%)	0.48	0.48	0.48	0.48	0.48	0.48	0.48
Sodium (%)	0.19	0.19	0.19	0.19	0.19	0.19	0.19
Digestible Tryptophan	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Digestible Threonine	0.89	0.89	0.91	0.92	0.89	0.91	0.92
Digestible Methine and Cystine (%)	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Digestible Lysine	1.28	1.28	1.30	1.32	1.28	1.30	1.32

a: The brown seaweed product name supplied by Acadian seaplants, b: The butyric acid product name supplied by ProBiotech, ME=metabolizable energy, DL Methionine Premix is comprised 50% DL Methionine and 50% ground corn. MCBS7 is broiler vitamin premix comprised of: Vitamin A 0.156%, Vitamin D3 premix 32.00%, Vitamin E 2.00%, Vitamin K 0.194%, Riboflavin 0.215%, DL Ca-pantothenate 0.6%, Vitamin B12 0.46%, Niacin 0.808%, Folic acid 2.20%, Choline chloride 26.70%, Biotin 6.00%, Pyridoxine 0.109%, Thiamine 0.082%, Manganous Oxide 4.00%, Zinc oxide 3.05%, Copper sulfate 1.28%, Selenium premix 1.485%, Ethoxyquin 1.66%, Ground corn 7.001%, Ground limestone 10.00%.

Table 4.2: Nutrient analysis of starter diets containing Tasco, Proformix, and BMD

Parameter	Basal	Medicated	0.50% Tasco	1.00% Tasco	Proformix	0.50% Tasco + Proformix	1.00% Tasco + Proformix
Dry Matter (%)	89.80	90.20	89.70	90.50	89.43	90.76	89.02
Crude Protein (%)	24.13	24.93	24.03	23.65	25.12	23.45	23.88
Calcium (%)	1.14	1.11	1.13	1.08	1.03	1.15	1.15
Potassium (%)	1.05	1.07	1.05	1.04	1.03	1.03	1.06
Magnesium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.19
Phosphorous (%)	0.65	0.62	0.62	0.59	0.59	0.62	0.63
Sodium (%)	0.18	0.17	0.18	0.16	0.14	0.17	0.18
Copper (ppm)	41.74	21.40	22.90	25.88	22.54	21.64	23.60
Manganese (ppm)	144.03	143.07	145.74	128.78	132.50	145.18	142.66
Zinc (ppm)	158.52	148.00	148.18	146.32	131.94	155.07	144.52
Crude Fat (%)	5.56	5.62	5.92	5.94	5.52	6.52	6.26

Table 4.3: Grower (Day 14-25) diet formulations including BMD, Tasco, and Proformix

Ingredient	Basal	Medicated	0.50% Tasco	1.00% Tasco	Profromix	0.50% Tasco + Profromix	1.00% Tasco + Profromix
Corn	44.32	44.22	43.49	42.67	44.24	43.41	42.58
Soybean Meal	36.48	36.49	36.55	36.62	36.49	36.56	36.64
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Ani/Veg Fat	4.59	4.63	4.91	5.22	4.62	4.94	5.25
Limestone	1.65	1.65	1.63	1.61	1.65	1.63	1.61
Dicalcium Phosphorus	1.06	1.06	1.06	1.07	1.06	1.06	1.07
MCBF8	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL Methionine Premix	0.53	0.53	0.52	0.52	0.53	0.52	0.52
Pelleting Agent	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.37	0.37	0.33	0.29	0.37	0.33	0.29
BMD	0	0.05	0	0	0	0	0
Tasco[a]	0	0	0.50	1.00	0	0.50	1.00
Profromix[b]	0	0	0	0	0.04	0.04	0.04
Lysine HCl	0	0	0	0	0	0	0
Total	100	100	100	100	100	100	100
Calculated nutrient composition on a as fed basis							
ME (kcal/kg)	3100	3100	3100	3100	3100	3100	3100
Crude Protein (%)	21.50	21.50	21.50	21.50	21.50	21.50	21.50
Calcium (%)	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Available Phosphorus (%)	0.44	0.44	0.44	0.44	0.44	0.44	0.44
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Digestible Tryptophan	0.18	0.18	0.23	0.23	0.23	0.23	0.23
Digestible Threonine	0.82	0.82	0.82	0.84	0.82	0.83	0.84
Digestible Methine and Cystine (%)	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Digestible Lysine	1.16	1.16	1.18	1.21	1.16	1.18	1.21

a: The brown seaweed product name supplied by Acadian seaplants, b: The butyric acid product name supplied by ProBiotech, ME=metabolizable energy, DL Methionine Premix is comprised 50% DL Methionine and 50% ground corn. MCBS7 is broiler vitamin premix comprised of: Vitamin A 0.156%, Vitamin D3 premix 32.00%, Vitamin E 2.00%, Vitamin K 0.194%, Riboflavin 0.215%, DL Ca-pantothenate 0.6%, Vitamin B12 0.46%, Niacin 0.808%, Folic acid 2.20%, Choline chloride 26.70%, Biotin 6.00%, Pyridoxine 0.109%, Thiamine 0.082%, Manganous Oxide 4.00%, Zinc oxide 3.05%, Copper sulfate 1.28%, Selenium premix 1.485%, Ethoxyquin 1.66%, Ground corn 7.001%, Ground limestone 10.00%.

Table 4.4: Nutrient analysis of grower diets containing Tasco, Proformix, and BMD

Parameter	Basal	Medicated	0.50% Tasco	1.00% Tasco	Proformix	0.50% Tasco + Proformix	1.00% Tasco + Proformix
Dry Matter (%)	87.35	87.74	88.12	87.60	86.82	87.82	86.85
Crude Protein (%)	22.32	22.12	21.19	21.63	21.86	22.76	21.18
Calcium (%)	0.94	0.94	0.91	0.91	0.88	0.93	0.88
Potassium (%)	1.01	0.98	0.99	0.93	0.87	0.88	0.91
Magnesium (%)	0.17	0.18	0.18	0.17	0.17	0.17	0.17
Phosphorous (%)	0.59	0.60	0.58	0.59	0.57	0.58	0.58
Sodium (%)	0.15	0.15	0.17	0.17	0.15	0.17	0.15
Copper (ppm)	20.40	20.58	20.18	20.27	17.72	20.60	21.52
Manganese (ppm)	144.4	135.61	138.38	135.30	135.51	138.48	129.60
Zinc (ppm)	124.12	128.34	139.78	143.68	142.58	146.28	133.83
Crude Fat (%)	7.25	6.47	7.46	7.30	7.18	7.32	7.40

Table 4.5: Finisher (Day 25-34) diet formulations including BMD, Tasco, and Proformix

Ingredient	Basal	Medicated	0.50% Tasco	1.00% Tasco	Profromix	0.50% Tasco + Profromix	1.00% Tasco + Profromix
Corn	49.12	49.02	48.32	47.52	47.44	48.24	47.44
Soybean Meal	31.42	31.44	31.49	31.56	31.57	31.50	31.57
Wheat	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Ani/Veg Fat	5.19	5.22	5.48	5.76	5.79	5.50	5.79
Limestone	1.50	1.50	1.48	1.46	1.46	1.48	1.46
Dicalcium Phosphorus	0.90	0.90	0.91	0.91	0.91	0.91	0.91
MCBF8	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL Methionine Premix	0.49	0.49	0.49	0.48	0.48	0.49	0.48
Pelleting Agent	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.38	0.38	0.34	0.30	0.30	0.34	0.30
BMD	0	0.05	0	0	0	0	0
Tasco[a]	0	0	0.50	1.00	0	0.50	1.00
Profromix[b]	0	0	0	0	0.04	0.04	0.04
Lysine HCl	0	0	0	0	0	0	0
Total	100	100	100	100	100	100	100
Calculated nutrient composition on a as fed basis							
ME (kcal/kg)	3200	3200	3200	3200	3200	3200	3200
Crude Protein (%)	19.50	19.50	19.50	19.50	19.50	19.50	19.50
Calcium (%)	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Available Phosphorus (%)	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Digestible Tryptophan	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Digestible Threonine	0.74	0.74	0.74	0.74	0.77	0.76	0.77
Digestible Methine and Cystine (%)	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Digestible Lysine	1.02	1.02	1.02	1.02	1.07	1.05	1.07

a: The brown seaweed product name supplied by Acadian seaplants, b: The butyric acid product name supplied by ProBiotech, ME=metabolizable energy, DL Methionine Premix is comprised 50% DL Methionine and 50% ground corn. MCBS7 is broiler vitamin premix comprised of: Vitamin A 0.156%, Vitamin D3 premix 32.00%, Vitamin E 2.00%, Vitamin K 0.194%, Riboflavin 0.215%, DL Ca-pantothenate 0.6%, Vitamin B12 0.46%, Niacin 0.808%, Folic acid 2.20%, Choline chloride 26.70%, Biotin 6.00%, Pyridoxine 0.109%, Thiamine 0.082%, Manganous Oxide 4.00%, Zinc oxide 3.05%, Copper sulfate 1.28%, Selenium premix 1.485%, Ethoxyquin 1.66%, Ground corn 7.001%, Ground limestone 10.00%.

Table 4.6: Nutrient analysis of finisher diets containing Tasco, Proformix, and BMD

Parameter	Basal	Medicated	0.50% Tasco	1.00% Tasco	Proformix	0.50% Tasco + Proformix	1.00% Tasco + Proformix
Dry Matter (%)	86.62	87.68	87.72	87.57	87.06	88.04	87.39
Crude Protein (%)	20.04	20.26	20.08	20.27	20.88	20.79	20.13
Calcium (%)	0.83	0.78	0.81	0.78	0.79	0.80	0.81
Potassium (%)	0.80	0.85	0.85	0.82	0.87	0.85	0.92
Magnesium (%)	0.16	0.16	0.16	0.16	0.16	0.16	0.17
Phosphorous (%)	0.53	0.54	0.53	0.52	0.54	0.54	0.55
Sodium (%)	0.18	0.16	0.16	0.16	0.13	0.14	0.18
Copper (ppm)	21.00	13.22	20.44	20.54	20.18	19.40	22.50
Manganese (ppm)	123.06	76.80	123.30	127.90	134.40	131.70	119.97
Zinc (ppm)	139.96	81.10	131.07	123.19	123.10	135.64	133.02
Crude Fat (%)	7.90	7.73	8.19	7.78	8.54	8.08	8.22

4.3.3 Growth performance, intestinal measurements, and statistical analysis

The procedure used to weigh broilers and collect and calculate growth data is described in section 3.3.3. The only difference being that batch weights for grower and finisher periods were conducted on days 25 and 34. The procedure used to collect and measure digestive tract parameters is described in section 3.3.4. The only difference being that sampling was conducted on days 24 and 34. The procedure used to evaluate lesions severity on the ileum of broiler is described in section 3.3.4.1. The procedure used to collect and measure histology data is described in section 3.3.4.2. The procedure used to conduct goblet cell counts is described in section 3.3.4.3.

All growth data collected was analyzed via One-Way ANOVA with repeated measures, using mixed models through the PROC GLIMMIX package of the SAS software (version 9.4, 2012, SAS Institute Inc., Cary, NC, USA) with pens as the experimental unit. Treatment, bird age, and the interaction between treatment and bird age were fixed effects. Residuals were tested for normality to assure assumptions required for ANOVA were met. Least square means were determined using the LSMeans statement of SAS. If dietary treatment effect was shown to be significantly different, PDMIX 800 software of SAS was utilized to determine pairwise comparisons via Tukey's pair-wise test. For the intestinal histological, intestinal index, goblet cell counts, and intestinal length data, a Two-Way ANOVA was conducted. This was done using mixed models with the PROC GLIMMIX package of the SAS software with treatment, sex, and treatment/sex interaction as the main fixed effects. As stated previously, the influence of the sex of the broilers was not taken into consideration for growth data analysis as it was impossible to definitively verify the sex of the birds, as they were not easily sexable without euthanasia. Least square means were determined using the LSMeans statement of SAS. The same software and codes that were used to determine pairwise comparisons for the growth data were also used for the intestinal data if a difference between treatment and/or sex was detected. For the intestinal ileum lesion score data, a Chi square test of independence was conducted, with feed treatment and lesion score as the categorical variables of interest. This was performed using the Chi square test option in SPSS statistics software (IBM SPSS Statistics for Windows, Version 26.0). The p-value of significance was 0.05.

4.4 Results

Broilers fed the mixture of Tasco and Proformix resulted in similar growth performance, feed intake, and feed efficacy parameters to broilers fed diets including these additives individually or fed basal or medicated diets, as shown in Table 4.7 ($p>0.05$).

Table 4.8 exhibited that incorporating either Tasco or Proformix individually or in unison in broiler diets did not impact the frequency or the intensity of lesions as broilers fed diets including Tasco and/or Proformix had similar scores to broilers fed basal and medicated diets ($p>0.05$).

Table 4.9 exhibited that including Tasco and Proformix in broiler diets did not improve intestinal morphology compared to birds fed a basal or medicated diet. Villus length, villus width, villus surface area, crypt depth, and intestinal wall thickness were similar for all treatments ($p>0.05$). Tasco and Proformix inclusion in diets had no impact on increasing goblet cell numbers compared to basal and medicated diets ($p>0.05$).

The intestinal index data showcased in Table 4.10. conveyed that for day 24, the diet the broilers were fed did not lead to alterations in intestinal indexes ($p>0.05$), but the sex of the broilers did lead to differences. Females fed 0.50% Tasco diets had larger intestinal indexes than males fed 0.50% Tasco, Proformix, or 0.50% Tasco + Proformix diets ($p<0.05$). All other male and female broilers examined on day 24 did not have significantly different intestinal indexes from each other ($p>0.05$). For the day 34 broilers examined the intestinal indices were not influenced by feed treatment ($p>0.05$), but were influenced by sex. Females fed 0.50% Tasco diets had a larger intestinal index than males fed Medicated, 0.50% Tasco, Proformix, or 1.00% Tasco + Proformix diets ($p<0.05$). All other female broilers did not have different intestinal indexes from the males examined at day 34 of this trial ($p>0.05$).

Table 4.7: Growth data and related parameters of broilers fed feed containing varying mixtures of Tasco and Proformix (65%butyric acid) for a complete production cycle

Treatments	Basal	Med	0.50%	1.00%	Pro	0.50%+Pro	1.00%+Pro	SEM	p-value
Starter (D 0-D14)									
BW D0(g)	44	44	44	44	44	44	44	17.7	1.00
BW D14(g)	369	357	362	376	357	372	376	17.7	0.97
BWG(g)	325	313	318	331	313	331	332	12.3	0.81
FI(g)	460	457	458	474	456	467	463	19.1	0.99
FCR	1.42	1.47	1.44	1.43	1.46	1.41	1.40	0.03	0.45
Grower (D14-D24)									
BW D24(g)	1076	1049	1047	1095	1035	1080	1066	17.7	0.21
BWG(g)	707	692	685	719	679	713	690	12.3	0.18
FI(g)	981	945	962	990	945	1004	1012	19.1	0.08
FCR	1.39	1.36	1.40	1.38	1.39	1.41	1.47	0.03	0.24
Finisher (D24-D34)									
BW D34(g)	2174	2162	2128	2183	2104	2181	2162	17.7	0.01
BWG(g)	1099	1114	1081	1088	1069	1105	1096	12.3	0.19
FI(g)	1701	1643	1641	1682	1622	1691	1719	19.1	0.004
FCR	1.55	1.48	1.52	1.55	1.52	1.53	1.57	0.03	0.33

Body weight gain (BWG)= Body weight of latest day-body weight of previous weigh day.
Feed intake (FI)= the sum of all recorded feed addition days-feed weight back of last day of feed period/ number of birds.
Feed conversion ratio (FCR)= Feed intake/BWG.
Standard error of the mean (SEM)= standard deviation/number of birds.

Table 4.8: Lesion scoring for broilers fed Tasco, Proformix, BMD, or a mixture of Tasco and Proformix

Treatment	# of birds with score 0	# of birds with score 1	# of birds with score 2	# of birds with score 3	# of birds with score 4	# of birds with score 5	# of birds with score 6	Average score	p-value
Day 24									
Basal	4	8	0	0	0	0	0	0.67	0.82
Med	5	6	1	0	0	0	0	0.67	
0.5%	5	6	1	0	0	0	0	0.67	
1.00%	4	7	1	0	0	0	0	0.75	
Pro	4	6	2	0	0	0	0	0.83	
1.00%+Pro	7	5	0	0	0	0	0	0.42	
0.50%+Pro	4	8	0	0	0	0	0	0.67	
Day 35									
Basal	8	3	1	0	0	0	0	0.42	0.63
Med	9	3	0	0	0	0	0	0.25	
0.50%	10	1	1	0	0	0	0	0.25	
1.00%	8	3	1	0	0	0	0	0.42	
Pro	9	3	0	0	0	0	0	0.25	
1.00%+Pro	8	2	2	0	0	0	0	0.50	
0.50%+Pro	5	6	1	0	0	0	0	0.67	

Table 4.9: Intestinal morphological data for broilers fed Tasco, Proformix, BMD, or a mixture of Tasco and Proformix

Treatments	Basal		Med		0.50%		1.00%		Pro		1.00%+Pro		0.50%+Pro		SEM	p-value
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F		
VL (μm)	763	639	786	751	727	719	878	838	854	783	1035	800	766	778	84.0	0.79
VW (μm)	204	212	292	245	203	202	252	197	238	235	182	188	214	210	30.0	0.88
CD (μm)	104	123	138	146	116	126	133	148	117	110	162	118	136	133	13.0	0.30
IWT (μm)	205	254	232	234	230	220	294	282	248	224	246	272	291	236	29.0	0.68
VSA (um^2)	4916	4264	7275	5844	4592	4595	6876	5042	6064	5830	6000	4571	5160	5358	998.0	0.92
GCC (100um^2)	19	19	21	24	26	23	22	17	20	28.00	24	26	22	26	3.0	0.52

VL=villus length, VW=villus width, CD=crypt depth, IWT=intestinal wall thickness, VSA=villus surface area, GCC=goblet cell count

Table 4.10: Intestinal indices (%body weight) of broilers fed Tasco, Proformix, BMD, or a mixture of Tasco and Proformix

Treatments	Basal		Med		0.50%		1.00%		Pro		1.00% +Pro		0.50% +Pro		SEM	p-value
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F		
Day 24	14.48^{ab}	14.62^{ab}	14.05^{ab}	15.03^{ab}	13.62^{b}	16.68^{a}	14.20^{ab}	15.69^{ab}	13.19^{b}	14.47^{ab}	13.95^{ab}	14.61^{ab}	13.76^{b}	15.39^{ab}	0.55	0.27
Day 34	2.81^{ab}	3.30^{ab}	2.72^{b}	3.16^{ab}	2.59^{b}	3.66^{a}	2.79^{ab}	3.34^{ab}	2.77^{b}	3.47^{ab}	2.67^{b}	3.31^{ab}	3.02^{ab}	3.32^{ab}	0.18	0.48

4.5 Discussion

The purpose of this investigation was to evaluate whether including *Ascophyllum nodosum* (Tasco) in combination with butyric acid (Proformix) in broiler feed would result in a synergistic effect that would improve body weight and intestinal health. The hypothesis was that Tasco would act as a prebiotic, while Proformix would act as a bactericide. Based on the growth performance data and intestinal health measurements collected, the inclusion Tasco in combination with Proformix in broiler diets did not result in a synergistic effect on bird growth and intestinal performance. The growth and intestinal health parameters were similar to broilers fed a basal diet, medicated diet, or diets that included Tasco or Proformix individually ($p>0.05$). The inclusion levels selected for the test ingredients were based on manufacturer recommendations, and CFIA required levels for each of the ingredients. Performance of the birds fed the experimental diets in this study were similar, which could indicate that observing the test ingredients at different mixture levels may result in a significant synergistic effect on broiler performance.

Butyric acid is an ideal organic acid for feed additive use due to its ability to lower intestinal pH. Lowering intestinal pH often inhibits the growth of pathogenic bacteria, such as *E. coli* and *Clostridium* spp, and promotes the proliferation of beneficial bacteria (Nari et al., 2020). It is difficult to conclude that the inclusion of Proformix in broiler diets modifies intestinal pH, as the growth data and ileal morphology data suggests there was little modification of the GI environment. Further investigation into butyric acid’s direct impact on intestinal pH is required.

There have been studies on dietary inclusions of different butyric acid derivatives, that have observed beneficial effects on broiler growth and intestinal morphological characteristics. For example, Antongiovanni et al. (2007) examined the impact of dietary butyric acid glycerides on broiler growth, gut histology, and carcass composition. The birds were fed one of five dietary treatments, where a standard broiler feed was used as the control and the remaining diets contained butyric acid glycerides at inclusion levels of 0.2%, 0.35%, 0.50%, or 1%. Birds fed 0.2% butyric acid glycerides weighed more than the control-fed birds with improved overall weight gain ($p<0.05$). While the inclusion of butyric acid glycerides in broiler feed may improve growth performance, the butyric acid product used in this study did not ($p>0.05$). This could be attributed to the differences in inclusion levels of the butyric acid products between both studies. The highest dietary inclusion levels of Proformix in this study was 0.06% during the starter phase. The lowest inclusion levels of butyric glycerides in Antongiovanni et al. (2007) study, was more than three times greater than the highest butyric acid product inclusion level in this study. This could indicate that higher dietary inclusion levels of butyric acid are required to have a tangible effect on improved broiler growth and health parameters.

The form of butyric acid in the Proformix may be a reason why improvements in broiler production traits were not observed in chickens fed the mixture of Tasco and Proformix (Guilloteau et al., 2010). Moquet et al (2018) observed that different forms of butyric acid can have varying effects on intestinal tract parameters such as tract development, fatty acid content, and proteolytic activity, in different segments of the gastrointestinal tract. Studies that analyze the effect of butyric acid on the intestinal tract of broilers and other animals when used as a feed additive often incorporate butyric acid in the form of tributyrin (Antongiovanni et al., 2007). Tributyrin is a triglyceride comprised of three butyrate moieties and is more structurally sound than butyric acid because of the glycerol groups around the butyric acid components of this compound (Shi et al., 2020). Glycerol has ester bonds with the butyric acid in tributyrin, which are strong connections that require specific enzymes such as lipase to break. These bonds help keep the butyric acid in a functional state as it moves through the gastrointestinal tract (Zou et al., 2020).

While glycerol offers additional protection to butyric acid, often these bonds are not enough protection for butyric acid to reach the ileum of the intestinal tract. Tributyrin is often microencapsulated when fed to animals when the target of interest for observing the effect of tributyrin or derivatives of butyrate such as butyric acid is the hindgut or the colon (Tugnoli et al., 2020). Encapsulation or microencapsulation is formulating an active ingredient with secondary materials used in preparation of a capsule that protects the bioactive component from undesirable environmental stressors until it is released to its desirable stimulus. Capsule or protective barriers can be formed in several ways including: emulsion-based systems, solid lipid nanoparticles, and biopolymeric gelled microspheres to provide protection to the active ingredient (Augustin et al., 2011). Microencapsulation allows the butyrin portion of tributyrin to survive the breakdown process of the stomach and duodenum and be gradually be released throughout the intestinal tract instead of all of the butyrin being destroyed or released in the upper digestive tract (Tugnoli et al., 2020). If the butyric portion of Proformix was microencapsulated it may result in a beneficial effect on ileal health and minimize impact of necrotic enteritis (Nari et al., 2020). It is possible that the butyric acid portion of

Proformix was encapsulated, as one of the ingredients used in the production of this product is vegetable oil. Vegetable oils can be used as encapsulating agents as they contain triglycerides, which are prepared from esters of glycerol and three fatty acids (Ataei et al., 2019). These compounds are structurally dense, due to the presence of double bonds within the chemical makeup of the triglycerides. The chemical makeup, fatty acid content, and number of double bonds present in the vegetable oil can influence how effective the oil is as an encapsulating agent as some oils are better at maintaining structural integrity during digestion than others (Ataei et al., 2019). Since the plant source of the vegetable oil used in the production of Proformix is not specifically stated, it is difficult to determine if the butyric portion of Proformix was effectively encapsulated, as it is possible that the oil used to encapsulate butyric acid could have broken down early in the digestive process. This results in butyric acid being exposed to breakdown and absorption earlier in the digestive tract (Sagiri et al., 2016). Based on the results of this study, it is recommended that investigations into the potential of butyric acid as an ileal health promoter and necrotic enteritis controller. This could be done by conducting necrotic enteritis challenge trials with encapsulated butyric acid feed ingredients to increase the likelihood of butyric acid remaining functional in the ileum.

Tasco may have had an increased chance of reaching the ileum, as *Ascophyllum nodosum* is high in non-starch polysaccharides (Chen et al., 2018). Non-starch polysaccharides are structurally dense microstructures that often require microbial fermentation in addition to standard digestive activity in monogastric animals for maceration and utilization of nutrients these structures surround (Chen et al., 2018). Since the broilers fed the 0.50% and 1.00% Tasco diets had similar ileal health parameters to broilers fed the basal diet, it cannot be concluded that Tasco was completely intact by the time it reached the ileum and further investigations into Tasco digestibility are required. In the future, it is recommended that encapsulated butyric acid products are included in broiler diets when examining the influence of butyric acid on ileal morphology. This is to ensure that the functional butyric acid portion of the product reaches the ileum intact and be readily available to the ileum to utilize to improve and maintain sufficient health and function.

The broilers in this study may have met their genetic potential in terms of growth performance and residual feed intake, as broilers fed a basal diet were of similar size to those fed the experimental diets. Residual feed intake is the difference between actual feed intake and predicted feed intake based on energy requirements for production and is considered a heritable trait (Mebratie et al., 2019). The addition of Tasco, Proformix, or BMD did not improve residual feed intake. The growth data and FCR in Table 4.7 suggests that these broilers were capable of utilizing the feed to its full potential, regardless of the inclusion of feed additives (Mebratie et al., 2019).

Zhong et al. (2020) examined the effect of ɣ-amino butyric acid as a feed additive that could act as reliever of heat stress by examining the growth and intestinal histomorphology of heat-stressed broilers. ɣ-amino butyric acid is a nutritional element that can act as a neurotransmitter that can block brain signals (Dhakal et al., 2012). This action can regulate the action of other neurotransmitters such as triglycerides, cholesterol, creatine kinase, and lactate dehydrogenase. By modifying the activity of neurotransmitters in broilers, the addition of ɣ-amino butyric acid to feed can improve growth rate and FCR when digested (Zhong et al., 2020). Ross 308 broilers fed diets that include ɣ-amino butyric acid at dietary inclusion level of 0.5% can experience increase jejunal villus lengths in heat-stressed environments. This can lead to improved digestive capability and increased growth performances in a heat-stressed environment, and possible other stressful conditions, such as the presence of pathogens (Al Wakeel et al. 2017).

The birds grown by Zhong et al. (2020) were fed a basal diet or a feed containing 0.01% ɣ-amino butyric acid. On day 49 ɣ-amino butyric acid fed birds were bigger than the control fed birds, and had a better feed to gain ratio and less mortalities throughout the study ($p<0.05$). Compared to the growth results of this study (Table 4.7), the broilers raised were heavier than the broilers studied by Zhong et al. (2020). This is likely due to the birds being heat stressed in Zhong et al. (2020) study, while no stressor was introduced to broilers in this study. Both studies exhibit that birds raised for 35-36 days fed a butyric acid containing diet does not result in heavier broilers than broilers fed a basal diet ($p>0.05$).

While Zhong et al. (2020) exhibited that including ɣ-butyric acid in broiler diets in heat stressed conditions can improve FCR compared to a basal diet, the FCR results for all the diets in this study was better than the FCR results of Zhong et al. (2020) study. Similar to the growth results, this is likely contributed to the influence heat stress in Zhong et al. (2020) versus the lack of stressors implemented in this study. The difference in FCR observations between these studies is that the Proformix added diets in this study did not lead to improved FCR compared to the basal diet and medicated diet fed birds ($P>0.05$). Zhong et al. (2020) showed that if broilers are grown under stressful conditions, that is when they are likely to utilize the additional benefits of butyric acid as they are raised in conditions where the basic required nutrients are not enough to maintain sufficient growth performance (Choi et al., 2014). If the broilers raised in this study were raised in more challenging conditions such as environmental stressors or pathogenic stressors, then the beneficial impact Proformix has on broiler growth may have been better conveyed.

The ileum was studied in this trial as this is the intestinal site where necrotic enteritis is most prominent in *Clostridium perfringens* infected broilers. Ileal morphological characteristics and goblet cell data in Table 4.9, conveyed that diet had no impact on improving ileal health characteristics as broilers fed the basal diet or medicated diet had similar characteristics to the Tasco and Proformix diets ($p>0.05$). It is likely that the butyric acid portion of the feed was absorbed in the jejunum, as this is the major site of butyric acid absorption along the intestinal tract; while the ileum is the major site of microbial fermentation (Guilloteau et al., 2010; Zhong et al., 2020). By the time the remainder of the feed reached the ileum, there may have been little to no butyric acid for the ileum to utilize to improve intestinal tract characteristics such as villus surface area or crypt depth.

Zhong et al. (2020) observed that ɣ-amino butyric acid did have a positive impact on jejunum histomorphology with longer villus lengths than the control birds ($p<0.05$); but did not improve ileal villus length compared to the basal feed ($p>0.05$). The inclusion of ɣ-butyric acid in broiler feed did not impact crypt depth in either the jejunum or ileum sections of the small intestinal tract ($p>0.05$). The ileal histomorphology results in Zhong et al. (2020) study is similar to the results found in this trial in the birds fed Proformix (65% butyric acid) containing feeds in terms that the addition of butyric acid to broiler feed did not change crypt depth compared to broilers fed a basal diet. The crypts depths observed by Zhong et al. (2020) were deeper than the crypt depths in this study. This could be due to the broilers being challenged in Zhong et al. (2020) investigation.

Crypts of the intestinal tract are a source of stem cell production in broilers (Biasato et al., 2018). Stem cells replace damaged or dead cells within the intestinal tract that provide protection to the epithelial layer of the intestinal tract. Deeper crypts are a sign of increased cell turnover occurring within the intestinal tract, which occurs due to increased apoptosis, an indicator of health difficulty within broilers (Biasato et al., 2018; O'Reilly et al., 2017). The apoptosis rate within animals can be influenced by stressors such as unfavorable temperatures or presence of pathogens (O'Reilly et al., 2017). A strategy that is frequently evaluated is dietary treatments, as certain diets contain compounds that aid in maintaining cell health and reduce the impact of environmental stressors and pathogens (Biasato et al., 2018). Since the broilers raised in this study were not challenged by a stressor such as *Clostridium perfringens*, or heat, the data collected on intestinal health reported in Table 4.9 indicate that the addition of Tasco, Proformix, or BMD was not required to maintain adequate intestinal morphological performance ($p>0.05$).

Antongiovanni et al. (2007) reported that butyric acid glycerides can impair intestinal morphology characteristics. They observed shorter villus length in both the jejunum and ileum areas of the small intestine in birds fed butyric acid glycerides ($p<0.05$). Birds fed butyric acid glycerides at 0.2%, 0.35%, 0.5%, and 1% dietary inclusion levels also had decreased crypt depth in the ileum ($p<0.05$); but birds fed 0.2% butyric acid glycerides had increased crypt depth in the jejunum ($p<0.05$). The ileal histomorphology results in Antongiovanni et al. (2007) is similar to the results found in this trial in broilers fed Proformix (65% butyric acid) containing feeds. The villus lengths of the 0.2% 0.35% and 0.5% diets on day 35 were 857µm, 856µm and 697µm respectfully, while the villus lengths of broilers fed a 0.04% Proformix inclusion level as well of the Proformix + 1.00% Tasco and Proformix + 0.50% Tasco diets of male and female broilers at day 35 were 854µm, 783µm, 1035µm, 800µm, 766µm, and 778µm respectfully.

There was no difference between the basal diet-fed broilers and Proformix-fed broilers in regards to intestinal villi lengths, while there was between the control and butyric acid glyceride diets in the Antongiovanni et al. (2007) investigation. This could be a result of Antongiovanni et al. (2007) investigating only female Ross broilers, while in this study, both male and female Ross broilers were observed. Female chickens have a more complex reproductive system than male broilers. Therefore, the butyric acid portion of this feed that would have improved intestinal villus length, was diverted to improving reproductive performance (Jiang et al., 2020).

Data indicates that butyric acid can activate the cyclic adenosine monophosphate (cAMP) signaling pathway in female pigs, but this is not conclusive. The production and regulation of estradiol occurs via the action of gonadotropins and the activation of cAMP (Lu et al., 2017). Estradiol is a hormone that is essential for promoting female sexual characteristics and reproductive function in female organisms. This means that butyric acid ingested by the broilers raised by Antongiovanni et al. (2007) may have been used to stimulate the activation of cAMP and increase estradiol production (Lu et al., 2017). Increased activity of this hormone may have led to a divergence on energy within the broiler from improve digestive tract morphology to improving reproductive performance. The exact mechanisms butyric acid utilizes to activate cAMP are currently unknown, and there is no information currently available on butyric acid's impact of female broiler hormone production (Lu et al., 2017). Since neither diet or sex of the broilers examined in this study resulted in differences in ileal villus length and other intestinal and growth characteristics, it is difficult to conclude that butyric acid can influence hormone production that change physiological characteristics as the effect of diet on hormone production was not observed and growth parameters observed did not take sex of broiler into consideration. Further investigation into broiler diet influence on hormone production is required as determining if and how butyric acid and/or brown seaweed influences hormone production in broilers could aid in the understanding how these additives could influence the physique of broilers.

4.6 Conclusion

Mixing Tasco with Proformix did not improve broiler performance in terms of growth and intestinal morphology compared to broilers fed the control diets or diets containing each of these feed additives individually. It is possible that most of the butyric acid portion of the Proformix product was absorbed in the upper parts of the intestinal tract, therefore, exhibiting most of its potential benefits for improving intestinal tract performance in areas of the intestinal tract that were not observed in this study. The mixture of Tasco and Proformix may not have worked due to different mixture levels of these feed additives potentially being required to observe a tangible influence on broiler growth and ileal morphological parameters. Future research should analyze the mixture of Tasco and Proformix in other parts of the intestinal tract besides the ileum, specifically the jejunum, to have a more comprehensive observation on the mixture's impact on improving broiler performance. Investigations should also attempt to combine different forms of butyric acid and different species of brown seaweed to determine what may be optimum mixtures of these alternatives to in-feed antibiotics. Mixtures of the effect of brown seaweed and butyric acid on microbial populations in the intestinal tract should be observed in order to determine if the mixture improves the ratio of beneficial to pathogenic bacteria compared to each alternative being individually incorporated in broiler feed.

CHAPTER 5 GENERAL DISCUSSION

An objective of this research was to examine if Tasco is a suitable alternative to in-feed antibiotics for promoting growth and health of broiler chickens. A second objective was to observe if including Proformix with Tasco in broiler feed will lead to a synergistic effect on broiler performance compared to Tasco alone. The hypothesis was that a dietary mixture of Tasco and Proformix would be synergistic in improving broiler performance. The basis of this hypothesis is that Tasco would act as a prebiotic stimulating beneficial microbial colonies within the ileum, while Proformix would act as a bactericide and inhibit the activity of pathogenic bacteria (Klurfeld, 2001; Levine et al., 2016). Properties of butyric acid such as inducing apoptosis and inhibit proliferation of pathogens would eradicate pathogenic microbes, while the properties of brown seaweed would focus more on building up beneficial bacteria within the intestinal tract. This would have led to an overall improvement in intestinal health and function, which would result in feed ingredients being used more efficiently, and more energy/nutrients from the feed being available for broiler chicken growth. This would have led to bigger and heavier chickens when compared to chickens fed standard feed, feed containing an antibiotic as a growth promoter, and feed containing just brown seaweed. Direct measurements on these microbial colonies were not made in this study, but parameters that are influenced by microbial activity were measured. These ingredients were implemented into the feed at CFIA-approved levels to evaluate the hypothesis in an applicable setting. Previous research has found that butyric acid can be effective at lowering the severity of necrotic enteritis, but at much higher inclusion levels than what is acceptable.

Research results exhibit that including Tasco into broiler feed at 1.00% can enhance broiler growth performance ($p<0.05$). However, including Proformix with Tasco in feed does not have a synergistic effect with Tasco, as the mixtures did not lead to improved growth ($p>0.05$). It should be noted that there was a change in replicate numbers between the Tasco growth trial and the Tasco + Proformix growth trial, which could have potentially affected the growth found in the Tasco + Proformix trial. This was due to the change in number of diet treatments between Chapter 3 and Chapter 4 study, with the Chapter 3 growth trails having eight replicates per treatment, and Chapter 4 growth trial having six replicates per treatment. This was to ensure even distribution of dietary treatments between both rooms, as the space available between the studies did not change. Neither dietary Tasco nor Proformix influenced ileum morphological parameters or goblet cell content ($p>0.05$). The sex of the broilers impacted intestinal indices, as some of the female broilers sampled in all three trials had larger intestinal indices than some males ($p<0.05$). In general, female broilers are smaller than males and the intestinal tract would take up more body composition as a result (Goo et al., 2019). Most of the data between the two studies were fairly consistent, except that the addition of 1.00% Tasco by itself did not lead to improved growth performance in the Chapter 4 study, while it did in the Chapter 3 study. The broilers in both studies were housed in the same facility that provides a bio secure environment and should limit the number of factors that can cause illness or stress of the chickens (Fike et al., 2005). This inconsistency could be due to the broilers observed in the Chapter 4 study reaching their maximum potential for utilizing feed for growth performance while in the Chapter 3 study, the broilers required Tasco inclusion for their growth potential to be realized (Mebratie et al., 2019).

These studies have shown that Tasco is an effective alternative to in-feed antibiotic growth promoters in regards to improving broiler growth performance and maintain adequate intestinal health. It is also possible that the inclusion of Tasco in broiler feed can improve intestinal morphological parameters such as intestinal wall thickness as seen in Tasco trial 2 data Table 3.13 in chapter 3. This observation was not seen consistently through out this study, so it cannot be concluded that including Tasco in broiler feed will also lead to thicker intestinal walls, thus improving intestinal integrity. Further research investigating the mode of action Tasco undertakes to improve broiler growth is required, as the data obtained in this study did not convey the exact mechanisms Tasco utilizes to improve broiler performance. Research should investigate the ability of Tasco to modify microbial colonies along the intestinal tract of broilers. Tasco may stimulate the activity of beneficial species of bacteria within the intestinal tract that may aid in enhanced broiler growth due to the high amount of non-starch polysaccharides present within *Ascophyllum nodosum* (Cabrita et al., 2016). Investigations into the impact of Tasco on improving the morphology of other sections of the intestinal tract besides the ileum at different stages of growth should be performed, as it is possible Tasco may have been destroyed or absorbed in the upper parts of the digestive tract due to its low inclusion level (Opheim et al., 2016).

The investigation into Tasco's applicability in broiler feed in chapter 3 concluded that Tasco inclusion levels of 1.00% is the most effective for broiler chickens due to its consistency of improving growth performance, while lower inclusion levels were not consistent between the 2 Tasco trials. Acadian Seaplants Ltd recommend Tasco inclusion levels between 0.25-0.50%, but the data collected in chapter 3 would indicate otherwise. More growth trials may be useful to revaluate and increase these recommendations, as it is possible that the species, housing conditions, and location of each of the respective studies were different, and could have influenced the amount of Tasco required for optimum growth performance (Choi et al., 2014; Evans and Critchley, 2014).

Investigations on mixing Tasco and Proformix or mixtures of brown seaweed and butyric acid in general are necessary. Based on the data collected, it cannot be concluded the mixture of Tasco and Proformix acted in this manner, as indicators observed that are signs of changes in microbial activity such as growth performance, ileal histomorphology, and goblet cell counts were not different from broiler fed a basal diet or each of these additives individually ($p>0.05$). It is possible that Tasco does act as a prebiotic in broilers, as there was a difference in growth performance between broilers fed basal and medicated diets to those fed Tasco included diets in the Tasco trials. Stimulation of beneficial microbial activity in the ileum could have resulted in the production of beneficial compounds that when absorbed may stimulate growth activity within broilers (Ǿverland et al., 2019). However, signs of prebiotic activity are frequently associated with improved intestinal morphology, as larger intestinal villi are associated with increased nutrient absorption, and higher nutrient availability for growth performance utilization (Charoensiddhi et al., 2017). The diets seemed to have little influence on ileal morphological parameters in the three growth trials ran for this study. Broilers raised in a challenged environment may benefit more from these compounds to maintain and improve ileal morphological integrity or the extra nutrients and compounds supplied by Tasco and Proformix may have been used to improve morphological characteristics earlier in the digestion process (Guilloteau et al., 2010). Future studies could investigate the ability of Tasco and Proformix to influence microbial colony populations when included in broiler feed either individually or in combination, as this will help determine if there is any relationship between Tasco and Proformix on improving digestive performance.

The lesion score data collected in all studies conducted exhibited that the inclusion of Tasco or Proformix did not influence the frequency or intensity of lesions occurring on the ileum of broilers ($p>0.05$). Lesions are often a sign of necrotic enteritis in broilers as the breakdown of the epithelial wall of the intestinal tract via *Clostridium perfringens* produced toxins results in the occurrence of lesions (Keyburn et al., 2006). It is understandable that feed treatment would not influence the occurrence of lesions in this study, as the broilers grown in these studies were not challenged by necrotic enteritis. This means the benefits of including Tasco and Proformix in broiler diets that control of necrotic enteritis by modifying microbial colonies and reenforcing intestinal epithelial integrity were not required (Kim and Pangestuti, 2011).

The potential of Tasco and Proformix to influence the ratio of beneficial to pathogenic bacteria by increasing beneficial bacterial activity is relevant to necrotic enteritis control in broilers (Yang et al., 2019). Therefore, if Tasco can aid in maintaining sufficient populations of beneficial microbes, then the impact of necrotic enteritis on broiler production and health traits will be limited (Yang et al., 2019). While the growth data may indicate that Tasco has a beneficial effect on specific microbial population ratios with the GI, further investigation of the influence of Tasco on broiler microbial population ratios is required.

Necrotic enteritis challenge trials should be conducted to investigate the potential of brown seaweed and butyric acid as preventative treatments for necrotic enteritis in broiler flocks. These trials should investigate growth performance and intestinal immunity characteristics and performance such as intestinal morphology and goblet cell counts. These studies should also investigate if the addition of these active ingredients lower the population of *Clostridium perfringens* colonies within the intestinal tract of the broilers. Investigations in how these feed additives affect the population of beneficial species of microbes along the intestinal tract should be conducted, as if certain species activity is enhanced or impaired by the inclusion of these additives, then this may affect the intensity that necrotic enteritis has of broiler health in performance.

CHAPTER 6 CONCLUSIONS

Tasco is a candidate as an effective alternative to in-feed antibiotics, as including it in broiler diets improves growth performance and maintains adequate ileal health under bio secure conditions at CFIA-approved levels. The inclusion of Tasco and Proformix in combination to diets did not improve upon the benefits of including Tasco by itself, as the addition of Proformix did not lead to improved growth performance or changes in ileal health. While it is possible that Tasco acts as a prebiotic and Proformix has qualities similar to an antibiotic, further research is required to determine the mode of action these feed additives have in relation to broiler growth performance and intestinal health and function. Research efforts should investigate these feed additives influence of modifying microbial populations and intestinal morphology and health parameters in all areas of the digestive tract. In terms of Tasco and Proformix's influence on necrotic enteritis control in broilers, necrotic enteritis challenge trials should be conducted to observe if the benefits that Tasco and Proformix have of broiler performance are effective at minimizing the impact of necrotic enteritis on broiler production characteristics and health.

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