EVALUATION OF INTESTINAL DEVELOPMENT AND THE BACTERIAL COMMUNITY IN THE GASTROINTESTINAL TRACT OF POULTRY

by

BRETT STEPHEN LUMPKINS

(under the direction of Amy B. Batal)

ABSTRACT

With advances in molecular tools to identify bacteria and the growing concern about antibiotic use in livestock feeds, researchers have focused on understanding the normal bacterial community in the gastrointestinal tract (GIT) of animals and how the bacterial community can be changed to benefit the animal. Experiments evaluated the development of the GIT and the bacterial community of broilers under various conditions. In the first experiments when chicks were fed from 0 to 21 d of age, the impact of different diets with alternative protein sources and crystalline amino acids on the development of the GIT were evaluated. The bacterial populations were different due to diet even when no differences in intestinal development manifested, but when a diet was fed that also affected intestinal development, a clear separation in the bacterial populations of birds fed a corn-soybean meal or a crystalline amino acid diet appeared. When male and female broilers received the same diet, there was no difference in intestinal development between the males and females, but the bacterial populations separated into 2 clusters based on gender. When evaluating different genetic lines of broilers, the multipurpose broilers were found to have the highest body weight gain and fastest rate of GIT development, followed by the high yield and the Athens Canadian Random Bred (1957 line of broiler) broilers. The bacterial populations grouped together between the high yield and

multipurpose broilers (modern lines), while the bacterial community of the Athens Canadian Random Bred broilers clustered separately from the modern genetic lines. When novel species of *Bacteroidaceae* and *Clostridia* served as a possible probiotic, no overall difference in performance, carcass yield, intestinal development, and bacterial community was observed. But during early stages of development (0 to 3 d), broilers inoculated with the novel combination of *Bacteroidaceae* and *Clostridia* had increased villi height and goblet cell concentration. In all experiments, as broilers aged and developed, a change in the bacterial community occurred. When the rate of GIT development plateaued, the bacterial populations stabilized and became less diverse.

INDEX WORDS: bacterial community, gastrointestinal tract, villi, broiler, biodiversity, bacteria

EVALUATION OF INTESTINAL DEVELOPMENT AND THE BACTERIAL COMMUNITY IN THE GASTROINTESTINAL TRACT OF POULTRY

by

BRETT STEPHEN LUMPKINS

B.S., The University of Florida, 2002

M.S., The University of Georgia, 2004

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2007

EVALUATION OF INTESTINAL DEVELOPMENT AND THE BACTERIAL COMMUNITY IN THE GASTROINTESTINAL TRACT OF POULTRY

by

BRETT STEPHEN LUMPKINS

Major Professor: Amy Batal

Committee: Margie Lee
Nick Dale
Michael Lacy
Michael Azain

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2007

DEDICATION

The intense efforts put forth for my doctoral degree could not have been made possible without the love and devotion of my family. My parents, Debra and Arthur Meyerson, love and devotion gave me the encouragement and support to strive for success. My parents bestowed upon me the true importance of having a good education, which encouraged me to attend excellent universities and to further my accomplishments. They were always there to guide me and give me every opportunity to better my education and myself. I owe my achievements and happy upbringing to my wonderful parents who have meant so much to me and who I can never thank enough.

To my grandparents, Margaret and Sellards Lumpkins, who I was fortunate enough to have in my life to share in all of my accomplishments. Poppy, who was not here with us in body but will always be in spirit, was a great man that taught me disciple and dedication to my work. A man I looked up to who gave me so many great memories I will remember and cherish forever.

Kelly, who stood by me during my doctoral program and brought so much love and happiness into my life. A wonderful woman who has made me very happy and I cannot wait to share my life with.

ACKNOWLEDGEMENTS

To my advisor, mentor, and friend, Amy Batal, I thank you for all of your time and guidance. I appreciate the encouragement you always gave me and the extra push to do better. You have made my graduate experience a wonderful time and enriched it with knowledge. I will never forget you as my mentor and best friend.

To Margie Lee who gave me another way of looking at research and determining the results from an ecological standpoint. I appreciate the opportunity of working in the lab and the joy I have found in ecology. I have learned much about molecular techniques and gained a greater appreciation for biochemistry. Thank you for your time and assistance.

To Nick Dale, thank you for enlightening me and increasing my knowledge of culture and history around the world and especially in the Americas. I appreciate the time you have spent teaching me about nutrition in the field and helping with group events to make my graduate experience a fun and memorable one.

To Mike Lacy and all the faculty and staff members of the Poultry Science Department, I thank you for all your help and assistance in making my graduate career an enjoyable one.

To David Perry and all the farm crew, all of you help makes an invaluable contribution to making everything easier for graduate students.

TABLE OF CONTENTS

Page

INTRODUCTION

Recently, there has been growing interest in evaluating the interaction of the intestinal bacteria on the health of an animal and how additives in the feed affect the bacteria in the gastrointestinal tract. Concern about subtherapeutic antibiotic use has caused researchers and animal health companies to concentrate their focus on finding alternatives that would manipulate the bacteria of the gastrointestinal tract (GIT), or gut. The intestinal bacteria is part of a complex ecosystem that affects animal health and performance through its effects on gut morphology, digestion of nutrients, and the immune response through a symbiotic or intra-organismal mutualism. The impact of the intestinal bacteria on animal performance and the factors that contribute to manipulating the bacterial community are not well understood. Researchers are questioning how changes in feed ingredients impact the bacterial population and if gender or the genetic line of birds affects the bacterial communities.

The gastrointestinal tract is a complex micro-ecosystem, which provides a specific niche for the commensal bacteria to reside in and act in a symbiotic manner within the host. The physically and chemically changes in the GIT affect the micro-ecosystem. Variation in the environmental conditions can impact the bacterial community. The development of the GIT in poultry can differ between genetic lines or breeds of birds or even between the genders of a specific species, and the use of certain feed ingredients can also impact the development of the GIT. By affecting the development of the GIT, the commensal intestinal bacteria would be changed. Therefore, if researchers can increase the commensal bacteria, then bird health and performance could be improved.

Through the use of new molecular techniques, researchers are better able to identify the intestinal bacteria of birds and attempt to understand the symbiotic behavior that could improve the health and performance of the bird. Drugs such as antibiotics and coccidiostats can have a dramatic impact on the composition of the bacterial community, but the interaction remains unclear. The use of probiotics (a live bacterial culture) has been one attempt at changing the bacterial community in the GIT to increase commensal bacteria. The introduction of a probiotic to the GIT has been documented to improve or stimulate performance, increase crypt development, facilitate intestinal capillary angiogenesis, improve the migration of enterocytes, intestinal villi length, and stimulate secretion of defensins. Another method of changing the intestinal bacteria is through the use of prebiotics, which provide a substrate to encourage the growth of commensal bacteria. By experimenting with different ingredients, genders, and genetic lines of birds, one might be able to better understand the factors that affect the bacterial community.

A deeper understanding of the ecological basis of the intestinal bacterial population present in the gastrointestinal tract of poultry may help researchers to gain a better understanding of the factors that contribute to the increase or change in the commensal bacteria. A greater understanding of the interaction of the micro-ecosystem and the bacterial community can allow animal health and poultry production companies to develop feeding and drug programs that will most effectively combat pathogens without negatively impacting the bird's normal intestinal bacteria. Little is known about the intestinal bacterial community in poultry; therefore, the objective of this research will be to evaluate the difference in intestinal bacterial composition in the developing small intestine resulting from various factors such as feeding diets with various feed ingredients, gender, and genetic lines of broilers. The study will also attempt to posit how

the bacterial community changes in the presence of a probiotic, a novel bacteria isolated from the chicken intestine.

CHAPTER 1

LITERATURE REVIEW

Any study of the GIT of poultry must begin with a review of the foundational sources in the field. These authorities have explored many of the aspects of poultry science essential for this study and will, by their work, provide the prelude to this investigation. However, the work done with poultry has been focused either on intestinal development or intestinal bacteria, and there are no reports that compare intestinal development with the residing intestinal bacterial community.

Background Studies of the Gastrointestinal Tract

Bayer et al. (1974) in an early study and others have revealed that the gastrointestinal tract (GIT) is a complex system that primarily serves in nutrient digestion and absorption and secondarily provides protection from foreign pathogens that enter the body through exogenous feed sources. In poultry, the GIT starts dramatically developing a few days prior to hatch, and continues for several days following hatch until a stage of developmental maturation is reached (Uni et al., 2003a; Sklan, 2001; Geyra et al., 2001). Immediately following hatch, the chick's GIT will encounter a number of exogenous nutrient sources, an event which stimulates the GIT into a state of rapid development in order to adapt to the carbohydrate and protein-based diets. These studies show that the chick's exposure to the environment outside of the shell also results in contact with foreign bacteria from the air and from exogenous food sources. This exposure contributes to the establishment of the intestinal bacterial community.

Sklan (2001) tracked the development of the GIT. As the chick undergoes developmental changes that impact both the physical and chemical conditions (Sklan, 2001) of the GIT, there is also a bacterial community residing in the intestines that is changing to adapt to the micro-ecosystem (Apajalahti et al., 2004). As the bird develops, the changing GIT is affecting the bacteria that reside in specific niches, resulting in a change in the composition of the intestinal bacteria, as Lu et al. (2003) noted. The change in diversity of the bacterial community may also have an effect on the symbiotic role between the host and the intestinal bacteria.

Studies Focusing on Gastrointestinal Structure

The gastrointestinal tract is composed of organs extending from the mouth where food enters to the large intestine and ultimately to the cloaca where waste is excreted (Diagram 1.1). The esophagus allows for passage of food from the beak to the crop. In their 2004 study, Dibner and Richards observed that, once at the crop, the food is moistened and softened by the mucus secreted here and in the mouth. Food begins to undergo chemical digestion in the proventriculus where HCL, pepsin, and mucus are secreted; digestion continues into the gizzard (Leeson and Summers, 2001). Additionally, the grinding effect of the gizzard breaks down large particles of feed. Food from the gizzard enters the small intestine as digesta.

The small intestine serves an important function for nutrient digestion and absorption and overall health of the bird. There are three parts of the small intestine (duodenum, jejunum, and ileum), which are responsible for further enzymatic breakdown, digestion and absorption of nutrients. As Sklan (2001) has shown, digestive enzymes, such as trypsin, amylase, and lipase, are secreted by the pancreas located in the duodenal loop and play an important role in the breakdown of complex nutrients into their simpler form for absorption. These small molecules are further broken down by “brush border” enzymes, produced in the enterocytes of the small

intestine. The small intestine is lined with villi (Diagram 1.2), which are structures extending from the muscular layer on the intestine wall. On the outside of each villus are microscopic structures referred to as microvilli. Overton and Shoup (1964) and Uni et al. (1995) noted that the villi folds and microvilli increase the surface area of the intestinal lining, a feature which aides in improving nutrient digestion and absorption as the digesta passes through the small intestine. Located at the base of the villus is the crypt of lieberkuhn, which is the site of epithelial cell formation. The epithelial cells are rhomboidal structures comprising the outer structure of the villi. In the center of the villus is an interweaving system of capillaries that act as a delivery system to transport the absorbed nutrients to the general circulation (Imondi et al., 1969; Weiser, 1973; Ferraris et al., 1992; Traber et al., 1991; Thomson et al., 1994). In the lining of the outermost area of the villi are small round structures called goblet cells. Located inside the goblet cells are mucin granules, which as their name imply produce mucin. Geyra et al. (2001) proved that mucin provides a protective mucosal coating to the villi to improve nutrient absorption and digestion. Mucin has recently been deemed to serve a second function by providing a nutrient source for the residing normal intestinal bacterial community by Apajalahti, 2005. Leeson and Summers showed that once most of the nutrients in the digesta have been digested and absorbed in the small intestine, the digesta then passes into the large intestine or colon. The colon is mainly responsible for water absorption. At the junction of the colon are 2 cecal pouches, which harbor a large population of anaerobic microbes, in numbers far greater than any other area in the GIT, as documented by Dibner and Richards (2004). Prior to hatch, the GIT forms and undergoes a number of developmental changes.

Studies Concerning Development of the Gastrointestinal Tract

Pre-Hatch Development

During incubation, the embryo is growing and developing into a chick. Uni et al. (2003a) found that through the progression of incubation the embryo increases in body weight (BW), and the small intestine grows at a faster rate than the BW. As Grey noted in 1972, the earliest signs of development in the GIT occur at 1 d of incubation where there is an increase in duodenal epithelial cells. At 14 to 16 d of incubation the villi start to emerge from the muscular layer with the presence of pre-villus ridges, which later develop into the mature villus (Grey, 1972). Sklan (2001) found that at 17 d of incubation the small intestines are rapidly developing and make up 1% of the embryos BW.

In two important studies, Uni and colleagues observed the development of the crypt in early stages of a bird's life. According to Uni et al. (2003a), the villi and muscular layer of the small intestine at 15 d of incubation are rudimentary and very poorly developed with no indication of crypt formation. However, by 17 d of incubation the presence of a crypt is observed, and the villi are present in two different stages of development, small and large villi (Uni et al., 1996). The underdeveloped small villi are approximately 65% the size of the large villi, but as the intestines become more developed, villi height and surface area increase and the number of small villi decrease closer to the time of hatch. The trend of the two stage villi continues until day 19 of incubation when further villi development occurs through budding at the villus base. Uni et al. (2003b) observed that by 20 d of incubation the large villi compose approximately 70% of the total villi population. The goblet cells located along the villi also increase in number with the increase in villi height, but at different rates in various segments of the small intestine. The goblet cell concentration per villus in the duodenum, jejunum, and ileum

increases slightly from 18 to 20 d of incubation. After 20 d of incubation, the goblet cell concentration significantly increases in the jejunum and ileum, resulting in a greater production of mucin (Uni et al., 2003b). As the embryo develops and nears hatch, there is an increase in duodenal DNA concentrations and the number of enterocytes per villus (Uni et al., 1996). These studies lay the groundwork for further inspections of the early GIT.

Marchaim and Kulka (1967) discovered that, prior to hatch, pancreatic enzymes start to be produced and increase in concentration closer to the time of hatch. The amylase concentration increases in a four step-wise increases with the greatest increase after 19 d of incubation (Ikeno and Ikeno, 1991). As early as 13 d of incubation, chymotrypsin concentration can be detected, and this level increases steeply after 18 d of incubation (Marchaim and Kulka, 1967). At 18 d of incubation, pancreatic trypsin is detectable in chicken embryos (Moran, 1985). Siddons (1969) found intestinal disaccharidases are detected at 12 d of incubation. The enzyme activity of brush border enzymes (sucrase, maltase, and aminopeptidase) are low at 15 d of incubation and remain constant until 19 d of incubation, at which time there is a marked increase in concentration, according to Uni et al. (2003a). Sucrase activity is detected at 10 d of incubation and steadily increases until hatch (Matsushita, 1985). In chickens, the enzyme activity of the pancreatic and brush border enzymes follow an increasing trend up until hatch, while turkeys have low to almost undetectable activity levels of amylase, lipase, trypsin, and maltase during incubation and just prior to hatch (Sell et al., 1991). Immediately following hatch, the gastrointestinal tract undergoes the most significant changes in development.

Post-Hatch Development

After hatch, the chick is exposed to a variety of exogenous factors that differ significantly from what the chick embryo encounters during incubation. As Sklan (2001) noted, such factors

as feed, water access, and the type of diet have a profound impact on the development of the chick. One of the most critical changes the chick experiences is the change in diet from a lipid-rich yolk diet to an almost exclusively grain-based diet high in carbohydrates (Sklan, 2001).

Lambson's early study (1970) found that once the chick begins to ingest carbohydrate-based exogenous nutrients, the internalized yolk sac undergoes a transition in utilization. During the stages of embryonic development the yolk is transported readily into the circulatory system of the embryo (Lambson, 1970). As the embryo nears hatch, the yolk sac is internalized and the yolk lipid is then transported into the small intestine by means of the yolk stalk, according to Esteban et al. (1991). Noy and Sklan (1999) stated that at hatch, the yolk sac comprises approximately 15 to 25% of a chick's body weight. The maintenance of the chick and intestinal growth is dependent on the utilization of the internalized yolk as the chick makes the transition to exogenous feed sources. The early presence of feed helps stimulate intestinal motility and will increase the rate at which the yolk is fully absorbed through the intestine (Noy and Sklan, 2001). In addition to yolk absorption, the GIT of the chick undergoes rapid development and morphological changes immediately following hatch.

Sklan (2001) also comments that a rapid relative (a measurement related to the animal's body weight) growth of the small intestine occurs starting at hatch and continues until 6 to 10 d of age. On an absolute basis, the weight and length of the small intestine increase with the broiler's body weight. By hatch, the small intestine comprises 3.5% of the chick's BW (Sklan, 2001). Dror et al. (1977) found that immediately after hatch, the segment with the greatest rate of relative growth is the duodenum, followed by the ileum and then the jejunum. Two days after hatch there is a 2% increase in small intestine weight and by 8 d of age the small intestine has increased 13% compared to birds at 2 d of age (Katanbaf et al., 1988). However, at or before 4 d

of age, the rate at which relative small intestine weight increases begins to slow, suggesting the end of rapid development in the small intestine (Noy and Sklan, 1997). Katanbaf et al. (1988) state that by 12 d of age, the weight of the small intestine weights plateau relative to the chick's BW. The relative length of the small intestine continues to decrease following hatch (Noy and Sklan, 1997).

Sklan (2001) also found that along with physical growth there is also an increase in villi size, which doubles within the first 48 hr following hatch. Early scanning electron microscopy observations in morphological changes in post hatch broiler chicks were conducted by Bayer et al. (1975). In day-old chicks the villi in the duodenum and jejunum has the appearance of broad finger-like projections or are narrow and plate-like in shape. The villi in the ileum are finger-shaped in appearance. Immediately following hatch the longest villus can be found in the duodenum, which Baranyiova and Holman (1976) found to be about twice the height of villi in any other segment. Around 4 d of age, when the relative duodenum growth rate starts to decrease; Noy and Sklan (1997) discovered a major increase in villus volume. From 4 to 10 d of age the villi height and perimeter increases 34 to 100%, compared to villi size at hatch. Bayer et al. (1975) found that by 7 d of age there were no visible differences between the villi of the jejunum and ileum. A dense covering of microvilli on the villi is present at all ages, even at hatch. As noted by Noy and Sklan (1997), the rate of villi growth in the jejunum and ileum begins slowing down by 10 d of age.

Various researchers also discovered that changes were also occurring within the intestinal cells of the villi, enterocytes, during this period of rapid development. The enterocytes are produced in the crypts, and during the younger ages it takes approximately 72 hr for the enterocytes to migrate up the villi, as the bird ages it can take up to 96 hr for these cells to

migrate (Geyra et al., 2001; Uni et al., 1998). Geyra et al. (2001) reported that immediately after hatch the proliferating enterocytes increase rapidly, but by 24 to 48 hr post hatch the crypts consist of 50% proliferating enterocytes. By 10 d post hatch there are approximately 6-15% proliferating enterocytes. Similar to the slowing of intestinal growth rate, there is also a decrease in the number of proliferating enterocytes as the chick matures. Furthermore, the enterocytes numbers can also be related to crypt growth, since the growth of the crypt's plateaus at 2 to 3 d of age. Based on intestinal cell and crypt measurements, one could suggest that at 2 to 3 d post hatch there is a slowing in the rate of intestinal growth and at 10 d of age intestinal development plateaus.

Noy and Sklan (1995) also found that the concentrations of digestive enzymes increase rapidly immediately following hatch and are crucial for the breakdown and absorption of nutrients. Much of the hydrolysis of complex molecules in the small intestine results from the secretion of pancreatic enzymes. The more important enzymes secreted from the pancreas are amylase, lipase, and trypsin, which are responsible for the digestion of starch, triglycerides, and polypeptides, respectively. Nitsan et al. (1991) showed that in the pancreas, there is a decrease in specific activity of amylase from hatch to 5 d of age followed by an increase to 8 d of age when activity levels off. Relative activity of amylase starts a gradual decrease after 8 d of age. According to Uni et al. (1995), the concentration of amylase secreted into the duodenum is low at 4 d of age and increases to 7 d of age, after which point it plateaus. The enzyme lipase is the main pancreatic enzyme for lipid digestion and has a specific activity that decreases after hatch to 6 d of age. Nitsen et al. (1991) further commented that after 6 d of age there is an increase in lipase specific activity to 21 d of age. The relative lipase activity increases from hatch to 8 d of age, at which point levels plateau and remain constant until 17 d of age when levels begin to

slowly decrease. The secretion of lipase from the pancreas increases from 4 to 21 d of age (Noy and Sklan, 1995). In the intestines, lipase activity gradually increases (by a factor of 2.5) with age until 23 d of age (Nitsen et al., 1991). At hatch the specific activity of trypsin in the pancreas starts to decrease until 4 d of age, after which levels increase up to 20 d of age. In the intestine the trypsin activity increases ten-fold from hatch to 15 d of age and then becomes steady, while the relative activity reaches a maximum earlier at 11 d of age, according to Nitsen et al. (1991). Noy and Sklan (1995) found that the daily net level of trypsin secretion in the duodenum follows a similar trend to specific activity with low levels at 4 d of age and then an increase up to 21 d of age. Chymotrypsin was reported to increase reaching maximum levels at 2 d of age and remain steady thereafter by Marchaim and Kulka (1967) and Nitsen et al. (1991).

Brush border enzymes are responsible for the final breakdown of molecules to their simplest form. The brush border enzymes are produced inside the enterocytes or its outer membrane. Uni (1999) found that after hatch of broilers there is a two-fold increase in sucrase activity, which remained stable until 35 d of age, after which point activity declined. Uni et al. (1998) observed lower mucosal sucrase and maltase activities in the duodenum vs. the jejunum and ileum of broiler chicks at 2 d of age. Maltase activity reaches a maximum level at 2 d of age and slightly decreases to 4 d of age, after which point there is a plateau (Uni et al., 1998). Chotinsku et al. (2001) discovered that at 7 d of age only traces of lactase and trehalase activity in the enterocytes could be detected in chicks. Tarvid (1990, 1992) reported very high levels of relative activity of aminopeptidase and dipeptidase at hatch, followed by a rapid decrease to 10 d of age. In the case of aminopeptidase there is an increase in relative activity at 15 d of age with different rates of increase in various segments of the small intestine. The dipeptidase relative activity continues to decrease from 10 to 40 d of age (Tarvid 1990).

The development and growth of the GIT is of the utmost importance to ensure optimal growth in poultry. A well developed GIT is crucial for utilization of exogenous nutrients and proper nutrient digestion and absorption.

Studies of the Factors Affecting Gastrointestinal Tract Development

When discussing the GIT development of poultry, it is important to remember that there are different types and strains of birds, many of which grow at significantly different rates. The poultry industry utilizes several different breeds of broilers and layers depending on the company's production parameters. The main difference in poultry breeds is the age at which maximal growth is reached. According to Katanbaf et al. (1988) and Lilja (1983), considering different breeds have varying rates of growth, one could assume that there is also a difference in their GIT development. A number of studies have been conducted to compare and/or contrast the morphological differences between various strains of meat type poultry as well as between meat and egg type birds.

Studies of the Effects of Genetics on Gastrointestinal Tract Development

Some of the first studies to evaluate the differences in the GIT development between different types of birds were conducted by Dror et al. (1977), who compared male chicks of a light breed (New Hampshire x White Leghorn) and a heavy breed (White Rock) by measuring the relative weight of the organs of the GIT, heart, and sections of the brain. The authors reported significant differences between the two breeds with a 17% higher BW in the heavy breed. The light breed had heavier relative weights of the pancreas, heart, and brain sections. There was no difference in relative weights of the whole GIT between breeds, but the duodenum and jejunum were heavier in the light breed birds, while the heavy breed birds had heavier ileum and ceca at 21 d of age. Dunnington and Siegel (1995) experimented with male White Plymouth

Rock chicks selected for high body weight (HW) or low body weight (LW) from hatch to 42 d of age. The authors reported that the HW chicks had heavier relative GIT weights for the first 10 d following hatch; after this point the LW chicks had a heavier relative GIT weight from 10 to 42 d of age. When comparing broilers of different crosses or of the same cross but genetically selected for ability to gain weight, an impact in the rate GIT develops becomes obvious because of body weight gain. Comparing lines of laying hens, Mitchell and Smith (1991) conducted an experiment with 3 different breeds of 6 wk old birds: highly selected (common commercial line), relaxed selected (a second type of commercial line), and an unselected line (brown leghorn). The highly selected commercial bird had the heaviest BW as well as absolute weights of all segments of the small intestine. The highly selected line also had the greatest villus surface area, followed by the relaxed and then the unselected line. However, on a relative weight basis the unselected brown leghorns had the heaviest small intestine weights for all 3 segments. These experiments suggest an inverse relationship between bird body weight and relative small intestine weight; heavier birds have lighter relative small intestine weight when compared to lightweight birds.

Yamauchi and Isshiki (1991) found that broilers, which are bred for rapid weight growth, have a faster rate of small intestinal development and an increased rate of morphological growth as evidenced by villi as compared to slower growing egg type birds. Early observations were made using a scanning electron microscope in the GIT of newly hatched White Leghorn (WL) and broiler (BR) males. At hatch the authors reported that both the WL and BR had uniform finger-like villi. By 10 d of age the BR had more goblet cells and a more developed villi. Within the first 10 d of age, the villi concentration of the small intestine was greater in the BR,

indicating more rapid growth compared to the WL. However, after 10 d of age, the WL had a greater concentration of villi

Uni et al. (1995) experimented with meat and layer-type birds using Arbor Acres (AA) broilers and Lohmann (L) layer-type chicks to determine differences between strains from 0 to 14 d of age. Uni and colleagues (1995) reported that AA chicks had larger and more organized villi at all ages and in all segments of the small intestine compared to the L chicks. Furthermore, the L chicks had a more rapid rate of food passage through the small intestine, and enzyme activity of amylase, trypsin, and lipase was lower in the L chicks than in the AA chicks. Uni et al. (1996) reported no difference in DNA concentrations between strains from 0 to 7 d of age, but the AA chicks had higher villi, deeper crypts, thicker lamina propria, a greater villus volume, and number of enterocytes per villus compared to the L chicks at all ages. The findings of Uni and colleagues support previous research on differences in developmental changes between broiler and layer type chickens, with the broilers having a more rapid rate of development. The increased rate of GIT development, in broilers could be considered one of the main contributing factors for the increased rate of body weight gain compared to layer-type birds.

Research comparing GIT development has not been limited to chickens. Applegate et al. (2005) compared turkey poults and Pekin ducklings for the first 7 d following hatch. Turkeys and Pekin ducks have similar egg weights, the researchers discovered that the ducklings had heavier and longer jejunum and ileum measurements and more rapid jejunal villi growth compared to the poults after hatch. The increase in small intestine growth allowed for the ducklings to reach a heavier body weight during the 7 d period, further suggesting the importance of villi growth in relation to rapid intestinal development and growth (Applegate et al., 2005). When Watkins et al. (2004) compared wild (Mallard) and domestic (Pekin) ducks,

they found no differences in the relative weight of the GIT as a whole during the first two weeks. After 2 wk of age the domestic duck maintained higher absolute body and GIT weights, but the relative weights of the GIT were heavier in wild ducks. Furthermore, Watkins and colleagues reported that domestic ducks had increased enzymatic activity and villi surface area, which may be the reason for the greater BW of the domestic duck, even though the wild duck had greater relative GIT weights. The greater relative intestine weight in the wild duck suggests an inverse relation between relative intestine weight and body weight.

Jamroz et al. (2004) compared male Shaver-Starbro chickens, Mulard cross-line ducklings, and Landaise goslings from 0 to 42 d of age fed standard diets in accordance with Polish Recommendations and National Research Council based on species specificity. The relative length of the small intestine in chicks was significantly longer than that of the ducks and geese. Jamroz et al. (2004) concluded that the intestinal tract develops earlier in the chicken than in waterfowl. The development of the GIT is affected by the strain and species, but what is consistent across poultry is the relationship of relative intestine weight and villi growth to BW. Birds with heavier weights had larger villi during the first 2 wks of age and lighter relative intestine weights compared to lighter weight birds.

Studies of the Effects of Feed Access on Gastrointestinal Tract Development

The practice of keeping chicks in the hatcher for a 24 to 36 hr time period after hatching to obtain maximal hatch is common in almost all commercial hatcheries. Some chicks may not have access to feed or water for up to 48 hr due to processing at the hatchery and transport, as Hill and Green (1997) and Misra and Fanguy (1978) have noted. Delaying birds access to feed and water for an extended period of time results in a loss of BW (Moran, 1990; Pinchasov and Noy, 1993). Upon hatch, the bird's GIT appears to need some form of exogenous nutrient

source, whether sawdust, semi-liquid feed, or solid feed in order to stimulate GIT development; This development may lead to improved weight gain (Noy and Sklan, 1999). Noy and Sklan reported that in Ross broilers the early exposure to an exogenous nutrient source increases BW 8 to 10% over chicks held 48 hr without a nutrient source. Breast meat yield was improved by 7 to 9% in the early fed birds. Bigot et al. (2003) studied the BW growth of broiler chicks and concluded that BW is depressed by a 48 hr delay in access to feed post hatch. Bigot and colleagues then went on to report that by 6 d of age the birds that were delayed feed access for 48 hr were 25 % lighter than birds fed immediately after hatch. Geyra et al. (2001) observed that when male Ross chicks were fasted from, 0 to 48 hr, 48 to 96 hr, or 144 to 196 hr, growth was depressed. When birds were reintroduced to feed, the growth was parallel to early fed control group. Even with access to feed, energy restriction of 1.5x $BW^{0.67}$ kcal ME/d from 7 to 14 d of age depressed chick body weights. When restricted fed chicks were given ad libitum feed, they were not capable of achieving the weights of the non-restricted control birds at the end of 48 d, according to Palo et al. (1995). Delaying access to feed and water post hatch not only has a negative effect on body weight gain, but also affects the development of the GIT (Baranylova and Holman, 1976; Uni et al., 1998).

Maiorda et al. (2003) and Uni et al. (1998) studied the phenomena of withheld feed further. When male broiler chicks are given immediate access to feed and water, they have higher absolute jejunum and ileum weights, while chicks denied feed or water for 72 hr had a greater number of villi per area with shorter villi (Maiorka et al., 2003). Uni et al. (1998) also reported that holding AA broiler chicks for 36 hr depressed jejunal villi volume up to and after 11 d of age, while in the ileum no difference was observed in villi volume between birds denied feed for 36 hr and those fed immediately up to 11 d of age.

The delayed access to feed may depress villi growth due to lack of stimuli, but there is an increase in the number of villi; thus, the bird can compensate for the depressed villi growth and still maintain adequate nutrient digestion and absorption. In 0 to 48 hr delay fed birds when villus surface area was depressed, Geyra et al. (2001) found that the numbers of proliferating cells in all segments of the small intestine were decreased. In addition, crypt depth was also depressed in the delay fed birds until 8 to 10 d of age, at which point duodenum and jejunum crypt depths were similar to those of immediately fed birds. Delayed access to feed for 48 hr impacts the size and concentration of goblet cells, and increases intestinal intracellular mucins (Uni et al., 2003; Geyra et al., 2001). Even though delayed access to feed will depress villi growth, the presence of feed will stimulate the gut for compensatory villi growth 3 to 4 d after feed access, resulting in similar villi surface area between early fed and delay fed broilers by 8 to 10 d of age (Uni et al., 2003a). When male broilers were denied feed and water for 72 hr after hatch, Smirnov et al. (2004) noted a marked depression in villus surface area and goblet cell size in all three segments of the small intestine at 28 d compared to immediately fed birds. Smirnov and colleagues also reported negative effects on muscle integrity of the small intestine due to the decrease in the thickness of the mucus adherent layer when birds were delayed feed.

The small intestine's structural characteristics were not only affected, but also experienced a change in enzyme activity when birds were restricted or had delayed access to feed. During a 48 hr delayed access to feed, trypsin and amylase activity remained low and increased only after the start of feed consumption, while lipase activity in delayed access fed birds remained similar to that of immediate fed birds, according to Sklan (2004). Tarvid (1992) and Susbill et al. (2003) found that proventicular proteolytic activity was significantly decreased due to feed restriction. After a 3 to 4 d feed deprivation, there was a marked increase of

pancreatic carboxypeptidase A total and specific activity over the ad libitum fed broiler chicks (Tarvid, 1992). Dipeptidases are the most sensitive to feed restriction, with a 2 fold increase in activity after a 24 hr feed deprivation period. However, no significant difference in digestive enzymes between meal fed birds (fed twice a day for a 3 hour period) and birds fed ad libitum appeared, according to Susbilla et al. (2003).

Researchers found that the occurrence of delayed access to feed also impacted the digestive system of turkeys. The delayed access to feed also depressed body gain in poults, similar to findings in chickens, according to Corless and Sell (1999), Potturi et al. (2005), and Noy et al. (2001). Corless and Sell (1999) delayed poults' access to feed and water for 54 hr after hatch and reported a depression in absolute weight of the small intestine, pancreas, and length of the small intestine from 0 to 5 d of age. The relative weights of the small intestine and pancreas depressed until 4 d of age, compared to the poults fed immediately (Corless and Sell, 1999). However, Noy et al. (2001) reported that when poults were delayed access to feed for 48 hr immediately following hatch, there was a slight increase in relative small intestine weight at the end of the fasted period. The increase seen in relative intestine weight between Noy et al. (2001) and Corless and Sell (1999) may result from the difference in delaying feed and the use of a different turkey genetic stock. Noy and colleagues observed that within the first 2 d after hatch, absolute small intestine weights were significantly lighter in delay fed poults compared to early fed poults. In delay fed poults, there was a rapid compensatory growth of the small intestine from 2 to 4 d so great that the absolute weight of the small intestine surpassed the early access fed poults. Also, a 48 hr delay to feed and water depressed villi growth and enterocyte numbers in all three segments of the small intestine compared to early fed poults (Noy et al., 2001). As Potturi et al. (2005) and Noy et al. (2001) noted, the small intestine was greatly

benefited with longer and wider villi, deeper crypts and more goblet cells when poultry were immediately fed, and the introduction of feed stimulated the rate of proliferation in the delayed fed poults.

Since the delay of feed access has a negative impact on GIT development in poultry, researchers have questioned whether GIT development could be improved if the embryo were supplied with exogenous nutrients prior to hatch. In one study, the early introduction of a nutrient source into the embryonic amniotic fluid (in-ovo feeding) had positive effects on the development of the GIT pre and post hatch. Tako et al. (2004) injected Ross embryos at 17.5 d of incubation with a carbohydrate source (CHO) or beta-hydroxy-betamethylbutyrate (HMB). Within 48 hr post injection villi width and surface area were increased over the control non-injected embryos. Even beyond hatch, at 3 d of age, villi surface area was still increased, and there was a 50% increase in the number of goblet cells in the jejunum compared to non injected embryos (Tako et al., 2004; Smirnov et al., 2006). The use of in-ovo feeding also stimulated jejunal sucrase-isomaltase and maltase activity, by increasing enzymatic activity to almost 50% greater than non injected birds at 3 d of age, as reported by Tako et al. (2004).

Studies of the Effects of Diet on Gastrointestinal Tract Development

According to several researchers, the use of various ingredients in commercial poultry diets change constantly based on availability and price, and such changes can have diverse effects on the development of the GIT (Rosebrough et al., 1979; Moran, 1989; Carew et al., 1972). A variety of cereal grains, corn, wheat, rye, and barley, used in poultry diets serve as the main energy or carbohydrate source and these grains can impact GIT development. A major component of some of these cereal grains is non-starch polysaccharides (NSP), which are considered to have anti-nutritional factors and result in adverse effects in bird performance and

GIT development, as Smits and Annison (1996) noted. The presence of NSP has been reported to increase crypt depth and villi height in the ileum while reducing enterocyte numbers in the jejunum (Iji et al., 2001). Iji et al. (2001) also reported a decrease in jejunal maltase and alkaline phosphatase from 0 to 7 d of age in birds fed NSP. On the other hand, the insoluble fraction of the NSP seemed to have beneficial health factors in humans, but in chickens only a few studies provided evidence of any beneficial effects of NSP on intestinal development (Riddell, 1976; Hetland et al., 2004).

In exploring the affects of NSP in broiler diets further, Iji et al. (2001) fed mix sexed Steggles x Ross broiler chicks NSP components from 7 to 21 d of age. The birds were fed a standard broiler diet supplemented with a 10% (Exp 1) and a 5% level (Exp 2) of NSP, consisting of alginic acid (AL), guar gum (GG), gum arabic (GA), or gum xanthan (GX). Each of the NSP impacted the GIT differently compared to control birds fed diets lacking NSP supplementation. Guar gum resulted in heavier intestine weights, while there were no differences in other organs such as the proventriculus, gizzard, and liver. The supplementation of GX resulted in deeper jejunal crypts. Birds fed the GA had longer ileal villi, while GA and GG supplementation resulted in the greatest villus surface area. Brush border enzymes were also impacted differently by the NSPs. Alginic acid supplementation caused the highest jejunal maltase and sucrase activity, while birds fed the GX supplemented diets had the lowest levels of jejunal maltase and sucrase activity. As Read (1987) discovered, the uses of water solutions of xanthan, guar and locust bean gums, commercial NSP for human consumption, have been observed to decrease glucose motility when tested in vitro. However, in vivo testing failed to demonstrate any effect on amino acid uptake when presented with NSP (Iji et al., 2001). Thus,

Iji et al. (2001) speculated that supplementing a diet with NSP will result in increased villi measurements to compensate for the decrease in nutrient availability.

Several researchers observed that dietary fiber had a dramatic effect on GIT development post hatch. Jorgensen et al. (1996) fed three different fiber sources (Pea fiber, wheat bran and oat bran) each at the inclusion levels of 0, 18.7, and 37.5 % of the diet to Ross 208 chicks. The dietary treatments were fed for 4 wk starting at 12 d of age, and findings showed that with the increase in crude fiber there was also an increase in the weight of the digestive system. Of the three feed ingredients, the increase in digestive size was most affected by pea fiber, but feeding oat bran had resulted in the largest decrease in digestibility of the diet. Rakowska et al. (1993) believed that the decrease in digestibility was related to the higher percentage of crude fiber in the oat bran. Furthermore, when chickens received a diet of 80% rye, damage to the intestinal villi and epithelium of the small intestine occurred. Work was also done with domestic geese to evaluate the impact of fiber on the development of the GIT. Yu et al., (1998) fed 6 wk old (beyond the stages of early development from 0 to 14 d of age) female White Roman geese for a 4 wk period the following ingredients in order to obtain a better understanding of the effect of different fiber sources on the development of the GIT: alfalfa meal, barley bran, rice hulls, cellulose, pectin, or lignin at an inclusion rate of 27, 24, 13, 6.3, 6.2, and 6.2%, respectively. The authors reported no difference in the length of the small intestine or colon-rectum due to the various fiber sources. Small intestine weights were similar for all treatments except for the pectin groups that had lighter relative weights and cecal length. However, the fiber source had a significant effect on ceca and colon-rectum weight, with the geese fed the barley bran having the higher weights and lengths compared to the pectin group. Based on villi measurements of the small intestine segments, researchers found no differences due to fiber source, but the birds fed

the cellulose and rice hulls were observed to have flatter folds in the cecal mucosa. The similarities in small intestinal weight, length, and villi may result from the use of older birds (6 wk of age) that were beyond the stages of early development and at the point of a developed GIT. In other birds besides broilers, researchers have found that the feeding of fiber impacted the GIT differently. Jamroz et al. (2001) wanted to compare the effects of adding 40% barley to the diets of Shaver-Starbro broiler chickens, Mulard ducks, and Landaise geese in accordance to the species based on the 1996 Polish Academy of Science for nutritional requirements . The inclusion of barley from 0 to 42 d of age resulted in longer absolute intestine lengths in chickens and geese than those of ducks at 21 d of age, but at 42 d of age there was no difference in intestinal lengths among the three species. Borin et al. (2006) also compared the GIT of chickens and ducks when fed 0, 7, 14, and 20% cassava leaf meal to Cambodian chickens and ducks, commercial broiler chickens, and White Pekin ducks from 0 to 90 d of age were used in this study. Increased levels of cassava leaf meal, resulted in an increase in the relative weight of the small intestine, ceca, gizzard, and pancreas and absolute length of the small intestine and ceca in both chickens and ducks. The results from Borin et al. (2006) suggested an increase in GIT organ weights and length with increasing levels of fiber fed to birds.

Researchers have worked with supplying cereal grains in different forms such as wetting or keeping in whole or ground form, in attempts to improve bird performance. Yasar and Forbes (1999) experimented with male Ross chicks from 7 to 42 d of age. The birds were fed mash diets containing 60% to 70% ground wheat barley or oats, and water was added at 1.3 kg per kg of dry feed. By wetting the diet, researchers found that birds had an increase in the absolute weight of the intestine, but on a relative basis there was no difference in intestine weight or length. Additionally, the birds fed the wet diet had increased villi height in the small intestine,

ceca, and colon, but a reduction in crypt cell proliferation rate, according to Yasar and Forbes (1999). When male broilers were fed a pelleted diet consisting of either 20% wheat or triticale in a ground or whole state from 17 to 42 d of age, no differences in relative intestinal length measurements between treatments appeared, as Jones and Taylor (2001) discovered. Jones and Taylor (2001) observed an increase in the proventriculus weight in birds fed the ground form, while birds fed the whole grain form had heavier gizzard weights, indicating the ability of large fiber particles to stimulate development of different GIT segments. To magnify the changes in GIT developmental due to diet, Batal and Parsons (2002) experimented with New Hampshire x Columbian Plymouth Rock male chicks fed a corn-soybean meal diet and a crystalline amino acid (AA) diet. Absolute weights of the small intestine, pancreas, liver, and gizzard were depressed by feeding the crystalline AA diet from 0 to 21 d of age, while feeding the crystalline AA from 8 to 21 d of age resulted in organ and small intestine weights falling between the birds fed the corn-soybean meal and crystalline amino acid diets fed from 0 to 21 d of age. On a relative basis the small intestine, pancreas, and liver weights of chicks fed the crystalline AA diet were depressed up to 7 d of age, but at 21 d of age there was no difference in relative weights. Batal and Parsons believed that the poor growth in the early stages of development resulted from slower gut development in the chicks fed the crystalline AA diet. The villi height and crypt dept was significantly depressed in the crystalline AA fed birds, a finding which further supported the idea of the slower growth and development of the GIT due to the lack of particle stimulation.

Studies Concerning Intestinal Bacteria

In the GIT resides a massive bacterial population that is a highly evolved community which interacts with the host on a co-mutualistic level. The presence of the bacterial community has attracted the interest of many researchers for hundreds of years. The bacteria residing in the

GIT is a massive symbiotic population that researchers suggest may aid in nutrient digestion, immunity, and physiological development of the host (Berg and Savage, 1972; Berg, 1996). In 1719, Leeuwenhoek was the first to make a microscopic observation of fecal bacteria, and, in 1885, Escherich initiated some of the first research pertaining to intestinal micro ecology (Chierici et al., 2003). This early examination of the gut bacteria intrigued many researchers and triggered future examination of the impact of the intestinal bacteria, which in turn led to advances in technological detection methods. There is an enormous amount of bacteria present in the GIT and fecal matter, and 40 to 80% is uncultivable (Sarma-Rupavtarm et al., 2004). The large and uncultivable bacteria present a problem to researchers in their attempts to determine what bacterium is actually present in the GIT and in what percentage. On the other hand, the development of germ-free animals has increased our knowledge of the effect of the intestinal bacteria on the development and function of the mucosal immune system (Gaskins, 2005). The normal intestinal bacterial community resides in the micro-ecosystem of the GIT and undergoes many of the same ecological principles encountered by the host animals. Environmental conditions, food source, and competition for space and food are some of the ecological factors that shape the diversity of the intestinal bacteria in the GIT of the host. Many people associate bacteria with having only negative effects, but in actuality the number of "beneficial" bacteria acting in a symbiotic manner with the host far exceeds the pathogenic bacteria, according to Tellez et al. (2006). From a symbiotic standpoint, the GIT of the bird provides an environment and a substrate source for the bacterial community; in turn, the intestinal bacteria provides nutrients to the host and acts as a defense from pathogens through means of competitive exclusion, as Draser and Barrow (1985) discovered. The preferred substrate of the intestinal bacteria is carbohydrates, which the bacteria are able to ferment to produce volatile fatty acids

and protein in the source of bacterial biomass which can benefit the host (Apajalahti, 2005). The host provides a food source (substrate) to the bacterial community, which can dictate the biodiversity of the intestinal bacteria. Through substrate specificity and the use of prebiotic feedings, the percentage of "beneficial" bacteria in the host can be increased, as Sonnenburg et al. (2005) and Lan et al. (2005) suggest. Researchers want to understand the symbiotic interaction of the bacterial community to improve the health and performance of the host.

The intestinal bacterial populations are impacted by a number of factors, and how these factors affect the bacterial population is complex. Binek et al. (2000) found that immediately following the hatch of a chick, the GIT comes into contact with bacteria from the environment, which initiates the development of the intestinal bacteria (Binek et al., 2000). The microbial population can be affected due to the ingestion of various bacteria from hatchery debris, feed, and water; factors at farms such as litter management, cleaning of houses between flocks, and bio-security can also affect the populations, according to Smith (1965), Barnes et al. (1972), and Mead and Adams (1975). A number of researchers have done work on examining how different external factors impact the intestinal bacteria of poultry, but none of the studies have taken into account poultry development in association with the bacterial community.

Factors Affecting the Bacterial Community of the Gastrointestinal Tract

The bacterial population in the gut is a large and diverse population that is ever-changing and is affected by a number of factors, such as diet and environmental conditions (Apajalahti, 2005). The biodiversity of the intestinal bacteria changes with age and varies at different segments of the GIT (spatial variation). Thus, this subject provides continuing interest to many researchers.

Effects of Age and Gastrointestinal Tract Spatial Location on Bacterial Community

Lu et al. (2003) analyzed ileum contents from birds at various ages ranging from 3 to 49 d of age using genescan or terminal restriction fragment length polymorphism (T-RFLP). T-RFLP allows for the determination of percent concentration of a specific bacterium. Lu et al. (2003) reported dramatic changes in the bacterial community as birds aged. At 3 d of age the dominant intestinal bacterial population was *Lactobacilli delbrueckii*; from 7 to 21 d of age *L. acidophilus* was dominant; by 49 d of age *L. cripatus* and *L. salivarius* were the dominant species of *Lactobacilli* (Lu et al., 2003). *Lactobacilli* is the dominant bacterial genus within the ileum, but the dominant species of *Lactobacilli* was different at all ages, suggesting the importance of the composition of the bacterial community on a species level.

Hume et al. (2003) analyzed the intestinal bacteria of Hyline W-36 chicks fed the same diet at various ages in the jejunum, ileum, ceca, and colon using denaturing gradient gel electrophoresis (DGGE). The authors reported a clustering or similarity in the bacterial community in the ceca of chicks less than 20 d of age, while birds older than 20 d of age had a separate bacterial grouping that was similar within their age range. However, there was no similarity in the intestinal bacterial community between chicks less than 20 d of age and those greater than 20 d of age, and as the leghorn chicks aged, the bacterial community changed. Hume and colleagues found that the closer in proximity the bacterial community from one segment of the GIT is to the other; the greater the similarity will be in the bacterial community composition.

Amit-Romach et al. (2004) conducted a study with broiler chicks and observed the microbial population at 4, 14, and 25 d of age in the duodenum, jejunum, ileum, and ceca. They used the16S rDNA gene of bacteria and analyzed the concentration of certain bacteria groups

such as *Bifidobacterium Lactobacillus*, *E. coli*, *Clostridium,* and *Campylobacter* through genescan. In the ceca, the levels of *Lactobacilli, Campylobacter, E. coli*, and *Clostridium* remained fairly constant between 4 to 25 d of age. However, as birds aged, the levels of *Bifidobacterium* increased, while the levels of salmonella decreased. In the 3 segments of the small intestine at 4 d of age, the intestinal bacterial population was dominantly *Lactobacilli* with the presence of *Clostridium* in the jejunum and ileum. By 25 d of age, there was an increase in *Lactobacilli, E.coli*, and *Clostridium* in the duodenum, while in the jejunum and ileum, *Lactobacilli* levels changed very little. In the jejunum *Clostridium* levels had decreased, but *Clostridium* levels were constant in the ileum, and there was an increase in *E. coli* levels by 25 d of age. These results suggest the duodenal changes that occurred through development made the environment more suitable for *Clostridia* and *Lactobacilli*, while in the other segments the bacterial community was not impacted as greatly with only small changes in bacterial populations.

Lu et al. (2003) also studied changes in the bacteria populations between the ileum and cecum at specific ages from 0 to 49 d of age. An obvious change in bacteria populations occurred as birds aged and the ileum developed. The bacterial community differed significantly from 3 d of age to 49 d of age, even though the bacterial communities did not significantly change from 7 to 21 d of age and 21 to 28 d of age (Lu et al., 2003). The results from the previous reports indicate a dramatic change in the bacterial communities as the bird ages. Lu et al. (2003) reported a dominant population of *Lactobacilli* in the ileum. However, the ceca community was dominated by *Clostridium*, indicating a dramatic difference in intestinal bacterial biodiversity due to spatial variation with differences in pH and morphological structure between the ileum and ceca.

Bjerrum et al. (2006) examined the intestinal bacterial population of the ileum and reported a dominant population of *Lactobacilli*, a finding which agreed with Lu et al. (2003). Gong et al. (2002) found similar results in Ross/Ross birds at 6 wk of age, with the ileum having a less diverse intestinal bacterial community than did the ceca, *Lactobacilli* being one of the major bacterial populations in the ileum. The difference in the bacterial community in intestinal segments was observed in Cobb chicks older than 4 d of age. But prior to 4 d of age the crop, duodenum, and ileum had similar intestinal bacterial populations (Van der Wielen et al., 2002). The authors' results indicate similar intestinal bacterial populations during the early stages of development between segments of the GIT. However, as the bird ages and the GIT becomes more defined in its structures and environmental conditions, the bacterial community changes and becomes established based on the conditions of that particular segment of the GIT.

A study was conducted with the same line of broiler chicks from 5 different hatcheries to determine if there was a variation in microbial communities due to the hatchery (Pedroso et al., 2005). Pedroso et al. (2005) reported different microbial populations in 1 d old birds from different hatcheries. The authors suggest that the difference in bacterial communities may have resulted from bacteria introduced pre-hatch or post-hatch from bacteria in the hatchery environment or possibly from the bacteria acquired during transport. Beyond the possible effects of the hatchery, there is also an impact on the intestinal bacterial community from different location and growing environments of the farm. In a study conducted by Bjerrum et al. (2006), the bacterial community of the ileum and ceca in birds raised at a conventional farm (Ross 208) were compared to the intestinal bacteria of birds raised at an organic farm (Scan Labelle 657). Samples were taken at 41 d of age, and the organic birds had significantly higher levels of *Clostridium* perfringens than did the conventional birds. This difference was believed to result

from the feeding of salinomycin to the conventional birds. The 2 different broiler types also harbored very different species of *Lactobacilli* in the ileum. The use of different diets added too many confounding factors to determine what was causing the change in the intestinal bacterial community. The bacterial community is ever changing and can be affected by factors other than spatial variation and age, such as diet and the use of antibiotics, prebiotics, or probiotics.

Effects of Diet on the Bacterial Community

Researchers have questioned the ability of the diet to affect the intestinal bacterial community as well as how it may aid in improved digestion. Much of the early work used various carbohydrate sources as a means of altering intestinal bacteria, suggesting that bacterial populations could be changed through diet (Rettger and Cheplin, 1921; Evenson, 1947; Johansson et al., 1948). Johansson et al. (1948) worked with Single Comb White Leghorns and fed different rations containing sucrose, dextrin, lactose plus sucrose, or whey plus sucrose, which were the sole carbohydrate sources. The experiment lasted from 4 to 10 wks of age, and the fecal droppings were collected and analyzed using agar media plates. The authors concluded that there was a significant impact on the bacterial population due to various carbohydrate sources. Pullets fed the dextrin diet had a large population of coliform bacteria and in pullets fed the lactose-based diet there was a large population of lactic acid-producing bacteria. At the time researchers assumed that the only factor changing the bacterial populations was the carbohydrate source, because the bacteria in the gut have been observed to be substrate specific, according to Sonnenburg et al. (2005). However, it is possible that different carbohydrate sources changed the intestinal structures, which in turn created different environments or niches for specific bacteria to thrive. Furthermore, analyzing only the excreta is not taking into account the varying bacterial populations due to spatial variation of the intestinal segments, and advances in DNA

based technology have allowed for more precise and accurate detection of specific intestinal bacteria.

Researchers considered the idea of using the same substrate but in different forms, ground or whole, to evaluate the change the intestinal bacteria. Bjerrum et al. (2005) fed ground or whole wheat to Ross 208 chicks at levels of 0, 6, 10, 20, and 30 % of the diet, increasing with age from 0 to 41 d of age. At 15 d of age the birds were infected with *Salmonella typhimurium*. By increasing the levels of whole wheat in a diet with age, a decrease in gizzard pH and a significant reduction in *Salmonella typhimurium* and *Clostridium perfringens* counts in the gizzard and ileum resulted. Birds fed diets with only ground wheat had no reduction in bacterial counts of *Salmonella typhimurium* and *Clostridium perfringens* with age. The author suggested that the gizzard serves as a barrier organ to pathogens, preventing entrance into the distal end of the small intestine due to the low pH. A more in-depth examination of using different forms of wheat stored conventionally or in an air-tight silo and fed in a ground or whole state from 15 to 42 d of age was conducted by Engberg et al. (2004) to determine the impact of wheat on the bacterial community and the GIT environment. Engberg and coworkers also reported a decrease in gizzard pH and an increase in ileal viscosity when birds received diets with whole wheat, compared to the control birds that received ground wheat stored conventionally. Bjerrum et al. (2005) and Engberg et al. (2004) both concluded that the use of whole wheat in diets can stimulate gizzard function to lower pH and decrease pathogenic bacteria from entering the intestinal tract, a response which decreased the numbers of enterobacteria and *Clostridium perfringens* in the ileum and ceca.

In an earlier study, Engberg et al. (2002) fed wheat-based diets that were either coarsely or finely ground and in either pellet or mash forms to Ross 208 chicks from 1 to 42 d of age.

Engberg and colleagues reported that feeding pellets improved bodyweight gain, decreased gizzard weight, decreased intestinal pH, and significantly lowered relative pancreas weight. Additionally, the birds fed the pelleted feed had increased enterococci and coliform bacteria in the ileum, while reduced *Clostridium perfringens* and *Lactobacilli* in the ileum. The results suggest that the presence of large particles in the gut is more important to stimulate GIT function by lowering pH to decrease pathogenic bacteria. The findings from Bjerrum et al. (2004) and Engberg et al. (2002 and 2004) strongly support the finding that changing intestinal conditions by diet manipulation either through ingredients or diet form can alter the bacterial niche, resulting in changes in the overall bacterial community.

Anti-nutritional factors of certain feed ingredients are still an issue that poultry nutritionists face today. Non-Starch polysaccharides are common anti-nutritional factors found in poultry diets and must be held at a minimum. The addition of NSP decreased performance due to the increase in intestinal viscosity (Moran, 2006; Iji, 1999), which reduced the exchange rate of nutrients with the mucosa and altered the GIT microbial populations with a higher level of *Lactobacilli*, according to Moran (2006). In a study conducted by Van Leeuwen et al. (2004), broiler chicks were fed diets with and without the inclusion of pectin (a form of NSP). At 7 d of age the chicks were inoculated with non-virulent *Salmonella typhimurium* in order to observe microbial activity. At 21 d of age the chicks fed the diet containing pectin had depressed performance and villi that appeared to be poorly developed. Furthermore, the birds that received the pectin diet and then were inoculated with the *Salmonella typhimurium* resulted in intensifying the effects of depressed performance and poorly developed villi seen in birds fed the pectin diet alone. The authors suggest that when birds were fed a diet high in NSPs, intestinal

viscosity increases, allowing for the pathogenic bacteria to reside more effectively in the GIT of birds and decrease performance and development.

Langhout et al. (2000) conducted a 2 x 2 factorial experiment to further support the conclusion that NSP decreases performance and changes the bacterial community by testing the effects of intestinal viscosity in conventional and germ-free chicks due to the presence of NSP in a diet. To increase intestinal viscosity, half the chicks received a diet with 30g/kg of highly methylated citrus pectin (HMP) in a corn-based diet. The feeding of diets with HMP to conventional birds resulted in a significant decrease in the percent of classic zigzag-shaped villi, a decrease in ileum pH, and an increase in small intestine weight compared to conventional birds fed the HMP free diets. However, feeding the germ-free chicks diets with HMP had little effect on the percent appearance of villi, intestinal pH, and small intestine weight. Bacterial counts were not measured, but conventional birds fed the HMP diet had an increase in lactic acid concentration, suggesting an increase in microbial activity. In the germ-free birds fed the HMP diet, there was no lactic acid, formic acid or volatile fatty acid, suggesting the lack of microbial activity in germ-free birds. Langhout and coworkers concluded that the increase in intestinal viscosity due to feeding HMP decreased the passage rate of the digesta and changed GIT conditions, allowing other intestinal bacteria to reside and possibly compete for nutrients. Linseed is another feed ingredient that contains a number of anti-nutritional factors such as mucilage, linatine, trypsin inhibitors, and phytic acid (Madhusudhan et al., 1986; Bhatty, 1995), which can cause a depression in the growth rate of broilers (Ajuyah et al., 1991; Lee et al., 1991). In a study by Alzueta et al. (2003) with Cobb chicks that receiving whole and demucilaged linseed at 16% of the diet from 0 to 23 d of age, the birds fed the whole linseed had decreased growth and increased ileal viscosity, which the researchers believed resulted in a

change of the bacterial community by significantly increasing the count of *Lactobacilli* compared to the birds fed the demucilaged diet. The results from Alzueta et al. (2003) suggest that high levels of *Lactobacilli* in the GIT may be a positive sign and could be associated with a negative effect on body growth.

The inclusion of NSPs is not the only means of manipulating the intestinal bacteria through diet means. Researchers have also considered the fat source. Oviedo-Rondon et al. (2006) reported that the use of essential oil blends can help to maintain a constant bacterial community in the gut of birds when exposed to a challenge. However, by using different sources of fat, producers can change the biodiversity of the microbial community. Using DGGE analysis, Knarreborg et al. (2002) evaluated changes in the microbial community of the ileum of Ross 208 chicks when diets were supplemented with soybean oil or a mixture of lard and tallow at a 10% level. Knarreborg and colleagues took ileum samples at 7, 14, 21, and 35 d of age for microbial analysis and observed that the intestinal bacteria were dependent on age. The use of soybean oil in diets decreased the level of *Clostridium perfringens* in the ileum of broilers compared to birds fed diets with the lard and tallow mixture.

The differences in diets change intestinal development and the residing bacteria, but depriving the bird of feed also leads to changes in the intestinal bacterial community. To test the impact of feed deprivation on the bacterial community, Binek et al. (2000) housed birds in an isolation chamber to prevent external confounding factors. The concentration of intestinal bacteria increased greatly 1 to 3 hr after hatch as compared to the intestinal bacteria level 1 to 3 hr prior to hatch. There was a shift in the bacterial community present in the ceca from *Enterobacteriaceae* and *Enterococcus* spp. 1 to 3 hr prior to hatch to *E. coli, Lactobacillus,* and obligate anaerobes 1 to 3 hr post hatch (Binek et al., 2000). Furthermore, there was a significant

difference in bacterial populations between the fed birds and the delayed feed access birds, a finding which could suggest that chicks presented with feed had an earlier establishment of bacteria in the micro-ecosystem of the GIT.

Molting hens are also deprived of feed. The presence of bacteria, particularly *Salmonella enterica,* is a major issue in the egg industry (Holt et al. 1995). When feed is restricted during a molt, the levels of *S. enterica* can increase 100 to 1000-fold and result in bloody GIT secretions, according to Holt and Porter (1992). Furthermore, the GIT of molted hens infected with *S. enterica* had increased inflammation of the epithelium and lamina propia of the ceca and colon compared the non-molted infected hens. The results suggest that the commensal bacteria of the GIT are not suited for the change in intestinal structure during the feed withdrawal period, a result which changes the niche and thus affects the intestinal bacteria present. By not performing a complete feed withdrawal during molt, producers can retain the intestinal bacteria in the crop, fermentative activities remain similar to those of non-molted hens, and the chance of *S. enterica* infection can be minimized, according to Ricke (2003). The use of pre and probiotics are methods which can be implemented to maintain the bacteria of the GIT.

The principle of prebiotics in the livestock industry is to modulate a bacterial community by increasing the number of non-harmful bacteria for competitive exclusion, a condition which improves the health of the animal (Lan et al., 2005). Prebiotics are non-digestible feed ingredients that benefit the host by increasing the native intestinal bacteria by providing a substrate to encourage commensal bacterial growth. Gibson and Roberfriod (1995) decided that these result in improved host health. The use of non-digestible oligosaccharides that contain fructose, galactose, glucose, and xylose are the most common forms of prebiotics (Gibson and Roberfroid, 1995; Gibson, 1998). Fructooligosaccharides (FOS) were proven to be an excellent

example of a prebiotic for humans (Gibson, 1998). The use of prebiotics is aimed at targeting the growth of a particular bacterial population in the intestine, and a 0.4% addition of FOS to a broiler diet from 0 to 49 d of age promoted *Bifidobacterium* and *Lactobacillus* growth, while inhibiting *E. coli*, as Xu et al. (2003) found. With the change in bacterial community by feeding 0.4% FOS, Juskiewicz et al. (2006) found an improvement in body weight gain and feed efficiency and an increase in protease activity, jejunal and ileal villi height, and microvilli height. However, feeding of FOS at 0.5, 1 and 2% of the diet to turkeys from 3 d to 8 wk of age did not have the same improved weight gain or feed efficiency benefits as seen in broilers (Juskiewicz et al. 2006). Once again, feeding an ingredient to different types of poultry does not result in the same effect.

Beyond FOS, many other prebiotcs have been tested such as isomaltooligosaccharides, reported to increase *Bifidobacterium* and decrease *Salmonella* in an insitu environment by Chung and Day (2004). Another prebiotic that can provide a substrate for commensal bacteria in the GIT is the use of mannanoligosaccharides (MOS). When mannanoligosaccharides were fed to Ross chicks at a 0.2% level, no effect on bird performance from 0 to 21 d of age occurred. However, when MOS was fed from 0 to 42 d of age weight gain improved, according to Albino et al. (2006). The results suggest that the MOS prebiotic does not have an immediate impact on bird performance and needs to be fed throughout the birds' lives to provide substantial amounts of substrate to the commensal bacteria, improving performance. Sims et al. (2004) fed turkeys a diet of 0.1% MOS, 0.55% bacitracin methylene disalicylate, or a combination of the two from 0 to 18 wk of age. The birds fed either the MOS or BMD diet were heavier than the control birds, and, when fed the combination, the birds had the heaviest BW and lowest feed conversion of all the birds receiving the other treatments. Sims and colleagues reported that feeding either the

MOS or BMD diet lowered the level of *Clostridium perfringens* in the ceca, suggesting a performance benefit when levels of the bacterial community shifted to have lower levels of *Clostridium perfringens*. The results of prebiotic use indicate improved performance through a shift in the bacterial community, which decreases the counts of pathogenic bacteria. Feed ingredients and prebiotics change the bacterial populations, but there is also the use of antibiotics, which are specifically designed to target bacteria and have been commonly used in commercial diets at sub-therapeutic levels since the 1950s (Fuller, 1989).

Effect of Antibiotics and the Bacterial Community

The recent concern over antibiotic use has encouraged researchers to further examine the impact of antibiotics on the intestinal bacteria. Engberg et al. (2000) experimented with two forms of antibiotics (zinc bacitracin and salinomycin) to determine the changes in intestinal bacteria in Ross 208 broilers from 0 to 42 d of age. At the termination of the experiment the gizzard, segments of the small intestine, cecum, and rectum were collected for the bacterial analysis of anaerobic bacteria, coliforms, lactic acid bacteria, *Lactobacilli, enterococci,* and *Clostridium perfringens*. A significant decrease in the counts of *C. perfringens* and *Lactobacilli salivarius* in the ceca occurred when diets were supplemented with zinc bacitracin or salinomycin alone or combined, which may indicate that high counts of *Lactobacilli* (at least *Lactobacilli salivarius)* may not be as beneficial as once thought and could be playing a role in growth depression due to a possible competition for nutrients (Engberg et al., 2000). Pedroso et al. (2006) fed three different antibiotics (Avilamycin, bacitracin methylene disalicylate and enramycin) in two different rearing environments (battery pens vs. floor pens). Using DGGE to analyze the differences in microbial populations, Pedroso and colleagues discovered little difference in intestinal bacterial community due to rearing environment, but the use of antibiotics

significantly altered the bacterial community and improved weight gain. Knarreborg et al. (2002) also fed subtherapeutic levels of antibiotics (Avilamycin) to Ross 208 chickens from 0 to 35 d of age. The presence of Avilamycin in the diet changed the bacterial community by decreasing *Clostridium perfringens* levels compared to the control birds, and there was an increase in *streptococci*, *enterobacteria*, and *Clostridium perfringens* with age, regardless of treatment. Lu et al. (2006), using TRFLP to analyze the microbial population, reported a significant decrease in *Lactobacilli* levels when birds received diets containing monensin. Additionally, an increase in *Clostridium* spp. and the presence of *Clostridium lituseburense* and *Clostridium irregularis* in the monensin fed birds appeared a finding which was not seen in the control birds. Based on the high levels of *Clostridium* and the fact there was no incidence of intestinal disease or mucosal legions in birds supplemented with monensin, the authors concluded that the *Clostridia* present was non-virulent. Thus, antibiotic use can change the microbial population to improve performance, but the presence of live bacteria (probiotics) into the GIT might also cause a shift in the microbial population.

Effect of Probiotics on the Bacterial Community

Many of the studies with probiotics are based on the principle of competitive exclusion, which prevents adherence of other bacteria to the intestinal ecosystem. This exclusion results in an increase of a particular bacterial population in the GIT, according to Hofacre et al. (2002). Much of the early work with probiotics in poultry used the *Lactobacilli* bacteria, based on the positive health responses seen in humans when ingesting *Lactobacilli* cultures (Fuller, 1989). However, chicks are not normally exposed to *Lactobacilli* following hatch, considering that chicks have a difficult time digesting lactose, which is rich in lactic acid-producing bacteria such as *Lactobacilli* (Leesons and Summers, 2001). Unlike chickens, humans receive much of their

nutrients from breast milk until they are weaned (Caicedo et al., 2005). The first work with presenting bacteria to poultry with the supplementation of 10^6 cfu/g of *Lactobacilli acidophilli* in the broiler diets resulted in similar growth performance to that of birds fed antibiotics (Larousse, 1970). *Lactobacilli acidophilus* were further tested in a water application at a concentration of 10^6 cfu/l. Toruero (1973), in an early study, found that body weight gain and feed efficiency of birds exposed to *Lactobacilli acidophilus* were similar to those of antibiotic fed birds and had improved performance compared to antibiotic-free birds.

Branching out and trying other *Lactibacilli* cultures, Jin et al. (1998) isolated *Lactobacillus* cultures from adult chickens and fed them at 10^9 cfu/ g of feed at 0.05, 0.10, and 0.15% of the diet. Jin and colleagues reported improved body weight gain and feed efficiency compared to the control birds at the 0.05 and 0.10% levels of mixed *Lactibacilli* cultures, but not at 0.15%. The lack of improved performance suggests that feeding a bacterial culture can aid performance, but too high a level may prove pointless. Furthermore, the feeding of the *Lactobacilli* cultures at all levels had little effect on total aerobe, anaerobes, *Lactobacilli* and *Streptocci* counts in the small intestine.

Most of the early work with probiotics was based on growth performance. Yurong et al. (2005) exposed chickens to a much greater percentage of bacteria ($4x10^9$ cfu per chicken per day for 3 d) in the water using *Lactobacilli acidophilus* in combination with *Bacillus subtilis*. The researchers reported increases in IgA in intestinal fluid, IgM in Peyer's Patch and cecal tonsils, IgG cells in the intestinal fluid, and T lymphocytes in the cecal tonsils at 7 to 10 d of age. The microvilli density also increased at 3 d of age, suggesting improved health and nutrient absorption, a result which may be the basis behind the increase in growth performance seen in the past. For example, Fuller reported that probiotics have improved health in a state of heat

stress where the bacterial community is affected. The use of *Lactobacilli* strains fed at 10^7 cfu/ g of feed can restore the bacterial community of the GIT to a state comparable to non heat stressed birds. A diet containing a toxin can also have negative effects on poultry health when consumed. When a broiler is exposed to deoxynivalenol, there might be no impact in growth performance, but the villi in the duodenum and jejunum are shorter and thinner (Award et al., 2006). Award et al. (2006), reported that through the use of a probiotic such as *Eubacterium* sp., the negative effects on the villi due to deoxynivalenol can be counteracted. The use of certain probiotics may not work as effectively in other species of poultry. In the case of turkeys when given 1.9x 10^9 cfu/ liter through the water, Johannsen et al. (2004) found that an effect on performance or shift in intestinal bacterial population by decreasing *Salmonella enterica* counts became noticeable.

In an attempt to use different bacterial cultures besides *Lactobacilli* to affect the bacterial community for competitive exclusion, La Ragione and Woodward (2003) experimented with 1 and 20 d old pathogen-free chicks, inoculating the birds with *Bacillus subtilis* $PY79^{hr}$ 24 h prior to challenging with *Salmonella enterica* and *Clostridium perfringens*. The initial inoculation was sufficient to suppress the colonization of *S. enterica* and *C. perfringens*, determined through fecal shedding. Interestingly, the *B. subtilis* persisted in the GIT throughout the experiment, with decreasing numbers as time passed. The results from La Ragione and Woodward (2003) suggest that other bacterial cultures can be used to competitively exclude pathogens. The ability to decrease the counts of *Clostridium perfringens* through the use of *B. subtilis* is of great interest. It was reported recently by Koutsos and Arias (2006) that *B. subtilis* isolated from the GIT of chickens has the ability to create a zone of inhibition, further strengthening the concept of competitive exclusion to maintain commensal intestinal bacteria. Improving the commensal

bacteria populations may be involved in the inhibition of harmful bacteria populations that might enter the GIT (Koutsos and Arias, 2006).

The past research from professionals in their field has given much insight into the impact of the intestinal bacterial community and intestinal development in poultry, but to reiterate there is no current work comparing the bacterial community with intestinal development. My research has gone the next step in setting the ground for observing intestinal bacterial communities at different stages of development in poultry.

Intestinal Development and the Bacterial Community Relationship in Other Animals

Researchers have observed a relationship between the bacterial community and the structures of the small intestine in recent years. The presence of certain bacteria in the GIT of chickens may be beneficial in improving overall health, according to Lan et al. (2005). In order to maintain optimal growth, the commensal bacteria in the bacterial community outcompetes pathogens for nutrients as well as space. This situation decreases the chances of pathogen colonization, according to Mead (2000) and Metchnikoff (1993). The majority of bacterial species that colonize the GIT are specific to the conditions of the micro-ecosystem and are difficult to culture when removed from their niches, as Suau et al. (1999) and Salzman et al. (2002) noted. The diversity of the intestinal bacteria has also been documented to vary at different segments throughout the GIT. Sarma-Rupavtarm et al. (2004) experimented with mice and observed that the intestinal bacterial diversity differed in various segments of the small intestine, supporting the theory that specific bacteria reside in a particular niche.

There is relatively little known about the symbiotic host-microbial relationship and its contribution to animal development. The knowledge gained comes from a very limited number of animal models. Much of the work has been conducted with the use of germ-free and

conventional animals. Many of these experiments studied what might be commensal bacteria such as *Bacteriodes thetaiotaomcron*. When these bacteria are colonized in the germ-free animals, researchers noted the benefits to the intestine that might incur (Hooper et al., 2000; Hooper et al., 2001).

The research concerning actual comparisons of intestinal bacterial community present in correlation to a stage of development is rare. Rawls et al. (2004) conducted one of the accounts of trying to correlate the bacterial community with intestinal development. These researchers experimented with germ-free and conventional raised zebrafish. The transparent body of the zebrafish as well as hematoxylin and eosin staining of intestinal cross sections allowed for visual observations of intestinal development and epithelial proliferation, respectively. The results indicated an underdeveloped GIT in the germ-free fish because of the small body size, swim bladder ,and intestinal tract as well as the least amount of epithelial cells compared to the conventionally raised zebrafish,. Furthermore, the bacteria present in the conventional zebrafish were similar to the bacteria that stimulate epithelial proliferation in mice. The intestinal bacteria in the conventional zebrafish had no levels of aeromonas bacteria, unlike the germ free zebrafish, which had almost 50% of the bacterial community consumed by aeromonas bacteris. The results of Rawls and colleagues suggest that the intestinal bacterial population can aid in stimulating GIT development, which will improve the overall growth of the animal. In a recent study, Ley et al. (2005) used the same genetic line with similar genes, except for the one controlling fat deposition. The researchers reported that the impact of the one gene dictating whether the mouse is lean or obese can also change the intestinal bacterial community of mice at the same age. The results suggest that the difference in the bacterial populations impacted nutrient absorption and intestinal development, resulting in either a lean or obese mouse.

Even though Ley et al. (2005) and Rawls et al. (2004) indicated that changes in the intestinal bacterial population were related to differences in development, no definitive correlation between intestinal development and the intestinal bacterial diversity of animals became obvious. Many of the studies of intestinal bacterial population were based on observations at different ages and/or with varying diets, and few if any research studies have been done in which the GIT development is correlated or even measured alone with intestinal bacterial populations in chickens. The ability to determine the normal intestinal bacterial population present at a specific stage of GIT development would provide critical information to animal health companies and may allow for the proper drug treatment when poultry flocks are faced with intestinal pathogens without negatively impacting the birds' normal intestinal bacteria

References

Ajuyah, A. O., K. Lee, R. T. Hardin and J.S. Sims. 1991. Changes in the yield and in fatty acid composition of whole carcass and selected meat portions of broiler chickens fed full fat oilseeds. Poult. Sci. 70: 2304-2314.

Albino, L.T., F. A. Feres, M. A. Dionizio, H. S. Rostagno, J. Geraldo de Vergas, D. Carvalho, P. C. Gomes, and C. H. R. Costa. 2006. Use of mannaoligosaccharid based prebiotic in the broiler diets. R. Bras. Zootec. 35:742-749.

Alzueta, C., M. L. Rodriguez, M. T. Cutuli, A. Rebole, L. T. Ortiz, C. Centeno, and J. Trevino. 2003. Effect of whole and demucilaged linseed in broiler chicken diets on digesta viscosity, nutrient utilization and intestinal microflora. Br. Poult. Sci. 44: 67-74.

Amit-Romach, E., D. Sklan, and Z. Uni. 2004. Microflora ecology of the chicken intestine using 16s ribosomal DNA primers. Poult. Sci. 83: 1093-1098.

Apajalahti, J. 2005. Comparative gut microflora, metabolic challenges, and potential opportunities. J. Appl. Poult. Res. 14: 444-453.

Apajalahti, J., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poult. Sci. J. 60: 223-232.

Applegate, T. J., D. M. Karcher, and M. S. Lilburn. 2005. Comparative development of the small intestine in the turkey poult and pekin duckling. Poult. Sci. 84: 426-431.

Arias, V. J., and E. A. Koutsos. 2006. Effects of copper source and level on intestinal physiology and growth of broiler chickens. Poult. Sci. 85: 999-1007.

Awad, W. A., J. Bohm, E. Razzazi-Fazeli, K. Ghareeb, and J. Zentek. 2006. Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. Poult. Sci. 85: 974-979.

Baranylova, E. and J. Holman. 1976. Morphological changes in the intestinal wall in fed and fasted chickens in the first week after hatching. Acta Veterinaria 45:151-158.

Barnes, E. M. D. A. Barnum, E. G. Harry, and G. C. Mead. 1972. Intestinal flora of chicken in period 2 to 6 weeks of age, with particular reference to anaerobic bacteria. Br. Poult. Sci. 13:311-326.

Batal, A. B., and C. M. Parsons. 2002. Effects of age on development of digestive organs and performance of chicks fed a corn-soybean meal versus a crystalline amino acid diet. Poult. Sci. 81: 1338-1341.

Bayer, R. C. F. H. Bird, S. D. Musgrrave, and C. B. Chawan, 1974. A simple method of preparation of gastrointestinal tract tissue for scanning electron microscopy. J. Anim. Sci. 38:354-356.

Bayer, R. C., C. B. Chawan, F. H. Bird, and S. D. Musgrave. 1975. Characteristics of absorptive surface of small-intestine of chicken from 1 day to 14 weeks of age. Poult. Sci. 54: 155-169.

Berg, R. D. 1996. The indigenous gastrointestinal microflora. Trens Microbiol. 31:107-133.

Bhatty, R. S. 1995. Nutrient composition of whole flaxseed and flaxseed meal in : Cunnane, S. C. and Thompson, L. U. (eds) flaxseed in human nutrition, Champaign IL, Academic press).

Bigot, K., S. Mignon-Grasteau, M. Picard, and S. Tesseraud. 2003. Effects of delayed feed intake on body, intestine, and muscle development in neonate broilers. Poult. Sci. 82: 781-788.

Binek, M. W. Borzemska, R. Pisarki, B. Blaszczak, G. Kosowska, H. Malec, and E. Karpinska. 2000. Evaluation of the efficacy of feed providing on development of gastrointestinal microflora of newly hatched broiler chickens. Arch. Geflugelkd. 64: 147-151.

Bjerrum, L. R. M. Engberg, T. D. Leser, B. B. Jensen, K. Finster, and K. Pedersen. 2006. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. Poult. Sci. 85: 1151-1164.

Bjerrum, L., K. Pedersen, and R. M. Engberg. 2005. The influence of whole wheat feeding on salmonella infection and gut flora composition in broilers. Avian Dis. 49: 9-15.

Borin, K., J. E. Lindberg, and R. B. Ogle. 2006. Digestibility and digestive organ development in indigenous and improved chickens and ducks fed diets with increasing inclusion levels of cassava leaf meal. Journal of Animal Physiology and Animal Nutrition 90: 230-237.

Caicedo, R. A., R. J. Schanler, N. Li, and J. Neu. 2005. The developing intestinal ecosystem: implications for the neonate. Pediatric Res. 58:625-628.

Carew, L. B. R. H. Machemer, R. W. sharp and D. C. Foss. 1972. Fat absorption by the very young chick. Poult. Sci. 51:738-742.

Chierici, R. S. Fanaro, D. Saccomandi, and V. Vigi. 2003. Advances in the modulation of the microbial ecology of the gut in early infancy. ACTA Paediatrica 92:56-63.

Chotinsky, D., E. Toncheva and Y. Profirov. 2001. Development of dissacharidase activity in the small intestine of broiler chickens. Br. Poult. Sci. 42:389-393.

Chung, C. H. and D. F. Day. 2004. Efficacy of Leuconostoc mesenteroides (ATCC 13146) isomaltooligosaccharides as a poultry prebiotic. Poult. Sci. 83:1302-1306.

Corless, A. B., and J. L. Sell. 1999. The effects of delayed access to feed and water on the physical and functional development of the digestive system of young turkeys. Poult. Sci. 78: 1158-1169.

Dibner, J. J., and J. D. Richards. 2004. The digestive system: Challenges and opportunities. J. Appl. Poult. Res. 13: 86-93.

Dror, Y. I. Nir, and Z. Nitsan. 1977. The relative growth of internal organs in light and heavy breeds. Poult. Sci. 18:493-496.

Dror, Y., I. Nir, and Z. Nitsan. 1977. Relative growth of internal organs in light and heavy breeds. Br. Poult. Sci. 18: 493-496.

Dunnington, E. A., and P. B. Siegel. 1995. Enzyme-activity and organ development in newly-hatched chicks selected for high or low 8-week body-weight. Poult. Sci. 74: 761-770.

Engberg, R. M., M. S. Hedemann, and B. B. Jensen. 2002. The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. Br. Poult. Sci. 43: 569-579.

Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83: 925-938.

Engberg, R. M., M. S. Hedemann, T. D. Leser, and B. B. Jensen. 2000. Effect of zinc bacitracin and salinomycin on intestinal microflora and performance of broilers. Poult. Sci. 79: 1311-1319.

Esteban, S., M. Moreno, J. M. Rayo, and J. A. Tur. 1991. Gastrointestinal emptying in the final days of incubation of chick embryo. Br. Poult. Sci. 32:279-284.

Evenson, A. E. 1947. The intestinal flora of laboratory animals and its modification by diet and drugs. PhD. Thesis, University of Wisconsin.

Ferraris, R. P., S. A.Villenas, and J. Diamond. 1992. Regulation of brush border enzyme activities and enterocyte migration rates in mouse small intestine. Amer. J. Physiology 262:G1047-G1059.

Fuller, R. 1989. Probiotics in man and animals. J. appl. Bacteriology 66:365-378.

Fuller, R. 1999. Probiotics for farm animals, P15-22. In Tannock G. W. (ed), Probiotics: a critical review, Horizon Scientific Press, Norwich.

Geyra, A., Z. Uni, and D. Sklan. 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci. 80: 776-782.

Geyra, A., Z. Uni, and D. Sklan. 2001. The effect of fasting at different ages on growth and tissue dynamics in the small intestine of the young chick. Br. J. Nutr. 86: 53-61.

Gibson G. R. and M.B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125:1401-1412.

Gibson, G. R. 1998. Dietary modulation of the human gut microflora using prebiotics. Brit J. Nutr. 80:S209-S212.

Gong, J. R. J. Foster, H. Yu, J.R. Chambers R. Wheatcroft, P. M. Sabour, S. Chen. 2002. Molecular analysis of bacterial population in the ileum of broiler chickens and comparison with bacteria in the cecum. FEMS Microbiol. Ecology 41:171-179.

Grey, R. D. 1972. Morphogenesis of intestinal villi. I. Scanning electron microscopy of the duodenal epithelium of the developing chick embryo. J. Morphol. 137: 193-214.

Hetland, H. M., M. Choct, and B. Svihus. 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. World's Poult. Sci. J. 60:415-422.

Hofacre, C. L., A. C. Johnson, B. J. Kelly, and R. Froyman. 2002. Effect of a commercial competitive exclusion culture on reduction of colonization of an antibiotic-resistant pathogenic escherichia coli in day-old broiler chickens. Avian Dis. 46: 198-202.

Holt, P. S., and R. E. Porter. 1992. Microbiological and histopathological effects of an induced-molt fasting procedure on a salmonella enteritidis infection in chickens. Avian Dis. 36: 610-618.

Holt, P.S. N.P. Macri and R. E. Porter. 1995. Microbiological analysis of the early Salmonella enteridis infection in molted an unmolted hens. Avian Dis. 39:55-63.

Hooper, L. V., P. G. Falk, and J. I Gordon. 2000. Analyzing the molecular foundations of commensalism in the mouse intestine. Microbiology 3:79-85.

Hooper, L.V., M. H. Wong, A. Thelin, L. Hansson, P. G. Falk, and J.I. Gordon. 2001. Molecular analysis of commensal host-microbial relationships in the intestine. Science 291:881-884.

Hume, M. E., L. F. Kubena, T. S. Edrington, C. J. Donskey, R. W. Moore, S.C. Ricke, and D. J. Nisbet. 2003. Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. Poult. Sci. 82: 1100-1107.

Ija, P. A. 1999. The impact of cereal non starch polysaccharides on intestinal development and function in broiler chickens. World's Poult. Sci. J. 55:374-387.

Iji, P. A. 2001. A. A. Saki, and D. R. Tivey. 2001. Intestinal development and body growth of broiler chicks on diets supplemented with non starch polysaccharides. Anim. Feed Sci. Technol. 89:175-188.

Ikeno, T., and K. Ikeno. 1991. Amylase activity increases in the yolk of fertilized-eggs during incubation in chickens. Poult. Sci. 70: 2176-2179.

Imondi, A. R., and F. H. Bird. 1966. Turnover of intestinal epithelium in chick. Poult. Sci. 45: 142-147.

Jamroz, D. K. Jakobsen, J. Orda, J. Skorupinska, and A. Wiliczkiewicz. 2001. Development of the gastrointestinal tract and digestibility of dietary fibre and amino acids in young chickens, ducks and geese fed diets with high amounts of barley. Comparative Biochem. And Phys. A Molecular and Integrative Physiology 130:643-652.

Jamroz, D., T. Wertelecki, A. Wiliczkiewicz, J. Orda, and J. Skorupinska. 2004. Dynamics of yolk sac resorption and post-hatching development of the gastrointestinal tract in chickens, ducks and geese. Journal of Animal Physiology and Animal Nutrition 88: 239-250.

Jin, L. Z., Y. W. Ho, N. Abdullah, and S. Jalaludin. 1998. Growth performance, intestinal microbial populations, and serum cholesterol of broilers fed diets containing lactobacillus cultures. Poult. Sci. 77: 1259-1265.

Johannsen, S. A., R. W. Griffith, I. V. Wesley and C. G. Scanes. 2004. Salmonella enterica serovat typhimurium colonization of the crop in the domestic turkey: influence of probiotic and prebiotic treatment (Lactobacillus acidophilus and Lactose). Avian Dis. 48:279-286.

Johansson, K.R., W.B. Sarles, and S. K. Shapiro. 1948. The intestinal microflora of hens as influenced by various carbohydrates in a biotin deficient ration. J. Bacteriology 56:619-634.

Jones, G. P. D. and R. D. Taylor. 2001. The incorporation of whole grain into pelleted broiler chicken diets: production and physiology responses. Br. Poult. Sci. 42:477-283.

Jorgensen, H. X. Q. Zhao, K. E. B. Knudsen, and B. O. Eggum. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br. J. Nutr. 75:379-395.

Juskiewicz, J., J. Jankowski, Z. Zdunczyk, and D. Mikulski. 2006. Performance and gastrointestinal tract metabolism of turkeys fed diets with different contents of fructooligosaccharides. Poult. Sci. 85: 886-891.

Katanbaf, M. N., E.A. Dunnington, P. B. Siegel. 1988. Allomorphic relationships from hatching to 56 days in parental lines and F1 crosses of chickens selected over 27 generations for high or low body weight. Growth Development and Aging 52:11-12.

Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, and G. W. Tannock. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Applied and Environmental Microbiology 68: 5918-5924.

Koutsos, E. A., and V. J. Arias. 2006. Intestinal ecology: Interactions among the gastrointestinal tract, nutrition, and the microflora. J. Appl. Poult. Res. 15: 161-173.

La Ragione, R. M. and M. J. Woodward. 2003. Competitive exclusion by Bacillus subtilis spores of salmonella enterica serotype Enteritidis and Clostridium perfringens in young chickens. Veterinary Microbiology 94:245-256.

Lambson, R. O. 1970. An electron microscopic study of entodermal cells of yolk sac of chick during incubation and after hatching. Am. J. Anat. 129:1-19.

Lan, Y., M. W. A. Verstegen, S. Tamminga, and B. A. Williams. 2005. The role of the commensal gut microbial community in broiler chickens. World's Poult. Sci. J. 61: 95-104.

Langhout, D. J. et al. 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free chicks. Br. J. Nutr. 83: 533-540.

Larousse, J. 1970. Aspects des actions conjuguees on commutative des antibiotiques et des ferments lactiques stabilizes par lyophilisation. Les ind. De L'alim animal 212:15-25

Lee, K., J. M. Olomu, and J. S. Sim. 1991. Live performance carcass yield protein and energy retention of broiler chickens fed canola and flax full fat seeds and the restored mixtures of meal and oil. Canadian J. Anim. Sci. 71:897-903.

Leeson, S. and J. D. Summers. 2001. Nutrition of the chicken. 4th edition rev. ed. University books Guelph Ontario Canada.

Ley, RE., F. Backhead, P. Turnbaugh, CA. Lozupone, RD. Knight, and JI. Gordon. 2005. Obesity alters gut microbial ecology. Proc. Nat'l Acad. Sci. 102: 11070-11075.

Lilja, C. 1983. Comparative study of postnatal growth and organ development in some species of birds. Growth 47:317-329.

Lu, J. R. U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, and M. D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Applied and Environmental Microbiology 69: 6816-6824.

Lu, J., C. L. Hofacre, and M. D. Lee. 2006. Emerging technologies in microbial ecology aid in understanding the effect of monensin in the diets of broilers in regard to the complex disease necrotic enteritis. J. Appl. Poult. Res. 15: 145-153.

Madhusudhan, K. T., J. P. Ramesh, T. Ogawua, K. Sasaoka, and N. Singh. 1986. Detoxification of commercial linseed meal for use in broiler rations. Poult. Sci. 164-171.

Maiorka, A, E. Santin, F. Dahika, I. C. Boleli, R.L. Furlan, and M. Macari. 2003. Posthatching water and feed deprivation affect the gastrointestinal tract and intestinal mucosa development of broiler chicks. J. Appl. Poult. Res. 12: 483-492.

Marchain, U. and R. G. Kulka. 1967. The non parallel increase of amylase chymotrypsinogen and procarboxypeptidase in the developing chick pancreas. Biochimica et biophysica Acta 146:553-559.

Matsushita, S. 1985. Development of sucrose in the chick small intestine. J. Experimental Zoology 233:377-383.

Mead, G. C. 2002. Factors affecting intestinal colonization of poultry by campylobacter and role of microflora in control. World's Poult. Sci. J. 58: 169-178.

Mead, G. C. and B. W. Adams. 1975. Some observations on cecal microflora of chick during first 2 weeks of life. Br. Poult. Sci. 16:169-176.

Misra, L.K. and R. C. Fanguy. 1978. Effect of delayed chick placement on subsequent growth and mortality of commercial broiler chicks. Poult. Sci. 57:1158.

Mitchell, M. A., and M. W. Smith. 1991. The effects of genetic selection for increased growth-rate on mucosal and muscle weights in the different regions of the small-intestine of the domestic-fowl (gallus-domesticus). Comparative Biochemistry and Physiology a-Physiology 99: 251-258.

Moran, E. T. 1985. Digestion and absorption of carbohydrates in fowl and events through prenatal development. J. Nutr. 116:665-674.

Moran, E. T. 1989. Effects of posthatch glucose on poults fed and fasted during yolk sac depletion. Poult. Sci. 68:1141-1147.

Moran, E. T. 2006. Anatomy, microbes, and fiber: Small versus large intestine. J. Appl. Poult. Res. 15: 154-160.

Moran, E.T. 1990. Effects of egg weight, glucose administration at hatch and delayed access to feed and water on the poult at 2 weeks of age. Poult. Sci. 69:1718-1723.

Nitsan, Z., E. A. Dunnigton, and P. B. Siegel. 1991. Organ growth and digestive enzyme levels to fifteen days of age in lines of chickens differing in body weight. Poult. Sci. 70:2040-2048.

Noy, Y. and D. Sklan. 1995. Digestion and absorption in the young chicks. Poult. Sci. 74:366-373.

Noy, Y. and D. Sklan. 1997. Posthatch development in poultry. J Appl. Poult. Res. 344-354.

Noy, Y. and D. Sklan. 1999. Energy utilization in newly hatched chicks. Poult. Sci. 78:1750-1756.Noy, Y. and D. Sklan. 1997. Posthatch development in poultry. J. Appl. Poult. Res. 6:344-354.

Noy, Y., A. Geyra, and D. Sklan. 2001. The effect of early feeding on growth and small intestinal development in the posthatch poult. Poult. Sci. 80: 912-919.

Noy, Y., and D. Sklan. 1998. Metabolic responses to early nutrition. J. Appl. Poult. Res. 7: 437-451.

Overton, J. and J. Shoup. 1964. Fine structure of cell surface specializations in maturing duodenal mucosa of chick. J. Cell Bio. 21:75-85.

Oviedo-Rondon, E. O., M. E. Hume, C. Hernandez, and S. Clemente-Hernandez. 2006. Intestinal microbial ecology of broilers vaccinated and challenged with mixed eimeria species, and supplemented with essential oil blends. Poult. Sci. 85: 854-860.

Palo, P. E., J. S. Sell, F. J. Piquer, M. F. Soto-Salanova, and L. Vilaseca. 1995. Effect of early nutrient restriction on broiler chicks 1. Effect and development of the gastrointestinal tract. Poult. Sci. 74:88-101.

Pedroso, A. A. J. F. M. Menten, M. R. Lambais, A. M. C. Racanicci, F. A. Longo, J. O. B. Sorbara. 2006. Intestinal bacterial community and growth performance of chickens fed diets containing antibiotics. Poultry Science 85: 747-752.

Pedroso, A. A., J. F. M. Menten, and M. R. Lambais. 2005. The structure of bacterial community in the intestines of newly hatched chicks. J. Appl. Poult. Res. 14: 232-237.

Pinchasov, Y. and Y. Noy. 1993. Comparison of posthatch holding timeand subsequent early performance of broilerchicks and turkey poults. Br. Poult. Sci. 34:111-120.

Potturi, P. V. L., J. A. Patterson, and T. J. Applegate. 2005. Effects of delayed placement on intestinal characteristics in turkey poults. Poult. Sci. 84: 816-824.

Rakowska, M., B. Rek-Cieply, E. Lipinski, I. Barcz, and B. Afanasjew. 1993. The effect of rye, probiotics, and nisin on faecel flora and histology of the small intestine of chicks. J. Anim. Feed Sci. 2:73-81.

Rawls, J. F., B. S. Samuel, and J. I Gordon. 2004. Gnotobobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. Proc. Nat'l Acad. Sci. 101:4596-4601.

Read, N.W. 1993. Rhodimet nutrition guide. 2nd ed. (Rhone-Poulenc Animal Nutrition: Antony France.)

Rettger, L. F. and H. A. Cheplin. 1921. Atreatise on the transformation of the intestinal flora with special reference to the implantation of Bacillus acidophilus. Yale University Press, New Haven, Conn.

Ricke, S. C. 2003. The gastrointestinal tract ecology of salmonella enteritidis colonization in molting hens. Poult. Sci. 82: 1003-1007.

Riddell, C. 1976. Influence of fiber in diet on dilation of proventriculus in chickens. Avain Dis. 20:442-445.

Rosebrough, R. W. R. H. Rosebrough, E. Gels, and K. Henderson. 1979. Effect of supplemental glucose or sucrose on liver and carcass glycogen metabolism of young chicks. Poult. Sci. 58:1524-1528.

Salzman, N.H., H. de Jong, Y. Paterson, H.J.M. Harmsen, G. W. Welling, and N.A. Bos. 2002. Analysis of 16S libraries of mouse gastrointestinal microflora reveal a large new group of mouse intestinal bacteria. Microbiology 148: 3651-3660.

Sarma-Rupavtarm, R. B., Z. M. Ge, D. B. Schauer, J. G. Fox, and M. F. Polz. 2004. Spatial distribution and stability of the eight microbial species of the altered schaedler flora in the mouse gastrointestinal tract. Appl. Envir. Microbiol 70: 2791-2800.

Sell, J. L., C. R. Angel, F. J. Piquer, E. G. Mallarino, and H. A. Albatshan. 1991. Developmental patterns of selected characteristics of the gastrointestinal-tract of young turkeys. Poult. Sci. 70: 1200-1205.

Siddons, R. C. 1969. Intestinal disaccharidase activities in the chick. Biochem. Journal 112: 51-59.

Sims, M. D., K. A. Dawson, K. E. Newman, P. Spring, and D. M. Hooge. 2004. Effects of dietary mannan oligosaccharide, bacitracin methylene disalicylate, or both on the live performance and intestinal microbiology of turkeys. Poult. Sci. 83: 1148-1154.

Sklan, D. 2001. Development of the digestive tract of poultry. World's Poult. Sci. J. 57: 415-428.

Sklan, D. 2004. Early gut development: the interaction between feed, gut health and immunity. In L. A. Tucker and J. A. Taylor-Pickard (Eds), Interfacing, Immunity, Gut Health and Performance (P 9-31). Nottingham: Nottingham University Press.

Smirnov, A. D. Sklan, and Z. Uni. 2004. Mucin dynamics in the chick small intestine are altered by starvation. J. Nutr. 134:736-742.

Smirnov, A., E. Tako, P. R. Ferket, and Z. Uni. 2006. Mucin gene expression and mucin content in the chicken intestinal goblet cells are affected by in ovo feeding of carbohydrates. Poult. Sci. 85: 669-673.

Smith, H. W. 1965. Development of flora of alimentary tract in young animals. J. Pathology and Bacteriology 90:495-513.

Smits, C. H. M. and G. Annison. 1996. Non-starch plant polysaccharides in broiler nutrition towards a physiologically valid approach to their determination. World's Poult Sci. J. 52:203-221.

Sonnenburg, J. L. et al. 2005. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science 307: 1955-1959.

Suau, A., R. Bonnet, M. Sutren, J.J Gordon, G. R. Gibson, M. D. Collins, and J. Dore. 1999. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl. Envir. Microbiol. 65:4799-4807.

Susbilla, J. P., I. Tarvid, C. B. Gow, and T. L. Frankel. 2003. Quantitative feed restriction or meal-feeding of broiler chicks alter functional development of enzymes for protein digestion. Br. Poult. Sci. 44: 698-709.

Tako, E. P.R. Ferket, and Z. Uni. 2004. Effects of in ovo feedin of carbohydrates and beta-hydroxy-beta-methlbutyrate on the development of chicken intestine. Poult. Sci. 83:2023-2028.

Tarvid, I. 1992. Effect of early postnatal long-term fasting on the development of peptide hydrolysis in chicks. Comparative Biochemistry and Physiology a-Physiology 101: 161-166.

Tarvid, I. L. 1990. Peptide digestion in poultry in early ontogenesis. In: Assimilation of organic and inorganic compounds in animal organisms (Valman, A.R., ed), Institute of Biology, Latvian Academy of Science, Zinatne, Riga, pp. 265-304.

Tellez, G., S. E. Higgins, A. M. Donoghue, and B. M. Hargis. 2006. Digestive physiology and the role of microorganisms. J. Appl. Poult. Res. 15: 136-144.

Thomson, A.B.R., C. I. Cheeseman, M. Keelan, R. Fedorak, M.T. Clandinin. 1994. Crypt cell production rate, enterocytes turnover time and appearance of transport along the jejunal villus of the rat. Biochimia et Biophysica Acta 863:197-204.

Tortuero, F. 1973. Influences of the implantation of Lactobacillus acidophilus in chicks on growth feed conversion malabsorption of fat syndrome and intestinal flora. Poult. Sci. 52:197-203.

Traber, P. G., D. L. Gumucio and W. Wang. 1991. Isolation of intestinal epithelial calls for the study of differential gene expression along the crypt villus axis. Amer. J. of Physiology. G895-903.

Uni, Z. 1999. Functional development of the small intestine in domestic birds: Cellular and molecular aspects. Poultry and Avian Biology Reviews 10: 167-179.

Uni, Z., A. Smirnov, and D. Sklan. 2003a. Pre- and posthatch development of goblet cells in the broiler small intestine: Effect of delayed access to feed. Poult. Sci. 82: 320-327.

Uni, Z., E. Tako, O. Gal-Garber, and D. Sklan. 2003b. Morphological, molecular, and functional changes in the chicken small intestine of the late-term embryo. Poult. Sci. 82: 1747-1754.

Uni, Z., S. Ganot, and D. Sklan. 1998. Posthatch development of mucosal function in the broiler small intestine. Poult. Sci. 77: 75-82.

Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small-intestines in heavy-strain and light-strain chicks. Poult. Sci. 74: 1622-1629.

Uni, Z., Y. Noy, and D. Sklan. 1996. Development of the small intestine in heavy and light strain chicks before and after hatching. Br. Poult. Sci. 37: 63-71.

van der Wielen, P., D. A. Keuzenkamp, L. J. A. Lipman, F. van Knapen, and S. Biesterveld. 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. Microbial Ecology 44: 286-293.

Van Leeuwen, P., J. Mouwen, J. D. Van der Klis, and M. W. A. Verstegen. 2004. Morphology of the small intestinal mucosal surface of broilers in relation to age, diet formulation, small intestinal microflora and performance. Br. Poult. Sci. 45: 41-48.

Watkins, E. J., P. J. Butler, and B. P. Kenyon. 2004. Posthatch growth of the digestive system in wild and domesticated ducks. Br. Poult. Sci. 45: 331-341.

Wieser, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. J. Bio. Chem. 248:2536-2541.

Xu, Z. R., C. H. Hu, M. S. Xia, X. A. Zhan, and M. Q. Wang. 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci. 82: 1030-1036.

Yamauchi, K. E., and Y. Isshiki. 1991. Scanning electron-microscopic observations on the intestinal villi in growing white leghorn and broiler-chickens from 1 to 30 days of age. Br. Poult. Sci. 32: 67-78.

Yasar, S. And J. M. Forbes. 1999. Performance and gastrointestinal response of broiler chickens fed on cereal grains based foods soaked in water. Br. Poult. Sci. 40:65-76.

Yu, B. C. C. Tsai, J.C. Hsu, and P.W. S. Chiou. 1998. Effect of different sources of dietary fibre on growth performance intestinal morphology and caecal cabohydrases of domestic geese. Br. Poult. Sci. 39: 560-567.

Yurong, Y., S. Ruiping, Z. Shimin, and J. Yibao. 2005. Effect of probiotics on intestinal mucosal immunity and ultrastructure of cecal tonsils of chickens. Archives of Animal Nutrition 59:237-246.

Diagram 1.1. The gastrointestinal tract of a chicken (adapted from Leeson and Summers, 2001)

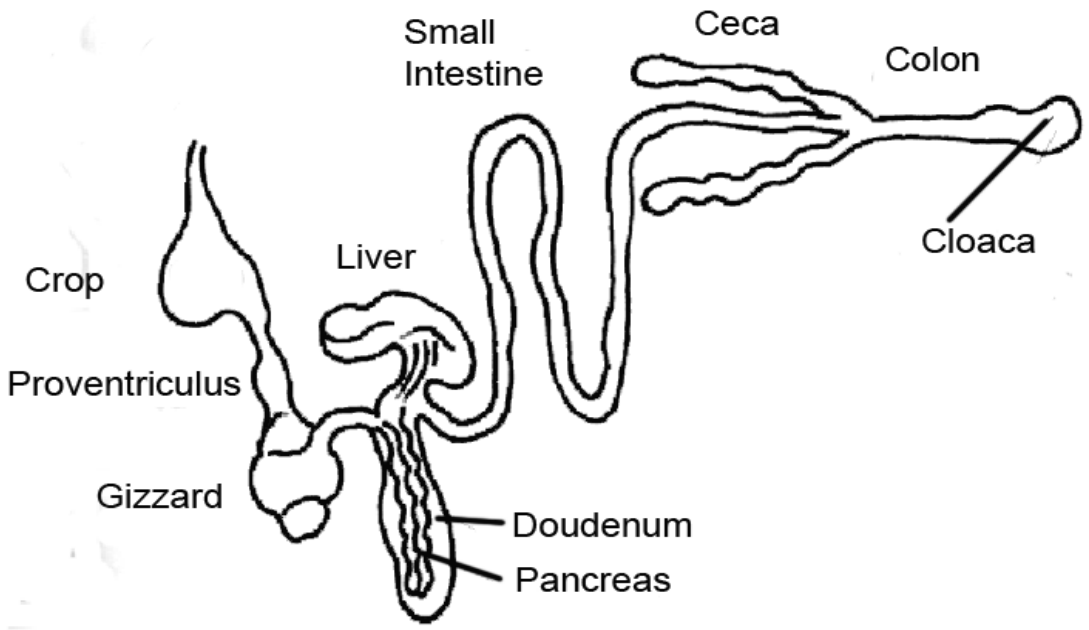
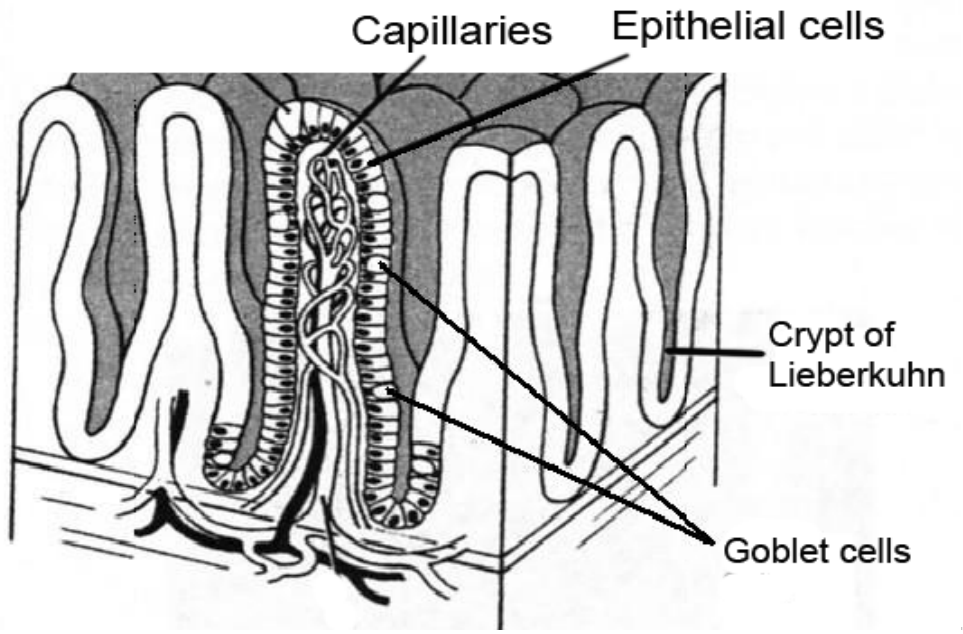


Diagram 1.2. Structural interpretation of villi lining the small intestine (adapted from Sklan, 2004).



CHAPTER 2

EFFECT OF FEED INGREDIENTS ON INTESTINAL DEVELOPMENT AND GUT BACTERIAL COMMUNITY OF BROILERS [1]

[1] Lumpkins, B. S., A.B. Batal, and M. D. Lee. To be submitted to *Poultry Science.*

ABSTRACT The bacterial population in the gastrointestinal tract (GIT) influences a variety of physiological processes; however, little is known about the true impact different intestinal bacterial communities have on GIT development in chickens. Two experiments (Exp.) were conducted with Cobb 500 chicks to evaluate the affect on intestinal development and the bacterial community when birds were fed diets with different feed ingredients. In Exp. 1, a corn-soybean meal (SBM) and a corn-fish/poultry by-product meal diet and in Exp. 2, a corn-SBM and a semi-purified corn gluten meal-casein-soy protein concentrate diet were fed to 9 replicate pens of 20 chicks each. At 4, 7, 14, and 21 d of age, chicks were randomly selected for jejunum and ileum sampling. Bacterial DNA was isolated from the digesta of the ileum, and denaturing gradient gel electrophoresis (DGGE) was used to examine the sequence diversity of the16s ribosomal community DNA. In Exp. 1, chicks fed the corn-fish/poultry by-product meal diet had greater jejunum weights and villi height at 4 and 14 d of age compared to the control fed birds, but at 7 and 21 d of age there was no difference in intestinal length, intestinal weight, villi height or villi width between treatments. A difference in bacterial community profiles was observed between treatments as well as with age, and the intestinal bacterial populations grouped together by treatment with greater than 80% similarity. In Exp. 2, the birds fed the semi-purified diets had longer relative intestinal lengths and shorter villi than the birds fed the corn-SBM diets. There was a clear separation in the composition of the intestinal bacterial population between the birds fed the semi-purified and corn-SBM diets. In Exp. 1 there was no overall difference in GIT development between diets; however, in Exp. 2 there was a difference in GIT development and performance between birds fed the corn-SBM diets and the semi-purified diets. Based on DGGE analysis, the use of different feed ingredients in poultry diets affects the composition of intestinal bacteria.

Key words: bacterial community, gastrointestinal tract, denaturing gradient gel electrophoresis, broilers

Introduction

The development of the gastrointestinal tract (**GIT**) is of great importance for proper nutrient absorption and growth (Noy and Sklan, 1998). The gastrointestinal tract is a micro-ecosystem in which intestinal bacteria thrive and flourish, and which potentially allows for symbiotic behavior between the bacterial community and their host (Xu et al., 2003). After hatch, the chick's GIT undergoes morphological changes in response to the change from a lipid rich yolk diet to a carbohydrate rich diet (Sklan, 2001). The gastrointestinal tract continues to change and develop as the bird ages, and the bacterial community also changes with the age of the bird (Lu et al., 2003). Diet also impacts GIT development and can alter the bacterial community (Freter et al., 1983; Sonnenburg et al., 2005) of the host.

Constant changes in feed ingredient cost and the producer's goal to formulate the lowest feed cost per unit of product have resulted in the use of alternative ingredients (Guevara, 2004; Korver et al., 2004). Researchers have been intrigued about the possible effects diet change can make on broiler performance, GIT development, and the bacterial community inhabiting the GIT. The use of different ingredients can either impair or benefit broiler performance and GIT development. The use of various ingredients such as cereal grains that provide some crude fiber to the poultry diet, have been reported to stimulate GIT development and improve bird performance (Moran, 1985). However, the use of excessive crude fiber or non-starch polysaccharides (water-soluble fiber) found in such ingredients as barley and rye results in increased intestinal viscosity, which decreases performance by impairing nutrient absorption (Jorgensen et al., 1996; Ija et al., 2001; Smits and Annison, 1996; Smulikowska et al., 2002). The increase in intestinal viscosity due to non-starch polysaccharides is believed to alter the bacterial population of the GIT, which increases the competition for nutrients with the host and

decreases broiler performance (Langhout et al., 2000). On the other extreme is the feeding of purified diets with crystalline amino acids (fed only for research purposes) which has no source of fiber and a powdery consistency resulting in poor performance and underdeveloped GIT in birds (Batal and Parsons, 2002). Once again, performance and intestinal development were impaired due to diet, but how the bacterial population was affected in this instance is not known.

As intestinal development can be altered by diet so can the composition of the bacterial community. Some of the earliest exposure of chicks to bacteria comes immediately following hatch, when the chick encounters a number of bacteria from the air in the hatchery (Pedroso et al., 2005). The bacterium moves through the GIT until a suitable environment or niche is found for the bacteria to competitively colonize. The bacterial community can act in an intra-organismal mutualism manner by improving nutrient absorption and protecting the host from pathogens through competitive exclusion (Apajalahti, 2005). The GIT is the first line of defense against many of the pathogens that an animal encounters, making the presence of specific bacteria in the GIT of great importance (Apajalahti, 2005). Therefore, a proper niche, including food source, is important to improve gut development and to aid in the residency of commensal bacteria.

Considering both GIT development and the composition or biodiversity of the intestinal bacteria can be manipulated through diet, the question is whether the change in the niche is due to GIT development or is it the actual substrate that impacts the composition and development of the intestinal bacterial community. Therefore, the objective of our experiment (**Exp**.) was to evaluate how the use of different feed ingredients affects intestinal development and the bacterial community.

Materials and Methods

Bird Husbandry and Dietary Treatments

All procedures were approved by the University of Georgia committee on Laboratory Animal Care. Chicks were reared on fresh litter wood shavings under standard management conditions with 24 hr lighting and ad libitum feed. In Exp. 1, six hundred-forty Cobb 500 chicks were randomly placed into 4 treatments each with 9 replications (160 chicks per treatment) in 36 pens with 96 x 229 cm dimension. The 4 dietary treatments were a standard corn-soybean meal (**SBM**) with and without the inclusion of soy hulls and dehydrated alfalfa increasing the fiber level from 2.0 to 4.4% (3,069 kcal ME_n/kg. and 22.6% crude protein), and an alternative protein diet consisting of a corn-fish/poultry by-product meal diet also with and without the inclusion of soy hulls and dehydrated alfalfa meal to increase the level of fiber from 2.0% to 4.4% (3,099 kcal ME_n/kg. and 22.6% crude protein) (Table 2.1). In Exp. 2, 4 dietary treatments were fed to 9 replications of 20 Cobb 500 male chicks. Chicks were randomly selected and allocated to 36 floor pens with a 96 x 229 cm dimension. The diets used in Exp. 1 did not result in dramatic differences in GIT measurements between treatments. Therefore, the diets in Exp. 2 were formulated to be more extreme in their difference in consistency, which would hopefully allow for a greater difference in GIT development. The 4 dietary treatments were a standard corn-SBM diet with and without the inclusion of wheat bran and barley and a semi-purified corn gluten meal-casein-soy protein concentrate diet with and without the inclusion of wheat bran and barley (Table2.2). The 4 diets were formulated to be isocaloric and isonitrogenous and met or exceeded the NRC requirements (NRC, 1994). The level of fiber for the corn-SBM diet with and without fiber was 2.6 and 2.0%, respectively, and for the semi-purified diets was 1.9 to 1.2%, respectively.

Animal sampling

In both Exp., two broiler chicks from each pen (18 birds per treatment) were euthanized and intestines were sampled at 4, 7, 14, and 21 d of age. For length and weight measurements, the small intestine was divided into three segments: duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the ducts to yolk stalk), and ileum (from yolk stalk to ileocecal junction). The 3 segments of the small intestine were flushed with 20 mL of physiological saline solution, and the empty weight was recorded. The contents of the jejunum and ileum following intestinal flushing of saline were collected in sterile bags and then stored at -80°C until isolation of bacterial DNA. Organ weights and lengths were expressed on a relative (g/kg of BW and mm/kg of BW, respectively) basis. For morphologic analysis, approximately 5 cm of the middle portion of the jejunum (from entry of the ducts to yolk stalk) was excised and fixed in 10% formalin. Six cross sections of 70% ethanol-preserved segments for jejunal sample were then prepared for staining with hematoxylin and eosin using standard paraffin embedding procedures (Uni *et al.*, 1995). A total of 3, intact well-oriented villi were selected in 8 replicates for each intestinal cross section (24 measurements for each intestinal sample with 288 measurements per treatment). Villus height was measured from the tip of the villus to the villus crypt junction; villus width was also measured as the distance across the middle of each villus. Morphological indices were determined using computer-aided light microscopy (16x magnification of the objective lens) image analysis.[1]

Bacterial DNA Isolation

The bacterial portion of the ileal contents was recovered by density gradient centrifugation through a gauze column and the DNA was extracted as described by Lu et al. (2003).

Denaturing Gradient Gel Electrophoresis

The V3-V4 region of the 16S ribosomal DNA (rDNA) of microorganisms of bacterial domain from contents of the chicken ileum were amplified with the primers HDA1-GC and HDA2 as described by Knarreborg et al. **(**2002). Amplicons were confirmed by electrophoresis using a 1.5% agarose gel containing ethidium bromide. DNA sequence polymorphisms of the amplicons were detected by resolving differences in molecular structure using denaturing gradient gel electrophoresis (**DGGE**). The DGGE was conducted using the D-Code Universal Mutation Detection system (BioRad, Hercules, CA) with 16 cm x 16 cm x 1 mm gels composed of 8% (wt/vol) polyacrylamide (acrylamide:bis 37.5:1) gels in 1x Tris-acetate EDTA buffer with a 15 to 55% linear denaturant gradient. The 100% denaturing solution contained 40% (vol/vol) formamide and 7.0 M urea. Electrophoresis was performed at a constant voltage of 200 V at 60°C for 3 h. Gels were fixed in 10% acetic acid for 15 min, washed 3 times in deionized water, then put in 50% methanol, washed and stained in 5µg of Sybr green I (FMC Bio products, Philadelphia, PA) /ml of 1x Tris-acetate EDTA buffer for 30 min.

Estimates of Diversity

After separation of amplicons by DGGE the profiles of the amplicons were compared using the coefficient of similarity (Cs), determined as: $Cs = (a + d)\ (a + b + c + d) - 1$, where Cs is the index of similarity between samples i and j, *a* is the number of amplicons common to samples i and j, *b* is the number of amplicons present in sample i, *c* is the number of amplicons present in sample j, and *d* is the number of distinct amplicons in samples i and j. If 2 profiles are identical, Cs equals 100%, if they are entirely different, Cs equals 0%. The presence or absence of amplicons was detected in the different samples, and the dendrogram was constructed using Treecon version 1.3b for Windows (Van de Peer and De Wachter, 1994, 1997). At the

[1] Image-Pro Plus Version 3.0, Media Cybernetics, Silver Spring, MD 20910.

occurrence of bacterial communities having a high level of similarity in their overall composition it will be referred to as a cluster. A cluster occurs when the composition of a bacterial community, based on treatment and/or age of the bird, group together with a level of similarity. Additionally, the composition of the bacterial communities differs from other bacterial communities due to a low level of similarity resulting in different clusters.

Statistical analysis

Data from both Exp. for performance and intestinal measurements were subjected to ANOVA procedure for a completely randomized design (Steel and Torrie, 1980) using the general linear model procedure of SAS (SAS Institute, 1990). Statistical significance of differences among treatments was assessed using the least significant difference test (Steel and Torrie, 1980). A probability level of $P < 0.05$ was used to define statistical significance.

Results and Discussions

Experiment 1

Based on the performance data, there was no dietary treatment that gave birds the best performance throughout the study (Table 2.3). During the early period (0 to 4 d of age) the birds fed the diets with alternative animal protein sources had improved body weight gain (**BWG**) and gain:feed ratio (**G:F**) compared to the control fed birds possibly due to increased nutrient absorption of the alternative protein ingredients. During the first 4 d of age, the inclusion of fiber had no effect on performance, but after 4 d of age the negative effects of increased fiber in the corn-SBM diet were clear due to a significant decrease in BWG and G:F in birds fed diets with fiber. The results herein agree with previous reports that also found fiber to have a negative effect on broiler performance (Langhout et al., 2000; Iji et al., 2001; Smulikowska et al. 2002). By 7 d of age, however, there was a similarity in performance between the 4 treatments. The

similarities in performance could be due to the fiber level only being moderately increased (4.4%) where other reports have increased fibers levels up to as high as 7% (Jorgensen et al., 1996). From 14 to 21 d of age the corn-SBM without fiber fed birds again had improved BWG and G:F over the corn-SBM with fiber fed birds and the alternative protein with and without fiber fed birds. These results might indicate the possible advantage of a pre-starter diet with alternative protein sources fed to 4 d of age to maximize performance.

In Exp. 1, each of the 3 intestinal segments had different trends for relative weight (g/kg of BW) and length (cm/kg of BW). In the duodenum, at 14 d of age there was no difference in relative weight and length (Table 2.4). However, the birds fed the alternative protein diet with and without fiber at 7 d of age and the alternative protein without fiber at 21 d of age had heavier and longer duodenum measurements compared to the corn-SBM fed birds. In the jejunum, there was no difference in relative weight or length between treatments at any of the measuring periods (Table 2.5). There was no difference in relative ileum length at any time between treatments, but the relative ileum weight was heaviest in birds fed the corn-SBM with fiber from 7 to 21 d of age (Table 2.6). At 4 and 7 d of age, the birds fed the control diet had the longest jejunal villi height (Table 2.7). At 14 d of age, there was no difference in villi height and by 21 d the birds fed the two diets with the increased fiber had the longest villi. These findings of increased intestinal measurements in the presence of cereal grains containing fiber, agrees with previous research (Williams et al., 1997; Ferket, 2000; Gabriel et al. 2003). The greater villi height and decreased performance, indicates that there may be a median point for villi height where maximal nutrient absorption is achieved that will result in the greatest performance. Another possibility could be that an excessive increase in villi height is not necessarily positive, considering the bird might not be achieving proper nutrient absorption and uses extra energy and

nutrients to develop the GIT in order to get proper nutrient absorption for growth. The measurement of the villi width was inconsistent between 7 and 21 d of age and at 4 and 14 d of age there was no difference between treatments. Therefore, it appears that there was no overall difference in GIT development among treatments.

Within dietary treatments the intestinal bacterial communities clustered together in several distinctly different groups (Figure 2.1). This clustering included treatments at specific ages that had a similarity in the composition of their bacterial community based on the dendogram's coefficient of similarity (%). The composition of the bacterial community had approximately 80% similarity within corn-SBM fed birds, and was approximately 63% similar in birds fed the alternative protein diets. The bacterial community in birds fed the corn-SBM and alternative protein was less than 60% similar. These results indicate that the bacterial community can be altered by feeding varying ingredients in a poultry diet, which agrees with previous reports where researchers fed different carbohydrates, enzymes, and fat sources and observed changes in the intestinal bacteria (Alzueta et al., 2003; Dibner and Buttin, 2002; Diebold et al., 2004; Knarreborg et al. 2002). Considering there was no overall difference in GIT development between treatments, it could be concluded that the bacterial population was altered due to the difference in feed ingredients, which are supplying different nutrient substrates to the bacterial community. The results further support the work of Sonnenburg et al. (2005) who reported that some intestinal bacteria are substrate specific based on research demonstrating *Bacteroides thetaiotaomicron* specificity for fructose. Furthermore, within each treatment, as the birds aged, there was a decrease in the percent similarity between intestinal bacterial populations, indicating an age-related change in the composition of the bacterial community. Studies that have reported bacterial community changes due to development focused on progression of age as

a developmental factor and also reported bacterial community changes with age (Lu et al., 2003; Amit-Romach et al., 2004; Smirnov et al., 2005).

Experiment 2

Since there was not a significant difference in performance and intestinal measurements due to the diets formulated in Exp. 1, a second Exp. was conducted looking at diets of extremely different ingredients formulated to determine if differences could be demonstrated in the GIT development between a standard corn-SBM diet a semi-purified diet. The feeding of a semi-purified diet resulted in a clear separation in performance and intestinal measurements compared to the corn-SBM fed birds at each of measuring periods (Table 2.3). The birds fed the corn-SBM diet with and without additional fiber, had similar BWG and G:F at each period. The BWG and G:F for birds fed the semi-purified diet were similar regardless of fiber content, but the semi-purified fed birds had lower BWG and G:F ($P < 0.05$) than the corn-SBM fed birds. However, adding a source of fiber to the semi-purified diet improved BWG and G:F ($P < 0.05$) over the birds fed just a semi-purified diet at each measuring period. Intestinal measurements were also different between birds fed the semi-purified and corn-SBM diets.

The birds fed the corn-SBM diet, with and without additional fiber, had similar ($P > 0.05$) relative weight and length measurements in the duodenum, jejunum and ileum, at each period. At 4 and 7 d of age, the relative duodenum weight was similar between birds fed the semi-purified diet with and without fiber (Table 2.4). However, at 14 and 21 d of age the birds fed the semi-purified diet had heavier relative duodenum weights then birds fed the semi-purified with fiber. The birds fed the semi-purified diet with fiber had similar relative duodenum weights and relative lengths with the corn-SBM fed birds at 14 and 21 d of age and 4 and 7d of age, respectively. The relative duodenum length of birds fed the semi-purified diet was consistently

longer than the other 3 treatments throughout the Exp. Additionally, there was no difference between treatments in relative jejunum weight and length throughout the Exp. ($P > 0.05$) (Table 2.5). The relative ileum weight was the lightest in birds fed the semi-purified with fiber diet, while birds fed the control had the heaviest weights at all periods (Table 2.6). There was no difference in ileum relative length between birds fed corn-SBM with and without fiber. There was a difference in ileum relative length between the semi-purified fed birds due to addition fiber. The birds fed the semi-purified diet had shorter ($P < 0.05$) ileum relative length than the semi-purified with fiber fed birds at all 4 measured periods indicating that fiber aids in gut stimulation. Once again, the jejunal villi length was different between semi-purified and corn-SBM fed birds regardless of fiber content ($P < 0.05$). At 4 d of age, the semi-purified fed birds had higher villi, but the corn-SBM fed birds had longer villi from 7 to 21 d of age (Table 2.7). The results from Exp. 2, indicated a much more underdeveloped small intestine in birds fed a semi-purified diet compared to birds fed a corn-SBM diet. The overall underdeveloped intestine of the semi-purified birds agrees with the finding of birds fed diets with crystalline amino acids by Batal and Parsons (2002).

The difference in performance and intestinal measurements is possibly due to the difference in the consistency between the two types of diets. Researchers have examined ingredients on a whole and ground basis and reported an improvement in performance when birds were fed ingredient in their whole form (Jones and Taylor, 2001; Annison, 1993; Wu et al., 2004). Based on previous findings and our results, it can be concluded that a coarser diet (i.e. fiber added to a semi-purified diet) may stimulate the gut and may improve nutrient absorption leading to greater gain and efficiency. Batal and Parsons (2002) reported similar finding with

performance in birds fed purified diets of a fine powdery consistency compared to a standard corn-SBM.

The bacterial populations were separated into two clear clusters between the birds fed the semi-purified diets and those fed the corn-SBM diets (Figure 2.2). The bacterial communities were at least 55% similar in birds fed the semi-purified diets, while the bacterial communities were > 60% similar in birds fed the corn-SBM diets. There was less than a 50% similarity in bacterial community between corn-SBM fed birds and the semi-purified fed birds. The birds fed the corn-SBM diets and those fed the semi-purified diets were consistently different in all measurements of GIT development and intestinal bacterial population. One possible reason for the separation in the bacterial community of the birds fed the two different type diets is the significant difference created in intestinal structures by the diet, which greatly affected the intestinal measurements and in turn the bacterial community. Beyond structural differences in the GIT there could also be an impact on the bacterial community due to the substrate, i.e. glucose added in the form of dextrose with birds fed the semi-purified diets. The separation in bacterial populations from birds fed the corn-SBM and semi-purified diets are amplified through the combination of changes in GIT measurements and differences in diet, which impact the environment of the micro-ecosystem and the substrate being supplied to the bacterial community. Even though, there was a separation in intestinal bacterial populations between the diets, once again age also had a large effect on the changes in bacterial populations within each treatment. Considering the small intestine is a micro ecosystem, changes in environment, in this case intestinal structures and food source, impact the biodiversity of the bacterial community.

In conclusion, when a diet contains an alternative protein source or an increased level of fiber, there is no overall impact on GIT development compared to a standard corn-SBM diet.

However, the substrate change has an affect the composition of the bacterial community. It is possible that the difference in diets, in order to effect intestinal development, could be a confounding factor in comparing intestinal bacterial population with various stages of intestinal development. However, when the birds were fed two completely different diets of either corn-SBM or semi-purified based there was a clear separation in GIT development and bacterial population. The results of Exp. 2 exemplified the impact of different diets on intestinal measurements, which altered the intestinal micro-ecosystem and change the composition of bacterial community. Therefore, the bacterial community in the intestines of broilers is affected by diet and intestinal development.

References

Alzueta, C. M. L. Rodriguez, M. T. Cutuli, A. Rebole, L. T. Ortiz, C. Centeno, J. Trevino. 2003. Effect of whole and demucilaged linseed in broiler chicken diets on digesta viscosity, nutrient utilization and intestinal microflora. Br. Poult. Sci. 44: 67-74.

Amit-Romach, E., D. Sklan, and Z. Uni. 2004. Microflora ecology of the chicken intestine using 16s ribosomal DNA primers. Poult. Sci. 83: 1093-1098.

Annison, G. 1993. The role of wheat non-starch polysaccharides in broiler nutrition. Australian J. Agric. Res. 44:405-422.

Apajalahti, J. 2005. Comparative gut microflora, metabolic challenges, and potential opportunities. J. Appl. Poult. Res. 14: 444-453.

Batal, A. B. and C. M. Parsons. 2002. Effects of age on development of digestive organs and performance of chicks fed a corn-soybean meal versus a crystalline amino acid diet. Poult. Sci. 81:1338-1341.

Corless, A. B., and J. L. Sell. 1999. The effects of delayed access to feed and water on the physical and functional development of the digestive system of young turkeys. Poult. Sci. 78: 1158-1169.

Dibner, J. J., and P. Buttin. 2002. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. J. Appl. Poult. Res. 11: 453-463.

Diebold, G., R. Mosenthin, H. P. Piepho, and W. C. Sauer. 2004. Effect of supplementation of xylanase and phospholipase to a wheat-based diet for weanling pigs on nutrient digestibility and concentrations of microbial metabolites in ileal digesta and feces. J. Anim. Sci. 82: 2647-2656.

Engberg, R. M., M. S. Hedemann, and B. B. Jensen. 2002. The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. Br. Poult. Sci. 43: 569-579.

Freter, R., H. Brickner, M. Botney, D. Cleven, A. Aranki. 1983. Mechanisms that control bacterial-populations in continuous-flow culture models of mouse large intestinal flora. Infection and Immunity 39:676-685.

Gabriel, I., S. Mallet, M. Leconte, G. Fort, and M. Naciri. 2003. Effects of whole wheat feeding on the development of coccidial infection in broiler chickens. Poult. Sci. 82: 1668-1676.

Guevara, V.R. 2004. Use of nonlinear programming to optimize performance response to energy density in broiler feed formulation. Poult. Sci. 83:147-151.

Hidalgo, M. A., A. J. Davis, N. M. Dale, and W. A. Dozier, III. 2004. Use of whole pearl millet in broiler diets. J. Appl. Poult. Res. 13:229-234.

Iji, P. A., A. A. Saki, and D. R. Tivey. 2001. Intestinal development and body growth of broiler chicks on diets supplemented with non-starch polysaccharides. Anim. Feed Sci. Technol. 89: 175-188.

Jones, G. P. D., and R. D. Taylor. 2001. The incorporation of whole grain into pelleted broiler chicken diets: production and physiological responses. Br. Poult. Sci. 42: 477-483.

Jorgensen, H., X. Zhao, K. Knudsen, and B. Eggum. 1996. The influence of dietary fibre source and level on the development of the gastrointestinal tract, digestibility and energy metabolism in broiler chickens. Br. J. Nutr. 75: 379-395.

Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, and G. W. Tannock. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microbiol. 68: 5918-5924.

Korver, D. R., M. J. Zuidhof, and K. R. Lawes. 2004. Performance characteristics and economic comparison of broiler chickens fed wheat and triticale-based diets. Poult. Sci. 83:716-725.

Langhout, D. J. J. B. Schutte, J. de Jong, H. Sloetjes, M. W. A. Verstegen, S. Tamminga. 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free chicks. Br. J. Nutr. 83: 533-540.

Lu, J. R. U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, M. D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69: 6816-6824.

Moran, E. T., Jr. 1985. Digestion and absorption of carbohydrates in fowl and events through prenatal development. J. Nutr. 115:665-674.

National Research Council. 1994. Nutrient requirements of poultry. 9th rev. ed. National Academy Press, Washington, DC.

Noy, Y., and D. Sklan. 1998. Metabolic responses to early nutrition. J. Appl. Poult. Res. 7: 437-451.

Pedroso, A. A., J. F. M. Menten, and M. R. Lambais. 2005. The structure of bacterial community in the intestines of newly hatched chicks. J. Appl. Poult. Res. 14: 232-237.

SAS Institute Inc., 1990. SAS STAT User's Guide Release 6.08. SAS Institute Inc., Cary NC.

Sklan, D. 2001. Development of the digestive tract of poultry. World's Poult. Sci. J. 57: 415-428.

Smirnov, A., E. Tako, P. R. Ferket, and Z. Uni. 2006. Mucin gene expression and mucin content in the chicken intestinal goblet cells are affected by in ovo feeding of carbohydrates. Poult. Sci. 85: 669-673.

Smits, C. H. M. and G. Annison. 1996. Non-starch plant polysaccharides in broiler nutrition-towards a physiologically valid approach to their determination. World's Poult. Sci. J. 52: 203-221.

Smulikowska, S., A. Mieczkowska, C. V. Nguyen, and M. Babelewska. 2002. The influence of digesta viscosity on the development of the stomach, on in vitro small intestinal motility and on digestion of nutrients in broiler chickens. J. Anim. Feed Sci. 11: 683-694.

Sonnenburg, J. L. et al. 2005. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science 307: 1955-1959.

Steel, R. G. D. and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.

Tako, E., P. R. Ferket, and Z. Uni. 2004. Effects of in ovo feeding of carbohydrates and beta-hydroxy-beta-methylbutyrate on the development of chicken intestine. Poult. Sci. 83: 2023-2028.

Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. Poult. Sci. 74:1622-1629.

Uni, Z., Y. Noy, and D. Sklan. 1999. Posthatch development of small intestinal function in the poult. Poult. Sci. 78: 215-222.

Van de Peer, Y. and R. De Wachter. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comut. Applic. Biosci. 10: 569-570.

Van de Peer, Y. and R. De Wachter. 1997. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. Comput. Applic. Biosci. 13: 227-230.

Xu J, Bjursell M. K, Himrod J, S, Deng, L. K. Carmichael, H. C. Chiang, L. V. Hooper, and J. I. Gordon. 2003. A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science 299: 2074-2076.

Williams, B. A. L. J. M. Van Osch, and R. P. Kwakkel. 1997. Fermentation characteristics of the cecal contents of broiler chickens fed fine and coarse particle diets. Br. Poult. Sci. 38:S41-S42.

Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles and W. H. Hendriks. 2004. Influences of method of whole wheat inclusion and xylanase supplementation on the performance,

apparent metabolizable energy, digestive tract measurements and gut morphology of broilers. Br. Poult. Sci. 45: 385-394.

Table 2.1. Composition of diets (as-fed basis) for Experiment 1

Ingredient	Trt. 1	Trt. 2	Trt. 3	Trt. 4
	----------%----------			
Corn, yellow	56.12	49.09	60.39	58.60
Soybean meal (48)	37.50	31.35	---	---
Alfalfa meal	---	4.25	---	9.51
Soy hulls	---	4.35	---	0.56
Fish meal	---	---	12.50	11.50
Poultry by-product	---	---	13.00	11.00
Peanut meal	---	---	6.00	6.00
Casein, dried	---	2.90	---	---
Fat, poultry	3.00	5.00	1.50	1.50
Limestone	0.80	0.58	---	0.25
Dicalcium phosphate	1.75	1.65	---	---
Salt	0.30	0.30	0.30	0.30
Vitamin mix[1]	0.25	0.25	0.25	0.25
Mineral mix[2]	0.08	0.08	0.08	0.08
DL-Met	0.20	0.20	0.20	0.20
L-Lys HCl	---	---	0.18	0.25
Solka floc	---	---	5.60	---
Contents by calculation				
ME, kcal/kg	3,069	3,069	3,099	3,099
Crude protein, %	22.6	22.6	22.6	22.6
Crude fiber, %	2.0	4.4	2.0	4.4
Lysine, %	1.14	1.27	1.11	1.13
Methionine, %	0.53	0.59	0.62	0.61
TSAA[3] , %	0.84	0.88	0.88	0.85
Threonine, %	0.80	0.85	0.75	0.74

[1]Vitamin mix provided the following (per kg of diet): thiamin•mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (cobalamin),12.0 µg; pyridoxine•HCL, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfite complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 ug; trans-retinyl acetate, 1,892 ug; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

[2]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4•H_2O$), 60 mg; iron ($FeSO_4•7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4•5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium ($NaSe0_3$), 0.3 mg.

[3] TSAA= Total sulfur amino acid

Table 2.2. Composition of diets (as-fed basis) for Experiment 2

Ingredient	Trt. 1	Trt. 2	Trt. 3	Trt. 4
	----------%----------			
Corn, yellow	56.12	46.02	---	---
Soybean meal (48)	37.50	36.65	---	---
Wheat bran	---	4.50	---	4.50
Barley	---	4.50	---	4.50
Dextrose	---	---	53.00	48.70
Corn gluten meal	---	---	12.08	12.82
Casein	---	---	5.00	5.00
Soy protein concentrate	---	---	6.00	4.00
Soybean oil	3.00	5.00	3.00	3.00
Limestone	0.80	0.75	1.50	1.50
Dicalcium phosphate	1.75	1.75	1.80	1.80
Salt	0.30	0.30	0.40	0.40
Vitamin mix[1]	0.25	0.25	0.50	0.50
Mineral mix[2]	0.08	0.08	0.08	0.08
DL-Met	0.20	0.20	0.10	0.09
L-Lys HCl	---	---	0.50	0.50
L-Glycine	---	---	2.00	2.00
L-Glutamic acid	---	---	4.92	4.92
L- Threonine	---	---	0.22	0.22
L- Tryptophan	---	---	0.11	0.05
L- Isoleucine	---	---	0.05	0.04
L-Arganine	---	---	0.55	0.51
L- Valine	---	---	0.05	0.03
L- Cystine	---	---	0.20	0.16
Solka floc	---	---	7.44	4.18
Na bicarbonate	---	---	0.50	0.50
Contents by calculation				
ME, kcal/kg	3,095	3,090	3,095	3,097
Crude protein, %	22.6	22.5	23.0	23.0
Crude fiber, %	2.0	2.6	1.2	1.9
Lysine, %	1.14	1.14	1.10	1.10
Methionine, %	0.53	0.53	0.52	0.50
TSAA[3] , %	0.84	0.83	0.92	0.87
Threonine, %	0.80	0.79	0.80	0.80

[1]Vitamin mix provided the following (per kg of diet): thiamin•mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (cobalamin),12.0 µg; pyridoxine•HCL, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfite complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 ug; trans-retinyl acetate, 1,892 ug; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

[2]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4•H_2O$), 60 mg; iron ($FeSO_4•7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4•5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium ($NaSe0_3$), 0.3 mg.

[3] TSAA= Total sulfur amino acid

Table 2.3. Effect of diet on body weight gain and feed efficiency (G:F)[1] for Exp. 1[2] and 2[2]
[1] G:F= gain to feed ratio.

Treatments	0 to4 d		4 to7 d		7 to14 d		0 to 21 d	
Exp. 1	Gain (g/chick)	G:F (g:kg)	Gain (g/chick)	G:F (g:kg)	Gain (g/chick)	G:F (g:kg)	Gain (g/chick)	G:F (g:kg)
Trt. 1 Control	31.5^{b}	483.2^{c}	50.1^{a}	614.0	227.6^{a}	684.3^{a}	711.5^{a}	683.0^{a}
Trt. 2 Corn-SBM w/ fiber[3]	35.0^{ab}	547.6^{b}	46.3^{a}	603.7	211.9^{b}	650.7^{b}	645.7^{b}	626.9^{b}
Trt. 3 Alternative protein[4]	38.6^{a}	596.8^{ab}	42.0^{b}	594.4	205.6^{ab}	605.9^{c}	652.7^{b}	639.7^{b}
Trt 4. Alternative protein w/ fiber[3]	38.5^{a}	628.0^{a}	40.7^{b}	568.7	208.0^{b}	617.0^{c}	665.0^{b}	629.2^{b}
Pooled SEM	0.78	19.58	1.36	20.00	4.42	8.22	8.60	20.40
Exp. 2								
Trt. 1 Control	32.5^{a}	875.1^{a}	56.0^{a}	757.8^{ab}	201.0^{a}	658.8	649.5^{a}	579.7^{a}
Trt. 2 Corn-SBM w/ fiber[5]	34.8^{a}	879.2^{a}	58.8^{a}	785.8^{a}	213.6^{a}	672.0	667.5^{ab}	618.1^{a}
Trt. 3 Semi purified[6]	11.3^{c}	416.5^{c}	15.3^{c}	728.5^{bc}	64.9^{c}	592.3	198.4^{c}	470.8^{b}
Trt 4. Semi purified w/ fiber[7]	23.8^{b}	681.3^{b}	26.5^{b}	703.7^{c}	99.5^{b}	660.5	321.7^{b}	518.1^{b}
Pooled SEM	0.78	19.58	1.14	17.37	3.32	27.23	8.60	20.40

[2] Means represent 9 pens per treatment, 20 chicks per pen.
[3] Alfalfa meal and soy hulls were added to increase the fiber level of the diet from 2.0 to 4.4%.
[4] Alternative protein diets= soybean meal was replaced with fishmeal, peanut meal, and poultry by-product meal to maintain isocaloric and isonitrogenous levels (3,099 kcal MEn/kg and 22.6% crude protein).
[5] Wheat bran and barley were added to increase the fiber level in the diet from 2.0 to 2.6%.
[6] Semi purified diets= soybean meal and corn was replaced with dextrose, corn gluten meal, casein, soy protein concentrate and synthetic amino acids to maintain isocaloric and isonitrogenous levels (3,095 kcal MEn/kg and 23.0% crude protein).
[7] Wheat bran and barley were added to increase the fiber level in the diet from 1.2 to 1.9.6%.
[a-d] Means within a column with no common superscript differ significantly ($P < 0.05$).

Table 2.4. Effect of diet on relative duodenum weight and length in Exp. 1[1] and Exp. 2[1]

	Relative Weight [2] (g/kg of BW)				Relative Length[3] (cm/kg of BW)			
Treatments	4d	7d	14d	21d	4d	7d	14d	21d
Exp. 1								
Trt. 1 Control	20.1[ab]	19.6[b]	16.1	12.0[b]	1549	1120[b]	577	321[a]
Trt. 2 Corn-SBM w/ fiber[4]	18.4[b]	19.5[b]	16.3	12.1[b]	1506	1149[b]	573	288[b]
Trt. 3 Alternative protein[5]	21.2[a]	22.8[a]	16.8	14.0[a]	1529	1216[ab]	621	347[a]
Trt 4. Alternative protein w/ fiber[4]	19.1[b]	22.3[a]	15.6	12.5[b]	1413	1264[a]	585	326[a]
Pooled SEM	0.94	0.55	0.43	0.42	61.6	38.6	27.9	10.3
Exp. 2								
Trt. 1 Control	24.0[a]	24.8	17.2[b]	11.9[b]	1667[b]	1193[b]	578[c]	79[c]
Trt. 2 Corn-SBM w/ fiber[6]	24.5[a]	23.5	17.5[b]	12.7[b]	1656[b]	1153[b]	562[c]	77[c]
Trt. 3 Semi purified[7]	20.8[b]	22.9	21.6[a]	14.3[a]	1863[a]	1585[a]	1162[a]	172[a]
Trt 4. Semi purified w/ fiber[8]	18.4[b]	24.8	18.8[b]	12.4[b]	1629[b]	1345[b]	836[b]	147[b]
Pooled SEM	0.65	0.76	0.63	0.44	44.5	64.7	35.6	7.1

[1]Means represent 9 pens per treatment, 2 randomly selected chicks per pen.
[2] Relative weight = g/kg of bodyweight.
[3] Relative length= cm/kg of bodyweight.
[4] Alfalfa meal and soy hulls were added to increase the fiber level of the diet from 2.0 to 4.4%.
[5] Alternative protein diets= soybean meal was replaced with fishmeal, peanut meal, and poultry by-product meal to maintain isocaloric and isonitrogenous levels (3,099 kcal MEn/kg and 22.6% crude protein).
[6] Wheat bran and barley were added to increase the fiber level in the diet from 2.0 to 2.6%.
[7] Semi purified diets= soybean meal and corn was replaced with dextrose, corn gluten meal, casein, soy protein concentrate and synthetic amino acids to maintain isocaloric and isonitrogenous levels (3,095 kcal MEn/kg and 23.0% crude protein).
[8] Wheat bran and barley were added to increase the fiber level in the diet from 1.2 to 1.9.6%.
[a-d] Means within a column with no common superscript differ significantly ($P < 0.05$).

Table 2.5. Effect of diet on relative jejunum weight and length in Exp. 1[1] and Exp. 2[1]

	Relative Weight [2] (g/kg of BW)				Relative Length[3] (cm/kg of BW)			
Treatments	4d	7d	14d	21d	4d	7d	14d	21d
Exp. 1								
Trt. 1 Control	27.8	29.5	25.0	20.6	3487	2381	1417	761
Trt. 2 Corn-SBM w/ fiber[4]	30.4	31.2	27.6	21.3	3665	2619	1532	750
Trt. 3 Alternative protein[5]	31.5	30.7	27.0	21.1	3482	2551	1425	782
Trt 4. Alternative protein w/ fiber[4]	28.7	30.2	26.7	21.3	3464	2824	1399	762
Pooled SEM	0.93	0.76	0.63	0.57	109.4	88.2	53.0	26.2
Exp. 2								
Trt. 1 Control	30.7	31.8	27.3	18.7	3695	2555	1380	186
Trt. 2 Corn-SBM w/ fiber[6]	34.6	33.4	27.2	20.0	3557	2538	1376	204
Trt. 3 Semi purified[7]	25.2	29.1	28.9	20.5	4034	3813	2477	367
Trt 4. Semi purified w/ fiber[8]	26.9	29.4	24.5	17.6	3331	3068	1834	262
Pooled SEM	1.17	1.00	0.87	0.47	108.1	147.1	66.9	10.1

[1]Means represent 9 pens per treatment, 2 randomly selected chicks per pen.
[2] Relative weight = g/kg of bodyweight.
[3] Relative length= cm/kg of bodyweight.
[4] Alfalfa meal and soy hulls were added to increase the fiber level of the diet from 2.0 to 4.4%.
[5] Alternative protein diets= soybean meal was replaced with fishmeal, peanut meal, and poultry by-product meal to maintain isocaloric and isonitrogenous levels (3,099 kcal MEn/kg and 22.6% crude protein).
[6] Wheat bran and barley were added to increase the fiber level in the diet from 2.0 to 2.6%.
[7] Semi purified diets= soybean meal and corn was replaced with dextrose, corn gluten meal, casein, soy protein concentrate and synthetic amino acids to maintain isocaloric and isonitrogenous levels (3,095 kcal MEn/kg and 23.0% crude protein).
[8] Wheat bran and barley were added to increase the fiber level in the diet from 1.2 to 1.9.6%.

Table 2.6. Effect of diet on relative ileum weight and length in Exp. 1[1] and Exp. 2[1]

	Relative Weight [2] (g/kg of BW)				Relative Length[3] (cm/kg of BW)			
Treatments	4d	7d	14d	21d	4d	7d	14d	21d
Exp. 1								
Trt. 1 Control	18.8	17.3^{bc}	15.6^{b}	13.9^{b}	3377	2269	1238	727
Trt. 2 Corn-SBM w/ fiber[4]	20.3	19.4^{a}	17.0^{a}	15.3^{a}	3146	2378	1309	732
Trt. 3 Alternative protein[5]	18.7	16.8^{c}	15.4^{b}	13.2^{b}	3207	2292	1275	712
Trt 4. Alternative protein w/ fiber[4]	19.0	18.7^{ab}	16.4^{ab}	13.6^{b}	3329	2555	1252	739
Pooled SEM	0.73	0.53	0.49	0.38	84.9	85.0	48.8	18.0
Exp. 2								
Trt. 1 Control	21.7^{a}	21.6^{a}	15.7^{a}	14.6^{a}	3302^{a}	2385^{b}	1219^{c}	198^{c}
Trt. 2 Corn-SBM w/ fiber[6]	21.9^{a}	20.1^{b}	16.7^{a}	14.2^{a}	3194^{a}	2290^{b}	1195^{c}	191^{c}
Trt. 3 Semi purified[7]	15.5^{b}	20.2^{b}	16.7^{a}	13.0^{b}	3219^{a}	3071^{a}	1947^{a}	342^{a}
Trt 4. Semi purified w/ fiber[8]	14.0^{b}	14.5^{c}	11.9^{b}	10.8^{c}	2652^{b}	2346^{b}	1478^{b}	232^{b}
Pooled SEM	0.55	0.66	0.60	0.51	81.2	93.6	48.6	11.6

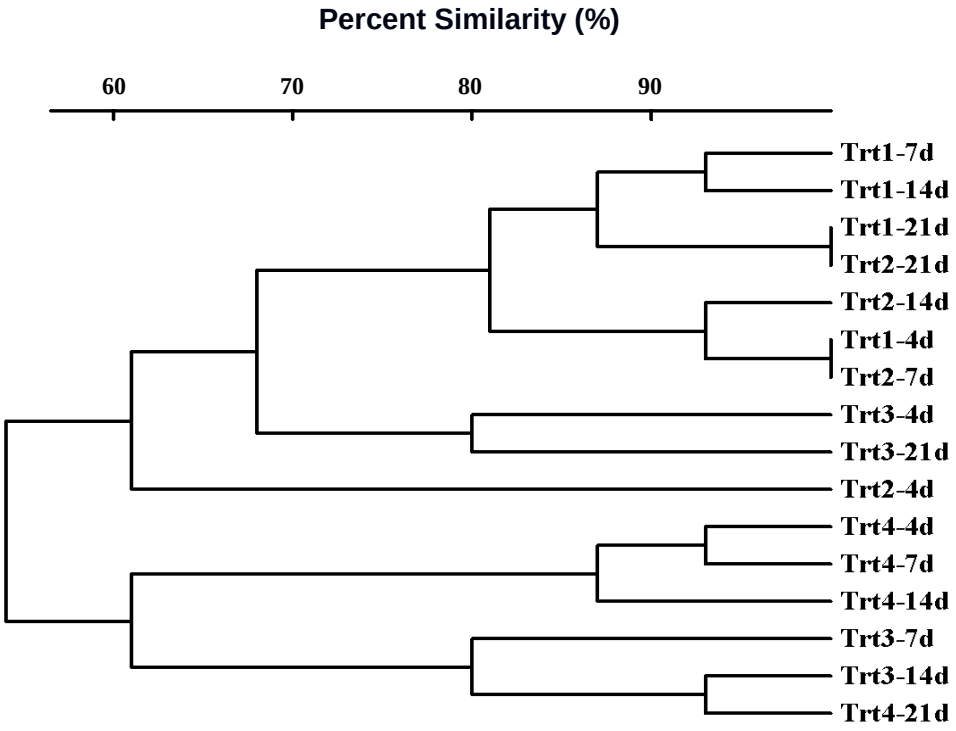
[1]Means represent 9 pens per treatment, 2 randomly selected chicks per pen.
[2] Relative weight = g/kg of bodyweight.
[3] Relative length= cm/kg of bodyweight.
[4] Alfalfa meal and soy hulls were added to increase the fiber level of the diet from 2.0 to 4.4%.
[5] Alternative protein diets= soybean meal was replaced with fishmeal, peanut meal, and poultry by-product meal to maintain isocaloric and isonitrogenous levels (3,099 kcal MEn/kg and 22.6% crude protein).
[6] Wheat bran and barley were added to increase the fiber level in the diet from 2.0 to 2.6%.
[7] Semi purified diets= soybean meal and corn was replaced with dextrose, corn gluten meal, casein, soy protein concentrate and synthetic amino acids to maintain isocaloric and isonitrogenous levels (3,095 kcal MEn/kg and 23.0% crude protein).
[8] Wheat bran and barley were added to increase the fiber level in the diet from 1.2 to 1.9.6%.
[a-d] Means within a column with no common superscript differ significantly ($P < 0.05$).

Table 2.7. Effects of diet on jejunum villi measurements in Exp. 1[1] and Exp. 2[1]

	Height (μm)				Width (μm)			
Treatments	4d	7d	14d	21d	4d	7d	14d	21d
Exp. 1								
Trt. 1 Control	298[a]	436[a]	576	635[b]	48	78[a]	94	91[b]
Trt. 2 Corn-SBM w/ fiber[2]	239[b]	371[b]	572	755[a]	52	68[b]	80	89[b]
Trt. 3 Alternative protein[3]	278[a]	367[b]	623	647[b]	52	79[a]	93	88[b]
Trt 4. Alternative protein w/ fiber[2]	220[b]	344[b]	567	746[a]	48	81[a]	90	110[a]
Pooled SEM	13.6	9.7	23.2	30.4	1.8	3.3	6.6	4.6
Exp. 2								
Trt. 1 Control	269[b]	340[b]	506[a]	631[a]	43[c]	52[b]	55[b]	70
Trt. 2 Corn-SBM w/ fiber[4]	223[c]	347[b]	530[a]	636[a]	58[a]	64[a]	76[a]	65
Trt. 3 Semi purified[5]	305[a]	292[c]	431[b]	504[b]	43[c]	50[b]	63[b]	61
Trt 4. Semi purified w/ fiber[6]	319[a]	379[a]	427[b]	544[b]	49[b]	56[b]	61[b]	66
Pooled SEM	2.7	9.0	13.6	21.0	1.9	2.5	3.7	9.3

[1]Means represent 9 pens per treatment, 2 randomly selected chicks per pen, 3 villi per chick.
[2] Alfalfa meal and soy hulls were added to increase the fiber level of the diet from 2.0 to 4.4%.
[3] Alternative protein diets= soybean meal was replaced with fishmeal, peanut meal, and poultry by-product meal to maintain isocaloric and isonitrogenous levels (3,099 kcal MEn/kg and 22.6% crude protein).
[4] Wheat bran and barley were added to increase the fiber level in the diet from 2.0 to 2.6%.
[5] Semi purified diets= soybean meal and corn was replaced with dextrose, corn gluten meal, casein, soy protein concentrate and synthetic amino acids to maintain isocaloric and isonitrogenous levels (3,095 kcal MEn/kg and 23.0% crude protein).
[6] Wheat bran and barley were added to increase the fiber level in the diet from 1.2 to 1.9.6%.
[a-d] Means within a column with no common superscript differ significantly ($P < 0.05$).

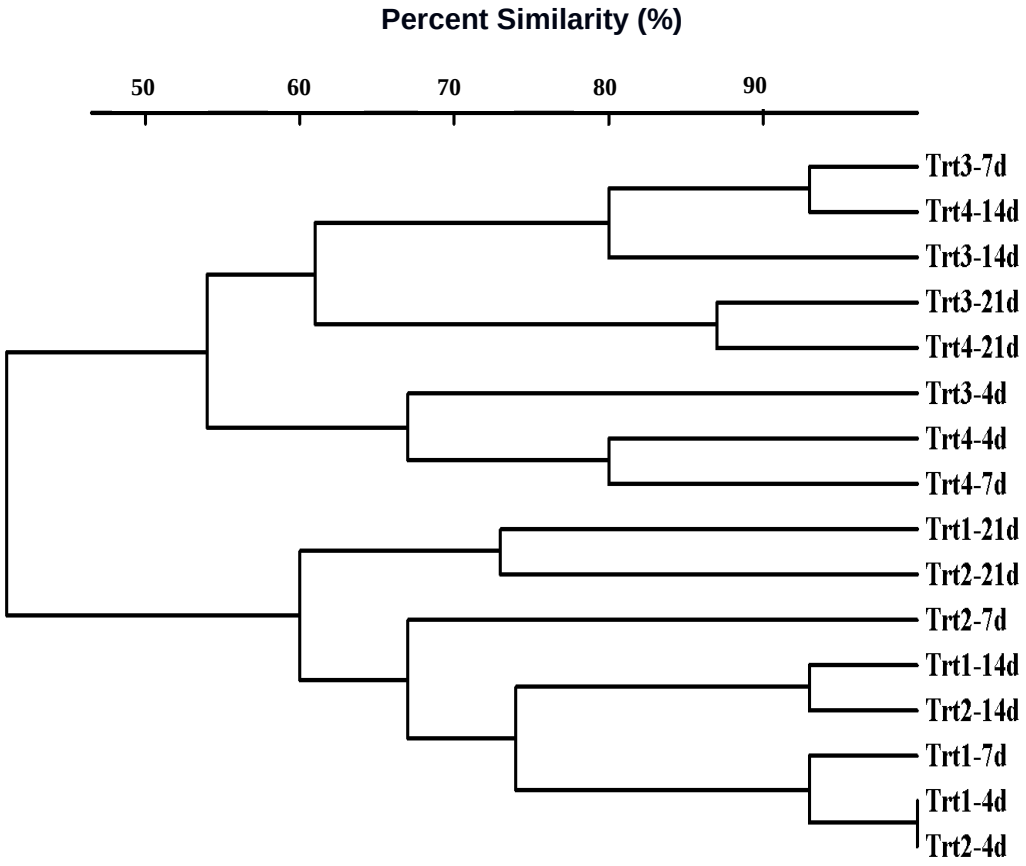
Figure 2.1. Dendogram showing the relatedness[1] of ileal bacterial community among birds fed different treatments from 0 to 21 d of age (Exp. 1[2])



[1] Simple matching using clustering method of Treecon® to produce dendogram representing the relationship between DGGE band patterns of V3-V4 region of 16s rDNA of the intestinal community DNA.

[2]Contents for bacterial analysis pooled from 16 birds per treatment per age period. Trt 1= control, Trt 2= corn-soybean meal with fiber, Trt 3= corn-fish/poultry by-product meal diet, and Trt 4= corn-fish/poultry by-product meal diet with fiber.

Figure 2.2. Dendogram showing the relatedness[1] of ileal bacterial community among birds fed different treatments from 0 to 21 d of age (Exp. 2[2])



[1] Simple matching using clustering method of Treecon® to produce dendogram representing the relationship between DGGE band patterns of V3-V4 region of 16s rDNA of the intestinal community DNA.

[2]Contents for bacterial analysis pooled from 16 birds per treatment per age period. Trt 1= control, Trt 2= corn-soybean meal with fiber, Trt 3= semi-purified corn gluten meal-casein-soy protein concentrate diet, and Trt 4= semi-purified corn gluten meal-casein-soy protein concentrate diet with fiber.

CHAPTER 3

THE EFFECT OF GENDER ON INTESTINAL DEVELOPMENT AND THE BACTERIAL COMMUNITY OF BROILERS [1]

[1] Lumpkins, B. S., A. B. Batal, and M. D. Lee. To be submitted to *Poultry Science.*

ABSTRACT The effect of gender impacting growth, carcass yield and nutritional requirements of chickens has been well documented, but little is known about how the sex of a chicken impacts the bacterial population of the gastrointestinal tract (GIT). Therefore, our objective was to evaluate the development of the GIT and biodiversity of the bacterial community between male and female broilers. An experiment was conducted with broiler chicks that were vent sexed at 0 d of age and allocated to 8 pens of 25 chicks per sex. All birds were fed a non-medicated corn soybean meal starter diet from 0 to 21 d of age. At 3, 7, 14, and 21 d of age, chicks were randomly selected for jejunum and ileum sampling. Bacterial DNA was isolated from the digesta of the ileum, and denaturing gradient gel electrophoresis (DGGE) was used to examine PCR amplified fragments of 16s ribosomal DNA. There was no difference in body weight gain ($P < 0.05$) between the males and females from 0 to 14 d of age. At 21 d of age males had greater body weight gain than females. Jejunal and ileal relative weight and length were similar ($P > 0.05$) at all measured periods except at 7 d of age where males had heavier and longer ileums ($P < 0.05$). At 14 d of age males had longer villi ($P < 0.05$) than females, but at all other ages villi height was similar. By 7 d of age there was the beginning of a divergence in intestinal development between males and females. Based on DGGE analysis there was a clear difference of the ileal bacterial community with less than 30% similarity of the bacterial community between males and females. Furthermore, as the birds aged the similarity of the intestinal bacteria decreased. The change in bacterial community between genders goes beyond intestinal development and may be impacted by other gender related factors.

Key words: bacterial community, gastrointestinal tract, denaturing gradient gel electrophoresis, gender

Introduction

The gastrointestinal tract (**GIT**) is obviously important for nutrient absorption and protection against many of the pathogens that enter the body (Mowat et al., 1997). The GIT also supports a micro-ecosystem that harbors a large and diverse population of bacteria (Drasar and Barrow, 1985; Franks, 1998) that create a symbiotic relationship with the host (Apajalahti, 2005). The intraorganismal mutualism of the intestinal bacteria requires unique structures and environmental conditions of the GIT in order to become established and aid in the improvement of bird health (Cook and Bird, 1973; Lan et al., 2005; Xu et al., 2003; Fuller, 1989) and nutrient utilization for optimal growth (Lee, 1999). Therefore, not only is the structure of the GIT important for bird development, but so is the understanding of the symbiotic relationship with the bacterial community.

Male and female broilers have been reported differ in growth rates, deposition of muscle and bone minerals, and nutrient requirements (Havenstein et al., 1994a, 1994b; Horsted et al., 2005; Han and Baker, 1993; Rose et al.1996). Furthermore, males have been reported to have leaner carcasses than females (Suto et al.1998). In mice the amount of carcass fat has been observed to be a contributing factor to the type of bacterial community that resides in the GIT (Ley et al., 2005). The ability of nutrients to be absorbed for utilization, resulting in increased fat, has been speculated to be associated with certain bacterial communities. The difference in dietary requirements of male and female broilers (Han and Baker, 1994) may also be contributed to the impact on bacterial populations further signifying the importance of trying to understand the bacterial community. Considering male and female broilers have different growth and development rates, one could hypothesize that micro-ecosystem of the GIT may also be different which would influence the bacterial community.

Research comparing the differences in intestinal bacterial communities between genders across different animal species is scarce, and there are no reports evaluating the bacterial populations in male and female broilers. Therefore, the objective of this experiment was to evaluate the influence of gender on growth rate, intestinal measurements and bacterial population of broilers from 0 to 21 d of age.

Materials and Methods

Bird Husbandry and Dietary Treatments

All procedures were approved by the University of Georgia Animal Use and Care Committee. Chicks were reared under standard commercial conditions and were provided with feed and water ad libitum. At hatch, broiler chicks (Cobb 500) were obtained from a local hatchery. Chicks were vent sexed and 25 of each sex were randomly allocated to 8 replicate (200 chicks per treatment) floor pens with litter shavings, with a dimension of 122 x 310 cm (density of 1,511 cm^2 per bird). The birds were housed in an environmentally controlled room under 24 h lighting conditions with nipple drinkers and pan feeders. Chicks were fed a non-medicated conventional corn-soybean meal starter diet (23% CP, 3,096 kcalME/kg, 1.14% total lysine, 0.53% total methionine, and 0.90% total sulfur amino acids) from 0 to 21 d of age.

Bird Sampling

Two birds per pen were euthanized and intestines were examined at 3, 7, 14, and 21 d of age. For length and weight measurements, the small intestine was divided into three segments: duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the ducts to yolk stalk), and ileum (from yolk stalk to ileocecal junction). The 3 segments of the small intestine were flushed with 20 mL of physiological saline solution, and the empty weight was recorded. The contents of the jejunum and ileum following intestinal flushing of saline were

collected in sterile bags and then stored at -80°C until isolation of bacterial DNA. Organ weights and lengths were expressed on a relative (grams/kg of body weight and mm/kg of body weight, respectively) basis. For morphologic analysis, approximately 5 cm of the middle portion of the jejunum (from entry of the bile ducts to yolk stalk) was excised and fixed in 10% formalin. Six cross sections of 70% ethanol-preserved segments per jejunal sample were then prepared for staining with hematoxylin and eosin using standard paraffin embedding procedures (Uni *et al.*, 1995). A total of 3, intact well-oriented villi were selected in 8 replicates for each intestinal cross section (24 measurements for each intestinal sample with 288 measurements per treatment). Villus height was measured from the tip of the villus to the crypt junction; villus width was measured as the distance across the middle of each villus. Morphological indices were determined using computer-aided light microscopy (16x magnification of the objective lens) with image software analysis.[1]

Bacterial DNA Isolation

The bacterial portion of the ileal contents was recovered by density gradient centrifugation through a gauze column, and the DNA was extracted as described by Lu et al. (2003).

Denaturing Gradient Gel Electrophoresis (DGGE)

The V3-V4 region of the 16S ribosomal DNA (rDNA) of microorganisms of bacterial domain from contents of the chicken ileum were amplified with the primers HDA1-GC and HDA2 as described by Knarreborg et al **(**2002). Amplicons were confirmed by electrophoresis using a 1.5% agarose gel containing ethidium bromide. DNA sequence polymorphisms of the amplicons were detected by resolving differences in molecular structure using denaturing gradient gel electrophoresis (**DGGE**). The DGGE was conducted using the D-Code Universal

[1] Image-Pro Plus Version 3.0, Media Cybernetics, Silver Spring, MD 20910.

Mutation Detection system (BioRad, Hercules, CA) with 16 cm x 16 cm x 1 mm gels composed of 8% (wt/vol) polyacrylamide (acrylamide:bis 37.5:1) gels in 1x Tris-acetate EDTA buffer with a 15 to 55% linear denaturant gradient. The 100% denaturing solution contained 40% (vol/vol) formamide and 7.0 M urea. Electrophoresis was performed at a constant voltage of 200 V at 60°C for 3 h. Gels were fixed in 10% acetic acid for 15 min, washed 3 times in deionized water, then put in 50% methanol, washed and stained in 5µg of Sybr green I (FMC Bio products, Philadelphia, PA) /ml of 1x Tris-acetate EDTA buffer for 30 min. After staining, the gels were analyzed using a laser densitometer FluorImage with the Fragment Analysis software.

Estimates of Diversity

After separation of amplicons by DGGE, the profiles of the amplicons were compared using the coefficient of similarity (Cs), determined as: $Cs = (a + d)\ (a + b + c + d) - 1$, where Cs is the index of similarity between samples i and j, a is the number of amplicons common to samples i and j, b is the number of amplicons present in sample i, c is the number of amplicons present in sample j, and d is the number of distinct amplicons in samples i and j. If 2 profiles are identical, Cs equals 100%, if they are entirely different, Cs equals 0%. The presence or absence of amplicons was detected in the different samples, and a dendrogram was constructed using Treecon version 1.3b for Windows (Van de Peer and De Wachter, 1994, 1997).

Statistical Analysis

Data from BWG and intestinal measurements within age were subjected to ANOVA procedure for a completely randomized design (Steel and Torrie, 1980) using the general linear model procedure of SAS (SAS Institute, 1990). Statistical significance of differences among sexes was assessed using the least significant difference test (Steel and Torrie, 1980). A probability level of $P < 0.05$ was used to determine statistical significance.

Results and Discussions

In this experiment, BWG (Table 3.1) and intestinal measurements were taken as a means of determining possible differences in development between the male and female broiler chicks, and DGGE allowed for analysis in predicting differences in the biodiversity of the bacterial community. Body weight gain was not significantly different ($P > 0.05$) from 0 to 14 d of age between sexes. However, by 21 d of age the males had an increase in growth indicated by an improved BWG ($P < 0.05$) compared to the females, representing the beginning of diverging growth rates between genders. These results agree with previous reports of males having higher BWG than females (Havenstein et al., 1994; Suto et al., 1993).

The relative weight and length of the 3 intestinal segments along with jejunal villi height (Table 3.2) were measured as a means of determining intestinal development. The relative duodenal weight was greater ($P < 0.05$) for females at 3 d of age and greater for males at 7 d of age. However, there was no difference ($P > 0.05$) in relative duodenum length between males and females at any measured periods. There were no differences ($P > 0.05$) in relative jejunal weight and length between genders at any of the measured periods. Males and females also had similar relative ileal weight and length at each measuring period except at 7 d of age when males had relatively greater and relatively longer ileums ($P < 0.05$) than the females. The morphological measurements of villi height in the jejunum were also similar ($P > 0.05$) between genders, except for at 14 d of age males had significantly higher villi than females (by approximately 50 micrometers). The jejunal villi width was greater for females than males at 3 d of ages, but males had wider villi than females from 7 to 21 d of age. The increase measurements of the villi in both height and width with age indicate an increase in surface area, which is associated with nutrient absorption and intestinal development (Sklan, 2001; Sklan and

Noy, 2003) and may correlate with the increase in male BWG. However, based on the intestinal measurements taken there were no strong consistent differences between genders, except in villi height and width after 14 d of age indicating a divergence in gender developmental rate.

Considering there was no overall difference in intestinal development or BWG between males and females during the early stages of development (0 to 14 d of age), one might conclude that the intestinal environment or micro ecosystem would also be similar allowing for similar bacterial communities, regardless of gender. However, bacterial analysis demonstrated that there was less than a 30% similarity in the bacterial populations of the two genders and indicating that the communities are different based on gender (Figure 3.1).

The reason for the difference in the bacterial communities between genders is not well understood and may involve a number of micro-environmental conditions such as pH, temperature, mucin composition or concentration that would provide various niches and impact the type of bacteria that successfully compete for nutrients in the intestine. There are few published reports on the composition of the intestinal bacteria of different genders of animals, none on poultry. A study of male and female Swiss Webster mice concurred with our findings that the bacterial communities are different between males and females (Ge et al., 2006). The female mice had a different species of *Lactobacilli* present in the bacterial community than the males, which was reported to be a contributing factor to the female's ability to immunologically respond to *H. hepaticus* infection. Therefore, differences in disease resistance between the sexes could be partly due to differences in the bacterial population present in the GIT. Furthermore certain species of bacteria present within the intestinal bacterial community could impact health by improving the animal's response to a specific infection, as suggested by Ge and colleagues, acting in an intraorganismal mutualism (Lan et al., 2005).

The bacterial community differences between genders could affect carcass composition. Male and female broilers differ in the amount of lean muscle mass and fat localized in the fat pad. As the bird ages, hormones are the major contributing factor in growth and development. The release of the hormones testosterone and estrogen allows for the development of secondary sex characteristics, which separates the appearance and further development between male and female broilers. These hormones are dictating factor for the difference in carcass composition. In a recent study with mice, intestinal bacterial populations were evaluated between lean and obese mice from the same genetic line (Ley et al., 2005). The authors reported a difference in bacterial populations between the lean and obese mice, suggesting that certain bacterial communities are capable of making exogenous nutrients more available to the host allowing for increased weight or in the case of the mice, obesity (Ley et al., 2005). Even prior to hatch, in certain genetic lines of birds, there is the beginning of a separation between genders with females have fast feathering primary wing feathers. These early differences in hormones due to their genetic difference could be impacting the intestinal micro-ecosystem resulting in the separation of the bacterial community. Therefore, the difference in sex-linked genes associated with carcass composition may be a contributing factor to the difference in bacterial community between male and female broiler chicks.

In addition to the impact of gender there was also an age effect on the bacterial community. In both the males and females, there was a difference in the composition of the bacterial community with age. In males, the bacterial community is 70% similar between 3 and 7 d of age and between 14 and 21 d of age, but there was a decrease in similarity to 45% when comparing the bacterial community at either 3 or 7 d of age with older birds at either 14 or 21 d of age. The bacterial community in the females followed a more stepwise trend, with the

bacterial community becoming less similar as the bird aged starting at 90% similarity between 3 and 7d of age to 50% similarity between 3 and 21 d of age. Previous studies have looked at the intestinal bacterial population of broilers and reported a change in the composition of the bacterial community as the bird ages, but the gender of the birds was not compared (Lu et al., 2003; Amit-Romach et al., 2004).

In conclusion, there was no difference in BWG or intestinal development between genders during the early stages of development (0 to 14 d of age), but after 14 d of age the rate of development changed between genders. Additionally, the biodiversity of the intestinal bacteria in both sexes changed as the birds aged. However, the intestinal bacterial population clearly separated into 2 clusters defined by gender. The similarity in BWG and intestinal measurements between genders during the early stages of development would suggest that intestinal micro-ecosystems were similar resulting in similar bacterial communities, but from 7 to 21 d of age there was a divergence in intestinal development. The divergence in intestinal development and the difference in the composition of the bacterial communities between genders is a contributing factor in the greater weight of males at 21 d of age. The possibility that additional factors affect the composition of the bacterial community of male and female broilers is strong and further research would prove beneficial in gaining a more extensive understanding of the bacterial community in the GIT.

References

Amit-Romach, E., D. Sklan, and Z. Uni. 2004. Microflora ecology of the chicken intestine using 16s ribosomal DNA primers. Poult. Sci. 83: 1093-1098.

Apajalahti, J. 2005. Comparative gut microflora, metabolic challenges, and potential opportunities. J. Appl. Poult. Res. 14: 444-453.

Apajalahti, J. H. A. L. K. Sarkilahti, B. R. E. Maki, J. P. Heikkinen, P. H. Nurminen. W. E. Holben. 1998. Effective recovery of bacterial DNA and percent-guanine-plus-cytosine-based analysis of community structure in the gastrointestinal tract of broiler chickens. Appl. Environ. Microbiol. 64: 4084-4088.

Cook, R. H., and F. H. Bird. 1973. Duodenal villus area and epithelial cellular migration in conventional and germfree chicks. Poult. Sci. 52: 2276-2280.

Drasar, B.S., and P. A. Barrow.1985. Intestinal Microbiology. American Society for Microbiology, Washington, D.C.

Franks, A.H., H.J.M. Harmsen, G.C. Rangs, G.J. Jansen, F. Schut, and G.W. Welling. 1998. Variations of bacterial populations in human feces measured by fluorescent *in situ* hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. Appl. Environ. Microbiol. 64: 3336-3345.

Fuller, R. 1989. A review: Probiotics in man and animal. J. Appl. Bacteriol. 66:365-378.

Ge, Z. M., Y. Feng, N. S. Taylor, M. Ohtani, M F. Polz, D. B. Schauer, and J G. Fox. 2006. Colonization dynamics of altered schaedler flora is influenced by gender, aging, and helicobacter hepaticus infection in the intestines of swiss webster mice. Appl. Environ. Microbiol.72: 5100-5103.

Han, Y. and D. H. Baker. 1993. Effects of sex, heat stress, body weight and genetic strain on the lysine requirement of broiler chicks. Poult. Sci. 72:701-708.

Han, Y., and D. H. Baker. 1994. Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. Poult. Sci. 73:1739-1745.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and B. T. Larson. 1994a. Growth, livability, and feed conversion of 1957 vs. 1991 broilers when fed typical 1957 and 1991 broiler diets. Poult. Sci. 73: 1785-1794.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and B. T. Larson. 1994b. Carcass composition and yield of 1991 vs 1957 broilers when fed typical 1957 and 1991 broiler diets. Poult. Sci. 73: 1795-1804.

Homma, H. E. Hoy, D-Z. Xu, Q. Lu, R. Feinman, and E. A. Deitch. 2005. The female intestine is more resistant than male intestine to gut injury and inflammation when subjected to conditions associated with shock states. Am. J. Physiol. Gastointest. Liver Physiol. 288:G466-G472.

Horsted, K. J. Henning, and J. E. Hermansen. 2005. Growth and sensory characteristics or organically reared broilers differing in strain, sex and age at slaughter. ACTA Agriculture Scandinavica- Anim. Sci. 55: 149-157.

Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, G. W. Tannock. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microbiol. 68:5918-24.

Lan, Y., M. W. A Verstegen, S. Tamminga, and B. A. Williams. 2005. The role of the commensal gut microbial community in broiler chickens. World's Poult Sci. J. 61:95-104.

Lee, Y. K., K. Nomoto, S. Salminen, and S.L. Gorbach. 1999. Handbook of probiotics. John Wiley & Sons, Inc., New York.

Ley, R. E., F. Backhead, P. Turnbaugh, C. A. Lozupone, R. D. Knight, and J. I. Gordon. 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. 102: 11070-11075.

Lu. J., U. Idris, D. Harmon, C. Hofacre, J. J. Maurer, and M. D. Lee. 2003. Diversity and succession of the intestinal bacterial community of maturing broiler chicken. Appl. Environ. Microbiol. 69:6816-6824.

Mowat, A. M. and J. L. Viney. 1997. The anatomical basis of intestinal immunity. Immunol. Rev. 156: 145-166.

Rose, N., P. Constantin, and C. Leterrier.1996. Sex difference in bone growth of broiler chickens. Growth Dev. Aging 60: 49-59.

SAS Institute. 2001. SAS® User's guide: Statistics. Version 8 Edition. SAS Institute Inc., Cary, NC.

Sklan, D. 2001. Development of the digestive tract of poultry. World's Poult. Sci. J. 57: 415-428.

Sklan, D., and Y. Noy. 2003. Functional development and intestinal absorption in the young poult. Br. Poult. Sci. 44: 651-658.

Steel, R.G.D., and J.H. Torrie. 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.

Suto, Z. P. Horn, J. F. Jensen, P. Sorensen, and J. Csapo. 1998. Carcass Traits, abdominal fat deposition and chemical composition of commercial meat type chicken during a twenty week growing period. Arch. Geflugelkd. 62:21-25.

Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. Poult. Sci. 74:1622-1629.

Van de Peer, Y. and R. De Wachter. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Applic. Biosci. 10: 569-570.

Van de Peer, Y. and R. De Wachter. 1997. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. Comput. Applic. Biosci. 13: 227-230.

Xu, Z. R., C. H. Hu, M. S. Xia, X. A. Zhan, and M. Q. Wang. 2003. Effects of dietary fructooligosaccharide on digestive enzymes and morphology of male broilers. Poult. Sci. 82:1030-1036.

Table 3.1. Effect of gender on body weight gain of broilers

	Body Weight Gain			
	----------g/chick----------			
Treatment[1]	0 to3d	3 to7d	7 to14d	14 to 21d
Male	32.0	82.1	301.3	450.6[a]
Female	35.6	79.6	275.4	394.6[b]
Pooled SEM	1.11	1.17	10.98	8.60

[1] Means represent 8 pens per treatment, 25 chicks per pen.
[a-b] Means within a column and intestinal segment with no common superscript differ significantly ($P < 0.05$).

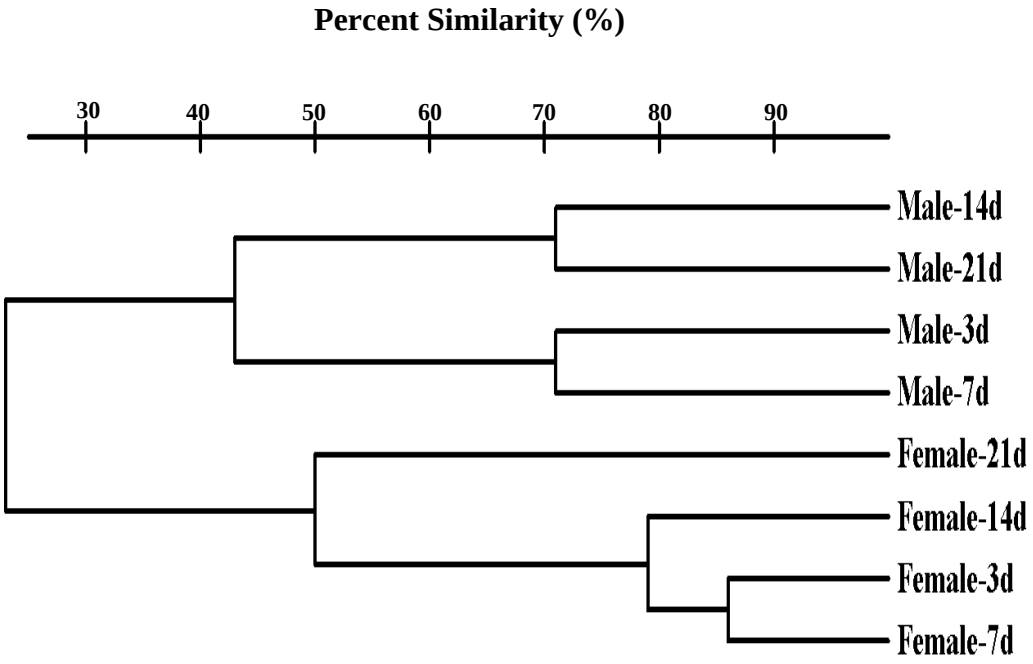
Table 3.2. Effect of gender on the relative weight and length of the small intestine and jejunal villi measurements of broilers

	Relative Intestinal Measurements							
	RelativeWeight[1]				Relative Length[2]			
	----------(g/kg of BW) ----------				----------(cm/kg of BW) ----------			
Treatments[3]	3d	7d	14d	21d	3d	7d	14d	21d
Duodenum								
Males	21.05[b]	21.17[a]	14.98	9.76	1582	1012	445	236
Females	22.70[a]	19.44[b]	14.67	10.36	1665	986	461	268
Pooled SEM	0.44	0.50	0.52	0.39	35.16	22.1	92.9	6.5
Jejunum								
Males	29.64	33.02	24.65	20.10	3448	2400	1112	669
Females	31.96	31.90	23.27	19.10	3696	2409	1147	710
Pooled SEM	1.01	0.57	0.73	0.60	103.0	58.2	31.7	14.0
Ileum								
Males	21.15	21.81[a]	15.48	13.16	3462	2204[a]	1003	574
Females	22.11	19.63[b]	14.99	11.94	3442	2027[b]	998	586
Pooled SEM	0.50	0.38	0.60	0.57	86.4	39.6	29.1	21.8

	Jejunum Villi Measurements							
	Height				Width			
	----------(μm)----------				----------(μm)----------			
Treatments[4]	3d	7d	14d	21d	3d	7d	14d	21d
Males	275.3	390.1	573.1[a]	683.2	42.9[b]	63.9[a]	66.0	89.4[a]
Females	275.2	396.1	522.1[b]	671.6	49.5[a]	57.3[b]	62.4	76.7[b]
Pooled SEM	6.50	15.27	14.41	21.37	1.72	2.26	4.88	3.97

[1] Relative weight = g/kg of bodyweight.
[2] Relative length= cm/kg of bodyweight.
[3] Means represent 8 pens per treatment, 2 randomly selected chicks per pen.
[4] Means represent 8 pens per treatment, 2 randomly selected chicks per pen, 3 villi per chick.
[a-b] Means within a column and intestinal segment with no common superscript differ significantly ($P < 0.05$).

Figure 3.1. Similarity[1] among ileal bacterial communities among male and female broilers from 0 to 21 d of age[2]



[1]Simple matching using clustering method of Treecon® to produce dendogram representing the relationship between DGGE band patterns of V3-V4 region of 16s rDNA of the intestinal community DNA.

[2]Contents for bacterial analysis pooled from 16 birds per sex per age period.

CHAPTER 4

EVALUATION OF THE BACTERIAL COMMUNITY AND INTESTINAL DEVELOPMENT OF DIFFERENT GENETIC LINES OF CHICKENS[1]

[1] Lumpkins, B. S., A.B. Batal, and M.D. Lee. To be submitted to *Poultry Science.*

ABSTRACT The gastrointestinal tract (GIT) provides an environment for a large and diverse population of intestinal bacteria, which is unique for each animal species. The poultry industry uses different genetic lines of chickens with varying rates of development, but little is known about how the bacterial community varies among these different genetic lines of chickens. Therefore, an experiment was conducted to observe and evaluate the changes in the bacterial community and GIT development of a modern multipurpose strain, high yield strain, and a historic strain (Athens Canadian Random Bred (ACR) of chicks. All birds were fed a standard non-medicated corn-soybean meal diet ad libitum from 0 to 35 d of age. Intestinal measurements and bacterial analysis of the ileum were conducted at 4, 8, 14, 21 and 35 d of age. Bacterial DNA was isolated from the digesta, and the distribution of bacterial 16S rRNA sequence polymorphisms were analyzed by a combination of denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphisms (T-RFLP). The multipurpose chicks performed the best from 0 to 14 d of age; however, overall performance was similar for the multipurpose and the high yield chicks. The ACR chicks had the poorest performance at all periods measured. The overall relative weight of the jejunum and ileum was not different between the 3 genetic lines, but the ACR chicks had the longest relative jejunum and ileum lengths. Furthermore, the multipurpose chicks had the longest villi height, while the ACR chicks had the shortest villi height in the jejunum and ileum at all measuring periods. Based on DGGE, the multipurpose and high yield chicks had similar bacterial communities at all ages. Regardless of the genetic line of chicks the bacterial community changed with age. The performance, GIT measurements, and bacterial community of the multipurpose and high yield chicks were similar to one another, while the performance, GIT measurements and bacterial community of the ACR differed compared to the multipurpose and high yield genetic lines. The results indicate that the

different genetic lines of broilers have varying rates of intestinal development, which may impact performance and the bacterial community.

Key words: Bacterial population, gastrointestinal tract, denaturing gradient gel electrophoresis, genetic lines, terminal restriction fragment length polymorphism

Introduction

A large and diverse population of commensal bacteria resides in the gastrointestinal tract (**GIT**). The intestinal bacterial community can act in an intra-organismal mutualism within the host (Xu et al., 2003; Apajalahti, 2005). Considering the GIT is a micro-ecosystem, changes in environmental conditions due to development of the GIT can result in changes in the intestinal bacteria (Apajalahti et al., 2004). Intestinal bacterial populations have been reported to be impacted by the age of the bird, which could be associated with GIT development (Lu et al., 2003). Furthermore, when the GIT has reached a steady state of development and environmental conditions stabilize, the composition of the bacterial community also stabilizes and becomes well established with a decrease in diversity (Gong et al., 2002).

The poultry industry utilizes a number of genetic lines of birds in order to meet production goals in both the broiler (meat) and laying hen (egg) industry. Broiler strains vary based on the growth rate. In the past, growth rate was much slower and the time it took to achieve a market weight of 2 kg was much longer compared today's modern breeds. Modern broiler breeds referred to as multipurpose broilers have a rapid growth rate immediately following hatch, and reach a market weight of about 2.2 kg in about 46 d. Modern high yield broilers reach a state of rapid growth later than the multipurpose broilers and achieve a heavier market weight between 2.8 and 3.3 kg in 49 d. These genetic lines of broilers have different rates of development, and the GIT reaches maturity at different ages (Corzo et al., 2005; Uni et al., 1995). Therefore, at comparable ages the stage of GIT development is different, and one would assume the bacterial community would also be different because of the difference in the micro-ecosystem of the gut.

Research comparing the differences in bacterial communities between different genetic lines of animals is scarce, and there are few if any reports evaluating the intestinal bacterial populations between broiler lines. The majority of the research conducted with the various genetic lines has been to evaluate GIT development. Therefore, it was our objective to evaluate differences in growth rate, intestinal development and the composition of the intestinal bacterial population of various genetic lines of broilers.

Materials and Methods

Bird Husbandry and Dietary Treatments

Chicks were reared under standard management conditions and fed and watered ad libitum. Multipurpose (**MP**) and high yield (**HY**) hatching eggs were acquired from local hatcheries and Athens Canadian Random Bred (**ACR**) eggs were obtained from stock maintained at the University of Georgia Poultry Research facility. The multipurpose broilers have a rapid rate of growth during the early stages of development (0 to 16 d of age) and reach a market weight of 2.22 kg by 45.8 d of age. The early growth rate of the HY broilers is not as rapid as that of MP broilers, but the growth rate of HY broilers increases during later ages and it typically surpasses the MP in body weight. The MP and HY broilers are modern genetic lines, but the ACR is a 1957 genetic line of broiler a much slower rate of development than modern broiler strains (Uni et al., 1995). The fertile eggs of the 3 genetic lines were incubated and hatched at the same time in the same facility. At hatch, 240 chicks of each genetic line were randomly allocated to 8 replications of 30 chicks (240 chicks per treatment) in a floor pen with litter shavings with a dimension of 122 x 310 cm (density of 1,259 cm^2 per bird). The birds were housed in an environmentally controlled room under 24 h lighting conditions with nipple drinkers and pan feeders, and were fed a conventional corn-soybean meal starter diet (23% CP,

3,096 kcalME/kg, 1.14% total lysine, 0.53% total methionine, and 0.89% total sulfur amino acids) (Table 4.1) Body weight and feed intake were recorded throughout the Exp, and body weight gain (**BWG**) and gain:feed (**G:F**) were calculated.

Bird Sampling

Two chicks from each pen (18 birds per treatment) were euthanized and intestines were sampled at 4, 8, 14, 21, and 35 d of age. For length and weight measurements, the small intestine was divided into three segments: duodenum (from gizzard to entry of the bile and pancreatic ducts), jejunum (from entry of the ducts to yolk stalk), and ileum (from yolk stalk to ileocecal junction). The 3 segments of the small intestine were flushed with 20 mL of physiological saline solution, and the empty weight of each section was recorded. From 2 additional chicks per pen (18 birds per treatment), jejunum and ileum contents were massaged out to include intestinal mucin and were collected in sterile bags and stored at -80°C until isolation of bacterial DNA. Organ weights and lengths were expressed on a relative (grams/kg of body weight and mm/kg of body weight, respectively) basis. For morphologic analysis, approximately 5 cm of the middle portion of the jejunum (from entry of the ducts to yolk stalk) was excised and fixed in 10% formalin. Six cross sections of 70% ethanol-preserved segments for jejunal sample were then prepared for staining with hematoxylin and eosin using standard paraffin embedding procedures (Uni *et al.*, 1995). A total of 3, intact well-oriented villi were selected in 8 replicates for each intestinal cross section (24 measurements for each intestinal sample with 288 measurements per treatment). Villus height was measured from the tip of the villus to the crypt junction; villus width was measured as the distance across the middle of each

villus. Morphological indices were determined using computer-aided light microscopy (16x magnification of the objective lens) with image software analysis.[1]

Bacterial DNA Isolation

The bacterial portion of the ileal contents was recovered by density gradient centrifugation through a gauze column and the DNA was extracted as described by Lu et al. (2003).

Denaturing Gradient Gel Electrophoresis (DGGE)

The V3-V4 region of the 16S ribosomal gene (rDNA) of microorganisms of the bacterial domain were amplified with the primers HDA1-GC and HDA2 as described by Knarreborg et al.(2002). Amplicons were confirmed by electrophoresis using a 1.5% agarose gel containing ethidium bromide. DNA sequence polymorphisms of the amplicons were detected by resolving differences in molecular structure using denaturing gradient gel electrophoresis (**DGGE**). The DGGE was conducted using the D-Code Universal Mutation Detection system (BioRad, Hercules, CA) with 16 cm x 16 cm x 1 mm gels composed of 8% (wt/vol) polyacrylamide (acrylamide:bis 37.5:1) gels in 1x Tris-acetate EDTA buffer with a 15 to 55% linear denaturant gradient. The 100% denaturing solution contained 40% (vol/vol) formamide and 7.0 M urea. Electrophoresis was performed at a constant voltage of 200 V at 60°C for 3 h. Gels were fixed in 10% acetic acid for 15 min, washed 3 times in deionized water, then put in 50% methanol, washed and stained in 5µg of Sybr green I (FMC Bio products, Philadelphia, PA) /ml of 1x Tris-acetate EDTA buffer for 30 min.

Estimates of Diversity

After separation by DGGE the profiles of the amplicons were compared using the coefficient of similarity (**Cs**), determined as: $Cs = (a + d)\,(a + b + c + d)^{-1}$, where Cs is the index

[1] Image-Pro Plus Version 3.0, Media Cybernetics, Silver Spring, MD 20910.

of similarity between samples i and j, *a* is the number of amplicons common to samples i and j, *b* is the number of amplicons present in sample i, *c* is the number of amplicons present in sample j, and *d* is the number of distinct amplicons in samples i and j. If 2 profiles are identical, Cs equals 100%, if they are entirely different, Cs equals 0%. The presence or absence of amplicons was detected in the different samples, and a dendrogram was constructed using Treecon version 1.3b for Windows (Van de Peer and De Wachter, 1994, 1997). At the occurrence of bacterial communities having a high level of similarity in their overall composition it will be referred to as a cluster. A cluster occurs when the composition of a bacterial community, based on treatment and/or age of the bird, group together with a level of similarity. Additionally, the composition of the bacterial communities differs from other bacterial communities due to a low level of similarity resulting in different clusters.

Terminal Restriction Length Polymorphism (T-RFLP)

The PCR for T-RFLP used universal 16s rDNA primers (8F and 1492R) as described by Lu et al 2006. Briefly, the 8F primer was labeled with 5'FAM (carboxyfluorescein-N-hydroxysuccinimide ester-dimethyl sulfoxide), the amplicons were digested with *Hae*III, and the T-RF were analyzed by electrophoresis on an automatic sequence analyzer in GeneScan mode (ABI PRISM 310 DNA sequencer, PE Biosystems, Foster City, CA). The T-RF peaks were identified by comparison to a 16s database of fragment sizes generated by silico analysis of *Hae*III cut sizes of the sequences obtained from the cloned libraries obtained from previous studies (Lu et al., 2003, 2006). The peak area in each T-RFLP profile was transformed to relative amount by taking the peak area for a phylotype and dividing by the total peak areas for all detectable phylotypes in the T-RFLP profile. Ratios were converted to percentages.

Statistical analysis

Data for performance and intestinal measurements were subjected to ANOVA procedure for a completely randomized design (Steel and Torrie, 1980) using the general linear model procedure of SAS (SAS Institute, 1990). Statistical significance of differences among treatments was assessed using the least significant difference test (Steel and Torrie, 1980). A probability level of $P < 0.05$ was used to determine statistical significance.

Results and Discussions

There were significant differences in BWG and G:F between the genetic lines during the early stages of growth (0 to 21 d of age) and between the modern genetic lines (MP and HY) and the 1957 ACR line after 21 d of age (Table 4.2). During the first 14 d of age, the MP chicks had the greatest BWG ($P < 0.05$), followed by the HY and then the ACR chicks. After 14 d of age the HY broilers caught up to the MP broilers and both the modern broiler lines had similar BWG and G:F ($P > 0.05$) through to the termination of the Exp. at 35 d of age. However, if the birds had been grown out further to 49 d of age, it is likely that the HY broilers would have had the greatest BWG during the later periods, due to their selection for growth rate at older ages. There was no difference in G:F at any measured period between the MP and HY broilers. The Athens Canadian Random Bred broilers had the lowest BWG and G:F ($P < 0.05$) at all of the given measuring periods. The difference in BWG and G:F between the modern genetic lines of broilers and those used in 1957 is of no surprise, and agrees with prior work by Havenstein et al. (1994a) who reported large improvements in BWG and G:F in 1991 Arbor Acre broilers compared to the ACR broilers.

The relative weights (Table 4.3) of the jejunum and ileum in general were similar ($P > 0.05$) for all genetic lines throughout the experiment. This is similar to the findings in Mallard

and Pekin ducks from Watkins et al. (2004). However, the duodenum relative weight was similar ($P > 0.05$) between the 2 modern lines and lighter ($P < 0.05$) compared to the ACR broilers from 14 to 35 d of age. Our results agree with the previous findings of Dror et al. (1977) who reported heavier relative duodenum weights in light breed broilers and Dunnigton and Siegel (1995) who reported a greater relative duodenal weight after 10 d of age in low weight selected birds versus high weight birds. The relative length for each of the 3 segments of small intestine was different between the genetic lines at each of the sampling periods, which corresponded to the differences in BWG during the first 2 wk of age. Beyond 2 wks of age the MP and HY broilers had similar relative intestinal lengths ($P > 0.05$). Both the MP and HY broilers had shorter relative intestine lengths ($P < 0.05$) than the ACR broilers. Our results agree with previous findings that observed heavy breeds having shorter relative intestinal lengths (Uni et al., 1995) suggesting an inverse relationship between BWG and relative intestinal length. Additionally, from a morphological standpoint there were differences in villi height at each measuring period. In the jejunum and ileum, the modern lines had greater villi height at all measured periods compared to the ACR broilers (Table 4.4). Uni et al. (1995) reported similar findings with the Arbor Acres broilers having greater villi height compared to the Lohmann laying type chicken from 0 to 14 d of age. In the jejunum the overall villi height was similar between the MP and HY broilers. The villi in the ACR broilers were shorter than the modern lines. The ileum villi height in the modern lines was different until after 21 d of age. Prior to 21 d the villi height was different between all 3 lines. This was the same time period in which BWG was different in the 3 lines. Overall, there was no difference in jejunal or ileal villi height-width ratio.

Based on the DGGE analysis, there was a difference in the bacterial population among the genetic lines. Two main clusters in the bacterial community separated the modern genetic lines (MP and HY) from the ACR lines (Figure 4.1). Furthermore, smaller clusters within the genetic lines grouped the bacterial populations together based on age. During the early ages (0 to 7 d of age) the GIT of all chicks are undergoing extremely dramatic changes resulting in unstable environmental conditions of the micro-ecosystem. This may be the reason the bacterial community of the ACR birds at 4 and 8 d of age clustered with the MP and HY broilers. However, by 2 wk of age the intestinal bacterial community became more stable and established allowing for the clear separation of the bacterial community of the ACR broilers from the modern genetic lines. There was less than a 50% similarity in the communities between the modern and 1957 ACR lines. The clustering of all 3 treatments at the early ages of development agrees with van der Wielen et al. (2002), who observed similar DGGE banding in broilers of the same genetic line at 4 d of age, but at older ages the bands were no longer similar between broilers. At 21 d of age when the MP and HY broilers had similar performance and intestinal measurements the bacterial populations was greater than 90% similar in the overall composition of the intestinal bacteria. One possible explanation for the difference in bacterial community between the modern and ACR broiler lines may be due to differences in villi height, which would result in increasing the distance away from the lumen providing a niche for certain bacterium. Villi height is just one factor contributing to the bacterial community's niche. Other possible contributors include the amount of mucin production, pH, and enzymes present in the GIT. However, the change in the bacterial community may also be affected by genetics, which has been reported to affect the intestinal bacteria in mice. In an experiment conducted with obese and lean mice, Ley et al. (2005) reported that the bacterial community was different

although the mice were genetically identical except for the gene associated with obesity. The authors contributed the change in bacterial community to the mice's obesity gene that appeared to have an effect on the intestinal bacteria allowing for exogenous nutrients to be more available to the host animal. Therefore, since modern broilers have a much greater carcass yield, heavier fat pads, and higher percentage of carcass fat than the 1957 ACR line (Havenstein et al., 1994b), it could be that genetics is a contributed in some way to the change in the intestinal bacterial population between the genetic lines.

Using terminal restriction fragment length polymorphism we were able to determine the composition of the bacterial community of the ileum (Figure 4.2). In the first 8 d of age, the MP broilers with the best performance had higher abundance of bacteria related to the *Clostridia* species than the other genetic lines, and a lower proportion of *Lactobacilli*. Furthermore *Staphylococcaceae* were detected in the bacterial community of the MP broilers but were not observed in the other lines. In all cases, as the birds aged, the level of *Lactobacilli* increased while the level of *Clostridia* decreased, but the MP broilers had higher levels of *Clostridia* at all sampled ages. By 21 d of age, it appeared that the intestinal bacterial populations became stable and established in the GIT micro-ecosystem. The MP and HY broilers had similar performance and GIT measurements at approximately the same age the bacterial community was becoming stable. Prior to establishing a stable bacterial community (21 d of age), the diversity of the bacterial community in all 3 genetic lines of broilers changed from one age period to the next, representing an age affect. This change in the bacterial community with age suggests a change in the intestinal micro-ecosystem, which agrees with previous reports (Lu et al., 2003; Amit-Romach et al., 2004; Smirnov et al., 2005

In conclusion, the MP broiler had the best performance and fastest rate of development during the first 21 d of age. These broilers also had higher levels of *Clostridia* and a presence of *Staphylococceae*, which were not present in the other genetics lines from 0 to 35 d of age. At 21 d of age, performance, intestinal measurements and bacterial composition were similar between the HY and MP broilers, while the ACR broilers had the lowest performance and slowest rate of development. It appears genetics selection for growth rate has not only provided the poultry industry with bigger and faster growing broilers, but also changes in the micro-ecosystem in the GIT that provides unique environmental conditions.

References

Amit-Romach, E., D. Sklan, and Z. Uni. 2004. Microflora ecology of the chicken intestine using 16s ribosomal DNA primers. Poult. Sci. 83: 1093-1098.

Apajalahti, J., A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poult. Sci. J. 60: 223-232.

Apajalahti, J. 2005. Comparative gut microflora, metabolic challenges, and potential opportunities. J. Appl. Poult. Res. 14: 444-453.

Corzo, A., M. T. Kidd, D. J. Burnham, E. R. Miller, S. L. Branton, R. Gonzalez. 2005. Dietary amino acid density effects on growth and carcass of broilers differing in strain cross and sex. J. Appl. Poult. Res. 14:1-9.

Dror, Y., I. Nir, and Z. Nitsan. 1977. Relative growth of internal organs in light and heavy breeds. Br. Poult. Sci. 18: 493-496.

Dunnington, E. A., and P. B. Siegel. 1995. Enzyme-activity and organ development in newly-hatched chicks selected for high or low 8-week body-weight. Poult. Sci. 74: 761-770.

Gong, J., R. J. Forster, H. Yu, J. R. Chambers, R. Wheatcroft, P. M. Sabour, and S. Chen. 2002. Molecular analysis of bacterial populations in the ileum of broiler chickens and comparison with bacteria in the cecum. FEMS Microbiol. Ecology 41: 171-179.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and B. T. Larson. 1994a. Growth, livability, and feed conversion of 1957 vs. 1991 broilers when fed typical 1957 and 1991 broiler diets. Poult. Sci. 73: 1785-1794.

Havenstein, G. B., P. R. Ferket, S. E. Scheideler, and D. V. Rives. 1994b. Carcass composition and yield of 1991 vs 1957 broilers when fed typical 1957 and 1991 broiler diets. Poult. Sci. 73: 1795-1804.

Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, and G. W. Tannock. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microbiol. 68: 5918-5924.

Ley, R. E., F. Backhead, P. Turnbaugh, C. A. Lozupone, R. D. Knight, and J. I. Gordon. 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. 102: 11070-11075.

Lu, J, U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, M. D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69: 6816-6824.

Lu, J., C. L. Hofacre, M. D. Lee. 2006. Emerging Technologies in Microbial Ecology Aid in Understanding the Complex Disease Necrotic Enteritis. J. Appl. Poult. Res. 15:145-153.

SAS Institute Inc., 1990. SAS STAT User's Guide Release 6.08. SAS Institute Inc., Cary NC.

Smirnov, A., E. Tako, P. R. Ferket, and Z. Uni. 2006. Mucin gene expression and mucin content in the chicken intestinal goblet cells are affected by in ovo feeding of carbohydrates. Poult. Sci. 85: 669-673.

Steel, R. G. D. and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.

Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. Poult. Sci. 74:1622-1629.

Van de Peer, Y. and R. De Wachter. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comut. Applic. Biosci. 10: 569-570.

Van de Peer, Y. and R. De Wachter. 1997. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. Comput. Applic. Biosci. 13: 227-230.

van der Wielen, P., D. A. Keuzenkamp, L. J. A. Lipman, F. van Knapen, and S. Biesterveld. 2002. Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. Microbial Ecology 44: 286-293.

Watkins, E. J., P. J. Butler, and B. P. Kenyon. 2004. Posthatch growth of the digestive system in wild and domesticated ducks. Br. Poult. Sci. 45: 331-341.

Xu J, Bjursell M. K, Himrod J, S, Deng, L. K. Carmichael, H. C. Chiang, L. V. Hooper, and J. I. Gordon. 2003. A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science 299: 2074-2076.

Table 4.1. Composition of the diet (as-fed basis)

Ingredients	%
Corn, yellow, ground	56.12
Soybean meal (48)	37.50
Fat, poultry	3.00
Dicalcium Phosphate	1.75
Limestone	0.80
Salt	0.30
Vitamin premix[1]	0.25
DL-Methionine	0.20
Trace mineral premix[2]	0.08
Contents by calculation	
ME, kcal/kg	3095
Crude protein, %	22.6
Lysine, %	1.14
Methionine, %	0.53
Met + Cys, %[3]	0.89

[1]Vitamin mix provided the following (per kg of diet): thiamin•mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (cobalamin),12.0 µg; pyridoxine•HCL, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfite complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 ug; trans-retinyl acetate, 1,892 ug; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.
[2]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4•H_2O$), 60 mg; iron ($FeSO_4•7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4•5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium ($NaSe0_3$), 0.3 mg.
[3]Met= methionine; Cys= cysteine.

Table 4.2. Weight gain and feed efficiency (G:F)[1] of 3 genetic lines of broilers

	0-8 d		8-14 d		14-21 d		21-35 d		0-35d	
Treatments[2]	Gain (g/chick)	G:F (g:kg)	Gain (g/chick)	G:F (g:kg)	Gain (g/chick)	G:F (g:kg)	Gain (g/chick)	G:F (g:kg)	Gain (g/chick)	G:F (g:kg)
Multipurpose	172.4[a]	819[a]	256.7[a]	767[a]	433.1[a]	643[b]	1221.5[a]	606[a]	2083.3[a]	635[a]
High yield	133.7[b]	705[a]	221.8[b]	713[a]	405.3[a]	688[b]	1112.9[b]	592[a]	1873.2[b]	635[a]
ACR[3]	44.2[c]	176[b]	59.1[c]	494[b]	102.3[b]	767[a]	262.4[c]	548[b]	468.0[c]	466[b]
Pooled SEM	2.94	22.89	3.82	40.80	12.06	53.66	20.04	13.52	18.49	9.35

[1] G:F = gain to feed ratio.
[2] Means represent 8 pens per treatment, 30 chicks per pen.
[3] ACR= Athens Canadian Random bred.
[a-c] Means within a column with no common superscript differ significantly ($P < 0.05$).

Table 4.3. Relative weight and length of the small intestine of 3 genetic lines of broilers

	RelativeWeight[2] (g/kg of BW)					Relative Length[3] (cm/kg of BW)				
Treatments[1]	4d	8d	14d	21d	35d	4d	8d	14d	21d	35d
Duodenum										
Multipurpose	21.7^{b}	19.4	15.2^{b}	11.1^{b}	6.3^{b}	1398^{b}	795^{c}	463^{b}	261^{c}	138^{b}
High yield	24.7^{a}	21.2	15.9^{b}	12.0^{b}	6.7^{b}	1723^{a}	1068^{b}	516^{b}	308^{b}	135^{b}
ACR[4]	17.0^{c}	20.7	18.2^{a}	15.4^{a}	10.3^{a}	1795^{a}	1548^{a}	1048^{a}	698^{a}	366^{a}
Pooled SEM	1.01	0.75	0.50	0.39	0.32	61.9	46.6	24.2	15.1	10.8
Jejunum										
Multipurpose	29.4	27.5	23.1	18.6	12.7^{b}	3260^{b}	1789^{c}	1035^{c}	657^{b}	350^{b}
High yield	29.2	29.5	25.4	18.8	13.0^{b}	3438^{b}	2283^{b}	1218^{b}	717^{b}	356^{b}
ACR	25.1	31.4	26.3	18.9	16.0^{a}	3926^{a}	3246^{a}	2323^{a}	1428^{a}	850^{a}
Pooled SEM	1.93	1.14	1.07	0.66	0.55	162.2	73.7	44.5	32.3	24.9
Ileum										
Multipurpose	24.8^{a}	19.8	15.3	13.3	10.3	2997^{c}	1717^{c}	1009^{c}	679^{b}	363^{b}
High yield	23.0^{a}	19.8	15.4	13.4	10.4	3397^{b}	2350^{b}	1184^{b}	733^{b}	387^{b}
ACR	17.0^{b}	17.9	16.7	13.0	10.3	3918^{a}	3105^{a}	2039^{a}	1400^{a}	858^{a}
Pooled SEM	1.47	1.31	0.52	0.41	0.39	136.7	88.37	52.6	23.9	25.5

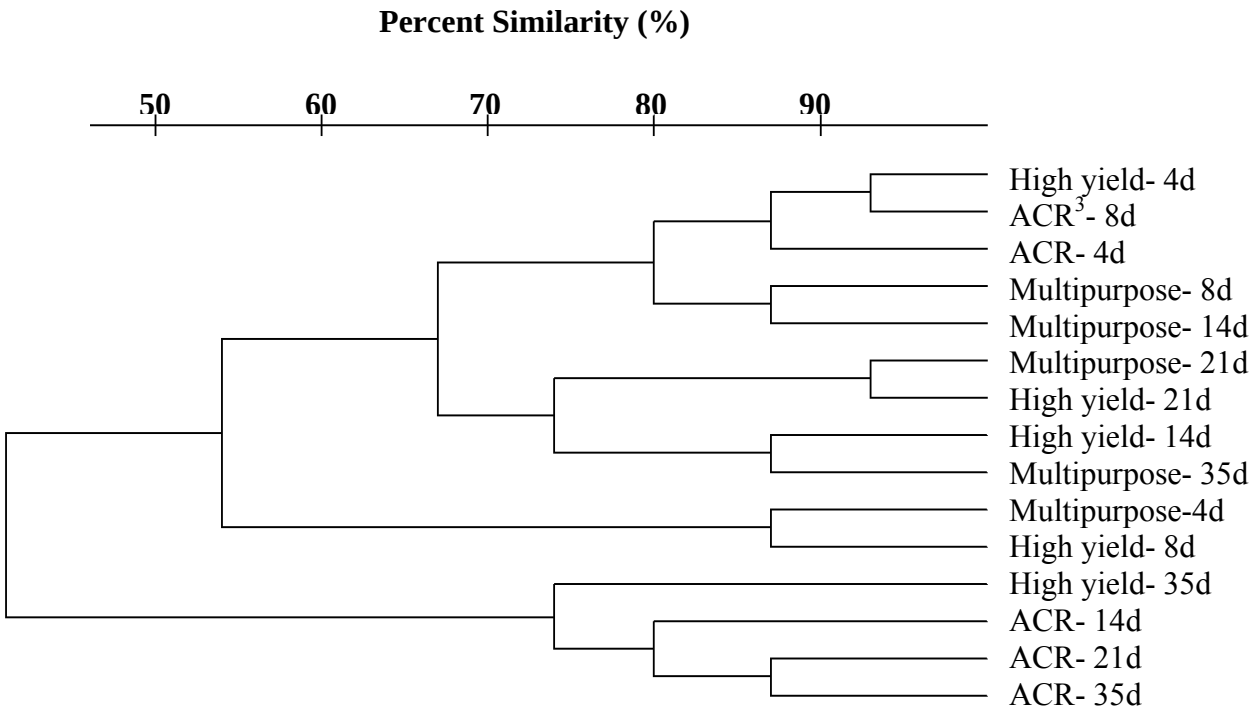
[1] Means represent 8 pens per treatment, 2 randomly selected chicks per pen.
[2] Weight = g/kg of bodyweight.
[3] Length= cm/kg of bodyweight.
[4] ACR= Athens Canadian Random Bred.
[a-c] Means within a column and intestinal segment with no common superscript differ significantly ($P < 0.05$).

Table 4.4. Jejunum and ileum villi measurements of 3 genetic lines of broilers

	Villi Height (μm)					Villi Height/Width Ratio (μm/μm)				
Treatments[1]	4d	8d	14d	21d	35 d	4d	8d	14d	21d	35d
Jejunum										
Multipurpose	279[a]	537[a]	638[a]	621[a]	781[a]	6.0	8.6	10.6	7.2[b]	12.9
High yield	274[a]	420[b]	636[a]	625[a]	851[a]	5.0	7.9	9.8	8.7[a]	12.8
ACR[2]	226[b]	332[c]	403[b]	492[b]	657[b]	5.9	7.8	8.6	9.3[a]	11.2
Pooled SEM	13.3	14.2	19.7	12.8	26.9	0.39	0.51	0.62	0.43	0.91
Ileum										
Multipurpose	207[a]	305[a]	362[a]	372[a]	442[a]	4.3	5.8	5.8[a]	5.2[a]	5.5
High yield	188[ab]	246[b]	307[b]	336[b]	432[a]	4.1	4.4	4.3[c]	4.2[b]	5.6
ACR	175[b]	237[b]	260[c]	292[c]	347[b]	4.5	5.2	5.1[b]	4.1[b]	5.6
Pooled SEM	6.9	11.7	8.4	10.7	15.2	0.33	0.49	0.22	0.30	0.42

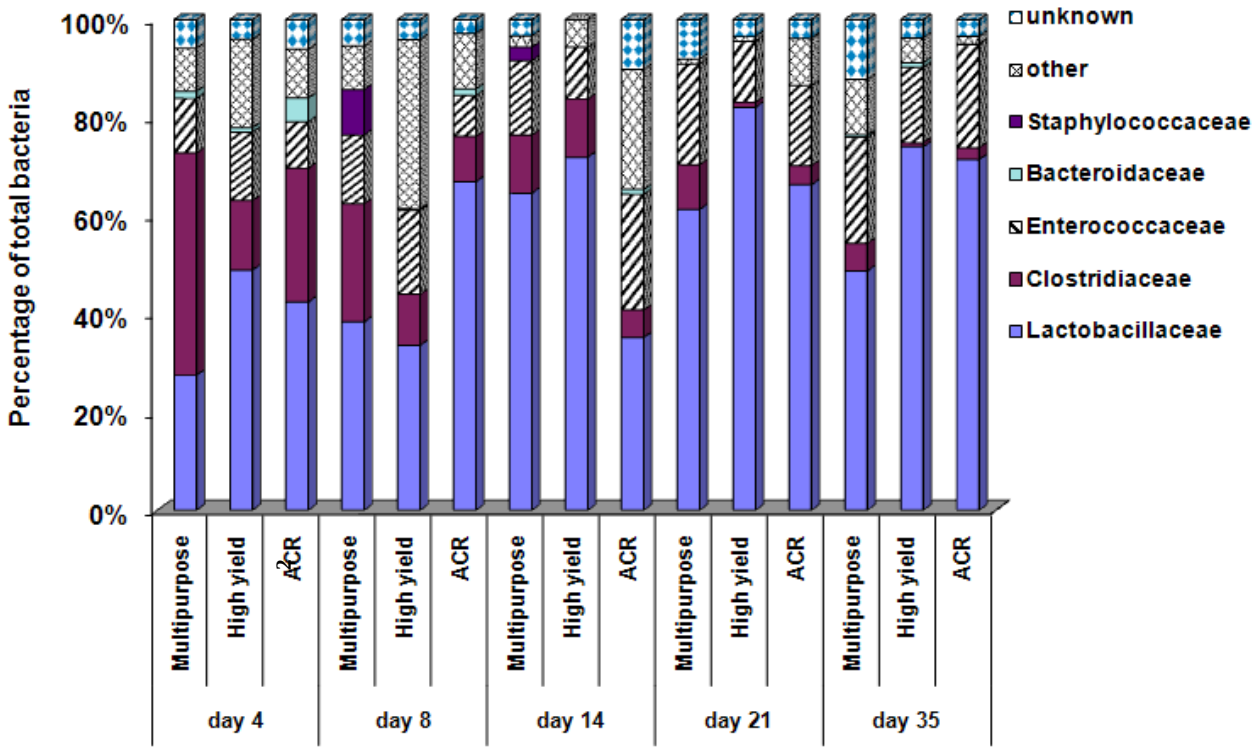
[1]Means represent 8 pens per treatment, 2 randomly selected chicks per pen, 3 villi per chick.
[2] ACR= Athens Canadian Random Bred.
[a-c] Means within a column and intestinal segment with no common superscript differ significantly ($P < 0.05$).

Figure 4.1. Dendogram showing the relatedness[1] of ileal bacterial community among 3 genetic lines of broilers[2]



[1] Simple matching using clustering method of Treecon® to produce dendogram representing the relationship[1] between DGGE band patterns of V3-V4 region of 16s rDNA of the intestinal community DNA (Van de Peer, Y**.** and R. De Wachter. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput. Applic. Biosci. 10, 569-570).
[2] Contents for bacterial analysis pooled from 16 birds per age period.
[3] ACR= Athens Canadian Random Bred.

Figure 4.2. Composition of ileal bacterial community of 3 genetic lines of broilers as determined by terminal restriction fragment length polymorphism analysis[1]



[1]Contents for bacterial analysis was pooled from 16 birds per treatment per age period.
[2] ACR= Athens Canadian Random Bred.

CHAPTER 5

INOCULATION OF NOVEL BACTERIA ON INTESTINAL DEVELOPMENT AND THE BACTERIAL COMMUNITY OF BROILERS[1]

[1] Lumpkins, B. S., A.B. Batal, and M.D. Lee. To be submitted to *Poultry Science.*

ABSTRACT The growing concern of feeding antibiotics to poultry has led researchers and animal health companies to investigate alternatives. An experiment was conducted to evaluate the effects of an oral inoculation with novel intestinal anaerobes (a possible probiotic) on the performance and development of the small intestine of broilers. At 0 d of age, male broiler chicks were separated into 4 treatments: a control and 3 test treatments that were orally inoculated with novel species of either *Bacteroidaceae* or *Clostridiaceae* or a combination of the two. Throughout the experiment all birds were fed a non-medicated corn-soybean meal diet. At 0, 1, 2, 3, 7, 16, and 42 d of age, performance parameters were measured and samples were taken for morphological and bacterial community analysis. For bacterial community analysis, community DNA isolated from small intestinal contents were amplified with universal 16s primers. Diversity and compositional changes were assessed using denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP). The performance parameters were similar among all 4 treatments from 0 to 16 d of age. At the end of the 42 d period the overall weight gain of the birds inoculated with *Clostridiaceae* was significantly lower than the control birds and the *Bacteroidaceae* inoculated birds. The birds inoculated with the combination of *Bacteroidaceae* and *Clostridiaceae* had increased villi height and goblet cell concentration during the first 3 d of age, but after 7 d of age there was no overall difference in morphological measurements between treatments. Based on the DGGE analysis, the intestinal bacterial populations clustered by age rather than treatment. Birds inoculated with either *Bacteroides* or *Clostridiaceae* had a higher proportion of lactobacilli in the ileum compared to the control birds, based on T-RFLP analysis at 42 d of age. The inoculation at 0 d of age with the combination of *Bacteroidaceae* and *Clostridiaceae* improved intestinal development

at young ages, but *Clostridiaceae* may have negative effects on weight gain during the latter stages of growth.

Key words: bacterial community, gastrointestinal tract, denaturing gradient gel electrophoresis, probiotic, terminal restriction fragment length polymorphism

Introduction

Over the years researchers have discovered a large diverse and novel bacterial population residing in the gastrointestinal tract of animals (Drasar and Barrow, 1985; Franks, 1998). These bacteria have colonized and formed their own ecosystem within the animal's intestine, and at the same time cave created a symbiotic relationship with the host. The bacterial populations in the gastrointestinal tract (GIT) occupy a complex micro-ecosystem where a number of ecological activities take place, such as intra-organismal mutualism or co-evolution. Understanding and exploiting these symbiotic relationships where there is a mutual benefit to the host and the organism residing in the host, one may be able to improve the intestinal and animal health. By introducing different substrates into the intestinal environment for the bacterial ecosystem, such as a feed ingredient that acts to stimulate growth or development of symbiotic organisms (prebiotic), or the supplementation of symbiotic bacteria themselves (probiotic) the bacterial community may be changed to optimize the amount of commensal bacteria.

Probiotics have been of great interest to the poultry health industry and researchers, due to an antibiotic ban in Europe and the growing concern in the US with feeding antibiotics at sub-therapeutic levels (Lee, 1999). By introducing a particular bacterial species to the gastrointestinal tract one may be able to increase the number of "beneficial" bacteria present and in turn further improve the development and integrity of the gastrointestinal tract, which would then aid in the overall performance of the bird (Apajalahti, 2005). Probiotics, composed of intestinal commensal bacteria, have been studied to determine their beneficial health effects (Lee, 1999). Many of the probiotics used have been *Lactobacilli* sp., and growth performance has been the main evaluating factor (Tortuero, 1972; Dilworth and Day, 1978; Jin et al., 1998). In addition, the ability to competitively exclude a particular bacterium (such as *Clostridium*

perfringens and *Salmonella Enteritidis)* from the GIT of the birds has also been of interest (Lan et al., 2004; Hofacre et al., 2002; La Ragione and Wood, 2003). Not all of the research using *Lactobacilli* in poultry has been conclusive (Jin et al., 1998; Johannsen et al., 2003).

Much of the past research using probiotics in poultry has been with the use of *Lactobacilli* cultures, and very little research has been conducted with other bacterial cultures isolated from the GIT of poultry. Therefore, the objective of our experiment was to determine how the introduction of novel bacterial species at 0 d of age affects the development and bacterial community of the GIT.

Materials and Methods

Bird Husbandry and Dietary Treatments

Chicks were reared under standard management conditions and fed and watered ad libitum. The objective of the experiment (**EXP**.) was to evaluate intestinal development and the composition of the residing intestinal bacterial population of male broiler chicks (Cobb 500) inoculated with novel bacteria at 0 d of age. At hatch 840 male chicks were transported from a local hatchery to the research facility. Upon arrival the birds were divided into 4 treatments of 3 replications each containing 70 chicks and were fed a non-medicated corn-soybean meal diet (Table 5.1). The birds were housed in an environmentally controlled room under 24 h lighting conditions with nipple drinkers and pan feeders. The treatments consisted of an oral inoculate with 50 microliters of 1 x 10^8 pooled *Bacteroidaceae* spp., 1 x 10^8 pooled *Clostridia* spp., and a combination of 1 x 10^8 *Bacteroidaceae* spp. and *Clostridia* spp. at 0 day of age, and a control group, which was not inoculated. The novel bacteria were selected based on previous work (Lu et al. 2003) where the bacterial community was evaluated in the ileum of 49 d old birds that were fed diets with and without the inclusion of monensin. The birds that were fed the monensin, a

common ionophore antibiotic, diet were observed to contain a novel form of *Clostridia* and *Bacteroidaceae*, which were not present in the bacterial community of the control birds. These anaerobic novel bacteria of *Clostridia* and *Bacteroidaceae* were isolated from the guts of the 49 d old chickens and orally inoculated to chicks at 0 d of age. At 0 (3 hours after inoculation), 1, 2, 3, 7, 16, and 42 d of age performance parameters were measured, and jejunum and ileum intestinal samples were taken from 4 birds in each replication for intestinal measurements (relative length and weight, villi height/width ratio, and goblet cell concentration per villi) and bacterial analysis. The intestinal segments were stained using Mayer's mucicarmine to allow for goblet cell measurement (Val-Bernal et al, 1999). Villi height, villi width and goblet cell numbers were measured and counted (Uni et al., 2003a,b). Body weight and feed intake were recorded throughout the Exp, and body weight gain (**BWG**) and gain:feed (**G:F**) were calculated. At the occurrence of mortality feed intake was adjusted based on bird days on feed. For processing yield evaluation, 15 birds per pen were randomly selected and wing-banded. After an overnight fast, the birds were individually weighed at the processing plant, slaughtered and eviscerated, after which carcasses were chilled for 12 h. The yield was obtained for the entire carcass, breast meat yield (**BMY**), wings and front and back halves. The carcasses were weighed and the back half, consisting of the leg quarters attached to the lower back, was removed and weighed, leaving the white meat front half. The wings and the breast meat (pectoralis major and minor) were removed from the front half and weighed.

Bird Sampling

Four male broilers from each pen were sacrificed and intestines were sampled at 0 (3 hours after inoculation), 1, 2, 3, 7, 16, and 42 d of age. For length and weight measurements, the small intestine was divided into three segments: duodenum (from gizzard to entry of the bile and

pancreatic ducts), jejunum (from entry of the ducts to yolk stalk), and ileum (from yolk stalk to ileocecal junction). The 3 segments of the small intestine were flushed with 20 mL of physiological saline solution, and the empty weight was recorded. From 2 additional chicks per pen (18 birds per treatment), jejunum and ileum contents were massaged out to include intestinal mucin and were collected in sterile bags and stored at -80°C until isolation of bacterial DNA. Organ weights and lengths were expressed on a relative (grams/kg of body weight and mm/kg of body weight, respectively) basis. For morphologic analysis, approximately 5 cm of the middle portion of the jejunum (from entry of the ducts to yolk stalk) was excised and fixed in 10% formalin. Six cross sections of 70% ethanol-preserved segments for jejunal sample were then prepared for staining with hematoxylin and eosin using standard paraffin embedding procedures (Uni *et al.*, 1995). A total of 3 intact well-oriented villi were selected in 8 replicates for each intestinal cross section (24 measurements for each intestinal sample with 288 measurements per treatment). Villus height was measured from the tip of the villus to the crypt junction; villus width was measured as the distance across the middle of each villus. Morphological indices were determined using computer-aided light microscopy (16x magnification of the objective lens) with image software analysis.[1]

Bacterial DNA Isolation

The bacterial portion of the ileal contents was recovered by density gradient centrifugation through a gauze column and the DNA was extracted as described by Lu et al. (2003).

Denaturing Gradient Gel Electrophoresis (DGGE)

The V3-V4 region of the 16S ribosomal DNA (rDNA) of microorganisms of bacterial domain from contents of the chicken ileum were amplified with the primers HDA1-GC and

[1] Image-Pro Plus Version 3.0, Media Cybernetics, Silver Spring, MD 20910.

HDA2 as described by Knarreborg **(**2002). Amplicons were confirmed by electrophoresis using a 1.5% agarose gel containing ethidium bromide. DNA sequence polymorphisms of the amplicons were detected by resolving differences in molecular structure using denaturing gradient gel electrophoresis (**DGGE**). The DGGE was conducted using the D-Code Universal Mutation Detection system (BioRad, Hercules, CA) with 16 cm x 16 cm x 1 mm gels composed of 8% (wt/vol) polyacrylamide (acrylamide:bis 37.5:1) gels in 1x Tris-acetate EDTA buffer with a 15 to 55% linear denaturant gradient. The 100% denaturing solution contained 40% (vol/vol) formamide and 7.0 M urea. Electrophoresis was performed at a constant voltage of 200 V at 60°C for 3 h. Gels were fixed in 10% acetic acid for 15 min, washed 3 times in deionized water, then put in 50% methanol, washed and stained in 5µg of Sybr green I (FMC Bio products, Philadelphia, PA) /ml of 1x Tris-acetate EDTA buffer for 30 min.

Estimates of Diversity

After separation by DGGE the profiles of the amplicons were compared using the coefficient of similarity (Cs), determined as: $Cs = (a + d)\ (a + b + c + d)^{-1}$, where Cs is the index of similarity between samples i and j, *a* is the number of amplicons common to samples i and j, *b* is the number of amplicons present in sample i, *c* is the number of amplicons present in sample j, and *d* is the number of distinct amplicons in samples i and j. If 2 profiles are identical, Cs equals 100%, if they are entirely different, Cs equals 0%. The presence or absence of amplicons was detected in the different samples, and a dendrogram was constructed using Treecon version 1.3b for windows (Van de Peer and De Wachter, 1994, 1997). At the occurrence of bacterial communities having a high level of similarity in their overall composition it will be referred to as a cluster. A cluster occurs when the composition of a bacterial community, based on treatment and/or age of the bird, group together with a level of similarity. Additionally, the composition of

the bacterial communities differs from other bacterial communities due to a low level of similarity resulting in different clusters.

Terminal Restriction Length Polymorphism (T-RFLP)

The PCR for T-RFLP used universal 16s rDNA primers (8F and 1492R) as described by Lu et al (2006). Briefly, the 8F primer was labeled with 5'FAM (carboxyfluorescein-N-hydroxysuccinimide ester-dimethyl sulfoxide), the amplicons were digested with *Hae*III, and the T-RF were analyzed by electrophoresis on an automatic sequence analyzer in GeneScan mode (ABI PRISM 310 DNA sequencer, PE Biosystems, Foster City, CA). The T-RF peaks were identified by comparison to a 16s database of fragment sizes generated by silico analysis of *Hae*III cut sizes of the sequences obtained from the cloned libraries obtained from previous studies (Lu et al., 2003, 2006). The peak area in each T-RFLP profile was transformed to relative amount by taking the peak area for a phylotype and dividing by the total peak areas for all detectable phylotypes in the T-RFLP profile. Ratios were converted to percentages.

Statistical Analysis

Data for performance and intestinal measurements were subjected to ANOVA procedure for a completely randomized design (Steel and Torrie, 1980) using the general linear model procedure of SAS (SAS Institute, 1990). Statistical significance of differences among treatments was assessed using the least significant difference test (Steel and Torrie, 1980). A probability level of $P < 0.05$ was used to determine statistical significance.

Results and Discussions

Chicks that were inoculated with *Clostridia* had the greatest BWG ($P < 0.05$) at 1 d of age (Table 5.2). However, from 2 to 7 d of age, there was no difference in BWG between the control and inoculated broilers, and at 16 d of age the inoculated broilers had greater BWG than

the control group. By 35 d of age the improvements in BWG in the inoculated broilers began to dissipate. At 42 d of age, the control broilers and *Bacteroidaceae* inoculated broilers had the greatest overall BWG. Since we were using a novel bacterium that has not been studied previously, we had to compare our results to studies done with other probiotics. In previous reports of feeding *Lactobacillus* at 10^9 cfu/g at 0.10% in the diet continuously (not a onetime dose) improved bird weight gain and feed efficiency were reported (Dilworth and Day, 1978; Watkins et al., 1982). High of levels (10^9 cfu/g at 0.15% of the diet) of *Lactobacillus* in the diet resulted in no improvements in performance (Jin et al., 1998). In our study the change in BWG from the early to latter ages between treatments may be due to the design of our experiment where chicks were only inoculated at 0 d of age. If the cultures could have been fed continuously, performance improvements may have persisted. Unfortunately, the novel bacteria were anaerobes and exposure to air resulted in death of the bacteria making continuous feeding problematic. It has been reported that supplementing chickens with *Clostridium butyricum*, (also an anaerobic bacteria, but different species used in our experiment) in the water lines resulted in significant improvements in BWG and G:F (Han et al., 1984). In our experiment, there were no overall differences in feed efficiency or carcass yield ($P > 0.05$) between treatments at any of the measuring periods.

The relative weight (Table 5.3) of each of the 3 segments of small intestine increased to 7 d of age and then decreased indicating the possible end of rapid intestinal development. During the first 2 d of age the birds inoculated with *Bacteroidaceae* had heavier duodenum and jejunum relative weights ($P < 0.05$) than the other treatments, while the other inoculated birds were similar to the control birds. In the duodenum, at 7 d of age the control and *Bacteroidaceae* inoculated birds had similar relative weights, which were heavier than the relative weights of the

broilers inoculated with *Clostridia* alone or in combination. By 16 d of age the birds inoculated with a combination of *Bacteroidaceae* and *Clostridia* had lower relative duodenum weights. In the jejunum, there was no difference between treatments from 3 to 16 d of age, but by 42 d of age the birds inoculated with *Clostridia* alone had the lightest relative weight ($P > 0.05$). Overall there were no consistent differences in the relative weight of the 3 segments of intestine, and at the same time BWG was similar for the first 16 d of age. At 42 d of age the differences between treatments in jejunum relative weight were the same as the differences between treatments in BWG. Our results do not agree with the findings of Awar et al. (2006), who fed $2.5x10^8$ cfu/kg of feed of an unspecified *Eubacterium* sp. product (Biomin) as a probiotic from 0 to 42 d of age and reported no difference in duodenum relative weight, while there was a significant increase in the relative weight of the jejunum and ileum compared to the control birds at 42 d of age. There was no difference in relative weight in the ileum between treatments at any of the measured periods. Additionally, the relative length measurements for all 3 segments of the small intestine were similar ($P > 0.05$) between treatments at any given measurement periods, regardless of inoculation (Table 5.4), which may relate to the similarity in BWG during the starter period (0 to 16 d of age) and G:F from 0 to 42 d of age. Our results on relative intestinal length agree with research conducted with another type of bacterium commonly found in the GIT (Onderci et al., 2006). Onderci et al. (2006) reported no difference in relative length of the duodenum, jejunum and ileum when continually using 10^6 cfu/ml of a strain of *E. coli* that produces α- amylase in the drinking water from 3 to 21 d. The presence of a probiotic appears to have an effect on the relative weight of certain segments of the small intestine, but not on relative length. This may indicate a change related to the physical structure of the GIT such as villi size or mucin production of the intestinal micro-ecosystem.

Broilers inoculated with *Clostridia* in combination with *Bacteroidaceae* had greater jejunal villi height from 0 to 7 d of age and at 16 d of age compared to the control birds. Furthermore, broilers in the inoculated treatment groups had greater villi height than the control birds (Table 5.5). By 42 d of age there was no difference in villi height between treatments ($P > 0.05$). During the study the birds with the greatest villi height/width ratio, a method of interpreting size relation of the villi for amount of absorptive surface area, varied between the *Clostridia* alone or the *Clostridia Bacteriodes* combination treatment, but the presence of *Clostridia* in the inoculation resulted in a greater ratio. The height/width ratio of the villi in the jejunum was very inconsistent between treatments at each measuring period throughout the study making it difficult to come to any conclusions. The ileal villi height and height/width ratio was statistically higher in birds inoculated with the combination of *Bacteroidaceae* and *Clostridia* during the early days of development (0 to 3 d of age) and at 42 d of age (Table 5.6). In attempting to relate villi height with performance, there was no consistent difference between treatments for these parameters, making it difficult to draw conclusions relative to treatment effect. However, at 42 d of age the broilers inoculated with *Clostridia* alone or in combination had the tallest villi height, but also had the lowest BWG. The shift in the bacterial community may have resulted in a decrease in nutrient absorption (Langhout, 2000), which the GIT may compensate for through increased villi height. Nutrient absorption has been reported to be affected by the intestinal bacteria (Ley et al., 2005). The increase of villi height during the early ages of development, close to time of inoculation, agrees with previous findings of increased jejunal villi height when birds were supplemented with an α amylase producing *E. coli* strain in the drinking water (Onderci et al., 2006). Onderci et al. (2006) observed villi increases in birds up to 42 d of age that were supplemented with an α amylase producing *E. coli* strain.

Chichlowski et al. (2006) also observed villi increases at 21 d of age when feeding PrimaLac at 0.3% of the diet. If we were able to supplement the birds with the novel bacteria for the duration of the study, there may have been a greater impact of the bacteria on GIT development. The goblet cell concentration per villi in the jejunum overall was similar between treatments, while in the ileum birds that were inoculated had higher goblet cell concentrations at 0, 2, 3, and 42 d of age compared to the control (Table 5.7). Overall, there was no similar trend between goblet cell concentration and performance or other GIT measurements. At 0 (3 hrs after inoculation) and 1 d after inoculation the increase in jejunum and ileum villi height followed the same trend as the increase in goblet cell concentration between treatments. However, in the latter ages there was no relationship between villi height and goblet cell concentration. At 42 d of age there was an inverse relation between villi height and goblet cell concentration, the broilers with the greater goblet cell numbers had shorter villi but improved BWG. The goblet cells produce mucin, which lines the intestine for protection and has been reported to be a substrate for the bacterial community (Smirnov et al., 2005). In a report by Cole et al. (2006) turkey poults were fed 250 mg of bacteriocins, a toxin produced by bacteria that inhibits growth of other bacteria, per kg of feed. The bacteriocins decreased Campylobacter concentrations, common bacterium found in poultry, and at the same time decreased goblet cell numbers compared to the control poults. Thus, a decrease in a particular bacterium common to the GIT decreased goblet cell concentration, so by inoculating with intestinal bacteria commonly found in the GIT there may be an increase in goblet cell concentration, which could benefit the bird through increased mucin production and aid in nutrient absorption (Geyra et al., 2001).

Based on DGGE (Figure 5.1), there appears to be no difference in bacterial communities between treatments. However, there was a clustering or similarity in bacterial communities

based on age. Regardless of the inoculation, the bacterial community changed as the broiler aged and developed. Two bacterial community clusterings occurred at 0 and 42 d of age, with each cluster having a similarity of approximately 77 to 100% between the 4 treatments. The clustering of the intestinal bacteria of birds at 3 d of age had an 83 to 92% similarity in bacterial community between treatments. These results agree with the findings of other researchers who also reported an intestinal bacterial population change with age (Lu et al., 2003; Amit-Romach et al., 2004; Smirnov et al., 2005). Since there was no overall difference between treatments in performance or intestinal measurements indicating a similar micro-ecosystem for all treatments, the clustering of bacterial communities based on age rather than treatment would be expected.

The use of DGGE only allows for determining the relationship of the bacterial community between treatments, but the use of T-RFLP gives a greater compositional basis for the intestinal bacteria biodiversity through the ability to detect specific bacteria. Based on T-RFLP analysis (Figure 5.2), there was a lower presence of *Lactobacilli* in birds 3 hours (0 d of age) after the inoculation of *Clostridia* alone or in combination with *Bacteroidaceae*. At 3 d of age the control birds had the lowest level of *Lactobacilli* in the bacterial community compared to the inoculated birds. By the end of the 42 d experiment, the birds inoculated with either *Bacteroidaceae* or *Clostridia* alone had the highest level of *Lactobacilli* suggesting a shift in the lactobacilli population of the bacterial community. The decrease in BWG at the end of 42 d may be due to the shift of the bacterial community in the *Clostridia* inoculated birds, but it is difficult to make a definitive conclusion. At 42 d of age there were high levels of *Lactobacilli* in all treatments except for the birds inoculated with the *Bacteroidaceae* and *Clostridia* combination. Birds fed other possible probiotics at a 0.2% level in the diet such as *Enterococcus faecium* or *Bacillus subtillis* PB6 were also reported to have elevated levels of lactic acid bacteria or

Lactobacilli (Samli et al., 2007; Teo and Tan, 2006) even though they were not novel anaerobes. The non elevated levels of *Lactobacilli* in the *Clostridia* and *Bacteroidaceae* inoculated broilers may be due to competitive exclusion and changes in early gut development. Competitive exclusion and GIT changes could prevent *Lactobacilli* from residing in the GIT at the high levels observed in the control group. Due to the variation in biodiversity among treatments at the different time periods it is difficult to draw any definitive conclusion on the impact of the bacterial community due to the novel bacteria. However, it is clear that the biodiversity of the intestinal bacteria changes as the bird grows and ages. This finding is supported by past research (Hume et al., 2003; Amit-Romach et al., 2004; Lu et al., 2003).

The use of probiotic feeding may provide an alternative to sub-therapeutic antibiotic use. However, in this experiment there was no real benefit of the inoculation of the novel bacteria at 0 d of age. Possibly the levels were either too high or too low, or the bird needed to be exposed to the novel bacteria throughout the experiment instead of the onetime dose. Another possibility may be that it is more important to understand the composition of the gut microflora, and not just the impact of one specific bacterium. The gut is a micro-ecosystem and with any ecosystem the biodiversity of the community is extremely important for the overall wellbeing of all bacterial organisms and the host. There is still much that is not known about the intestinal bacterial community and researchers must continue their endeavors to find new methods to detect bacteria, which will allow for further recognition of novel bacterial species, considering 90% of the bacteria in the gut are still unidentified (Apajalahti, 2004).

References

Amit-Romach, E. D. , D. Sklan, and Z. Uni. 2004. Microflora ecology of the chicken intestine using 16s ribosomal DNA primers. Poult. Sci. 83: 1093-1098.

Apajalahti, J. A. Kettunen, and H. Graham. 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. World's Poult. Sci. J. 60: 223-232.

Apajalahti, J. 2005. Comparative gut microflora, metabolic challenges, and potential opportunities. J. Appl. Poult. Res. 14: 444-453.

Awar, W. A., J. Bohm, E. Razzazi-Fazeli, K.Ghareeb, and J. Zentek. 2006. Effect of addition of a probiotic microorganism to broiler diets contaminated with deoxynivalenol on performance and histological alterations of intestinal villi of broiler chickens. Poult. Sci. 85: 974-979.

Bry L, PG Falk, T Midtvedt, JI Gordon. 1996. A model of host-microbial interactions in an open mammalian ecosystem. Science. 273:1380-1383.

Chichlowski, M., W. J. Croom, F. W. Edens, B. W. McBride, R. Qui, C. C. Chiang, L. R. Daniel, G. B. Havenstein, and M. D. Koci. 2007 Microarchitecture and spatial relationship between bacteria and ileal, cecal, and colonic epithelium in chicks fed a direct-fed microbial, PrimaLac, and salinomycin. Poult. Sci. 86:1121-1132.

Cole, K., M. B. Farnell, A. M. Donoghue, N. J. Sterns, E. A. Svetoch, B. N Eruslanov, L. I. Volodina, Y. N. Kovalev, V. V. Perelygin, E. V. Mitsevich, I. P. Mitsevich, V. P. Levchuk, V. D. Pokhilenko, V. N. Borzenkov, O. E. Svetoch, T. Y. Kudryavtseva, I. Reyes-Herrera, P. J. Blore, F. Solis de los Santos, and D. J. Donoghue. 2006.

Bacteriocins reduce Campylobacter colonization and alter gut morphology in turkey poults. Poult. Sci. 85:1570-1575.

Cook and Bird. 1973. Duodenal villus area and epithelial cellular migration in conventional and germ-free chicks. Poult. Sci. 52:2276-2280.

Dilworth, B. C. and E. J. Day. 1978. Lactobacillus cultures in broiler diets. Poult. Sci. 57:1101

Drasar, B.S., and P. A. Barrow.1985. Intestinal Microbiology. American Society for Microbiology, Washington, D.C.

Franks, A.H., H.J.M. Harmsen, G.C. Rangs, G.J. Jansen, F. Schut, and G.W. Welling. 1998. Variations of bacterial populations in human feces measured by fluorescent *in situ* hybridization with group-specific 16S rRNA-targeted oligonucleotide probes. Appl. Environ. Microbiol. 64: 3336-3345.

Geyra, A. Z. Uni, and D. Sklan. 2001. Enterocyte dynamics and mucosal development in the posthatch chick. Poult. Sci. 80:776-782.

Han, I.K., S.C. Lee, J.H. Lee, J.D. Kim, P.K. Jung, and J.C. Lee. 1984. Studies on the growth promoting effects of probiotics II. The effects of Clostridium butyricum ID on the performance and the changes in the microbial flora of the feces and intestinal contents of the broiler chicks. Korean J. Anim. Sci. 26:158-165.

Hofacre, C. L., A. C. Johnson, B. J. Kelly, and R. Froyman. 2002. Effect of a commercial competitive exclusion culture on reduction of colonization of an antibiotic-resistant pathogenic Escherichia coli in day old broiler chickens. Avian Dis.46:198-202.

Hume, M. E, L. F. Kubena, T. S. Edrington, C. J. Donskey, R. W. Moore, S. C. Ricke, and D. J. Nisbet. 2003. Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. Poult. Sci. 82:1100-1107.

Jin, L. Z., Y. W. Ho., N. Abdullah, and S. Jalaludin. 1998. Growth performance, intestinal microbial populations and serum cholesterol of broilers fed diets containing Lactobacillus cultures. Poult. Sci. 77: 1259-1265.

Knarreborg, A., M. A. Simon, R. M. Engberg, B. B. Jensen, and G. W. Tannock. 2002. Effects of dietary fat source and subtherapeutic levels of antibiotic on the bacterial community in the ileum of broiler chickens at various ages. Appl. Environ. Microbiol. 68: 5918-5924.

Langhout, D. J. J. B. Schutte, J. de Jong, H. Sloetjes, M. W. A. Verstegen, S. Tamminga. 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free chicks. Br. J. Nutr. 83: 533-540

Lee, Y. K., K. Nomoto, S. Salminen, and S.L. Gorbach. 1999. Handbook of probiotics. John Wiley & Sons, Inc., New York.

Ley, R. E., F. Backhead, P. Turnbaugh, C. A. Lozupone, R. D. Knight, and J. I. Gordon. 2005. Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. 102: 11070-11075.

Lu, J. R., U. Idris, B. Harmon, C. Hofacre, J. J. Maurer, and M. D. Lee. 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. Appl. Environ. Microbiol. 69: 6816-6824.

Lu, J., C. L. Hofacre, M. D. Lee. 2006. Emerging Technologies in Microbial Ecology Aid in Understanding the Complex Disease Necrotic Enteritis. J. Appl. Poult. Res. 15:145-153.

Onderci, M., N. Sahin, K. Sahin, G. Cikim, A. Aydin, I. Ozercan, and S. Aydin. 2006. Efficacy of supplementation of α- amylase- producing bacterial culture on the performance, nutrient use, and gut morphology of broiler chickens fed a corn-based diet. Poult. Sci. 85:505-510.

Samli, H. E., N. Senkoylu, F. Koc, M. Kanter, and A. Agma. 2007. Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. Archives Anim. Nutr. 61:42-49.

SAS Institute Inc., 1990. SAS STAT User's Guide Release 6.08. SAS Institute Inc., Cary NC.

Smirnov, A., R. Perez, E. Amit-Romach, D. Sklan, and Z. Uni. 2005. Mucin dynamics and microbial populations in chicken small intestine are changed by dietary probiotic and antibiotic growth promoter supplementation. J. Nutr. 135: 187-192.

Steel, R. G. D. and J. H. Torrie, 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill Book Co., Inc., New York, NY.

Teo, A. Y. and H. M. Tan. 2006. Effect of Bacillus subtilis PB6 (cloSTAT) on broilers infected with a pathogenic strain of Escherichia coli. J. Appl. Poult. Res. 15:229-235.

Uni, Z., Y. Noy, and D. Sklan. 1995. Posthatch changes in morphology and function of the small intestines in heavy- and light-strain chicks. Poult. Sci. 74:1622-1629.

Uni , Z., A. Smirnov, and D. Sklan. 2003a. Pre- and posthatch development of goblet cells in the broiler small intestine: effect of delayed access to feed. Poult. Sci. 82:320-327.

Uni, Z., E. Tako, O. Gal.Garber, and D. Sklan. 2003b. Morphological, molecular and functional changes in the chicken small intestine of the late embryo. Poult. Sci. 82:1747-1754.

Val-Bernal, J. F., J. J. Gomez-Roman, T. Vallina, F. Villoria, M. Mayorga, and P. Garcia-Arranz. 1999. Papillary (chromophil) renal cell carcinoma with mucinous secretion. Pathology Research and Practice 195:11-17.

Van de Peer, Y., De Wachter. R. 1994. TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comut. Applic. Biosci. 10: 569-570.

Van de Peer, Y., De Wachter, R. 1997. Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. Comput. Applic. Biosci. 13: 227-230.

Watkins, B. A., B. F. Miller and D. H. Neil 1982. In vivio effects of Lactobacillus acidophilis against pathogenic Escherichia coli in gnotobiotic chicks. Poult. Sci. 61: 1298-1308.

Xu, J. and J.I. Gordon. 2003. Inaugural Article: Honor thy symbionts. Proc. Natl. Acad. Sci. 100: 10452-10459.

Table 5.1. Composition of the diets (as fed basis)

	Starter	Grower	Finisher
Ingredients		%	
Corn, yellow, ground	56.12	60.80	68.00
Soybean meal (48)	37.50	32.61	26.22
Fat, poultry	3.00	3.43	2.99
Dicalcium phosphate	1.75	1.56	1.32
Limestone	0.80	0.78	0.62
Salt	0.30	0.32	0.35
Vitamin premix[1]	0.25	0.25	0.25
DL-Methionine	0.20	0.17	0.17
Trace mineral premix[2]	0.08	0.08	0.08
Contents by calculation			
ME, kcal/kg	3,096	3,140	3,191
Protein, %	22.3	20.6	18.1
Lysine, %	1.18	1.01	0.85
Methionine, %	0.53	0.48	0.45
Met + Cys, %[3]	0.89	0.76	0.70

[1]Vitamin mix provided the following (per kg of diet): thiamin•mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B_{12} (cobalamin),12.0 µg; pyridoxine•HCL, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfite complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 ug; trans-retinyl acetate, 1,892 ug; all-rac α tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

[2]Trace mineral mix provided the following (per kg of diet): manganese ($MnSO_4•H_2O$), 60 mg; iron ($FeSO_4•7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4•5H_2O$), 5 mg; iodine (ethylene diamine dihydroiodide), 0.15 mg; selenium ($NaSe0_3$), 0.3 mg.

[3] Met= methionine; Cys= cysteine.

Table 5.2. Effect of oral inoculation at 0 d of age with novel bacteria on the body weight gain and feed efficiency (G:F)[1] of broilers

	Gain						
	----------(g/chick)----------						
Treatments[2]	0 to 1d	1 to 2d	2 to 3d	3 to 7d	7 to 16d	16 to 35d	0 to 42d
Control	14.8[b]	13.9	16.5	101	411[b]	1585[a]	2551[a]
Bacteroidaceae	15.0[b]	13.4	16.7	103	429[ab]	1544[ab]	2527[a]
Clostridiaceae	15.7[a]	13.1	16.7	105	438[a]	1563[a]	2448[b]
Bacteroidaceae and *Clostridiaceae*	14.8[b]	13.6	16.4	103	421[a]	1507[b]	2427[b]
Pooled SEM	0.19	0.22	0.26	1.16	5.49	17.04	26.99
	Gain:feed						
	----------(g/kg)----------						
	0 to 1d	1 to 2d	2 to 3d	3 to 7d	7 to 16d	16 to 35d	0 to 42d
Control	1189[c]	1086	929	864	740	603	573
Bacteroidaceae	1225[bc]	999	916	871	787	605	580
Clostridiaceae	1372[ab]	1137	933	861	777	607	570
Bacteroidaceae and *Clostridiaceae*	1408[a]	1063	948	852	760	607	577
Pooled SEM	47.82	66.78	54.58	19.54	13.58	4.96	4.84

[1] G:F= gain to feed ratio.
[2] Means represent 3 pens per treatment, 70 chicks per pen.
[a-c] Means within a column and parameter with no common superscript differ significantly ($P < 0.05$).

Table 5.3. Effect of oral inoculation at 0 d of age with novel bacteria on the relative intestinal weights of broilers

Treatments[1]	Relative Weight[2] (g/kg of BW)						
Duodenum	0d	1d	2d	3d	7d	16d	42d
Control	8.2	14.9^{b}	17.2^{b}	21.9	21.0^{a}	12.9^{a}	4.9
Bacteroidaceae	10.7	16.9^{a}	19.6^{a}	18.6	19.8^{ab}	13.2^{a}	5.2
Clostridiaceae	9.0	12.7^{c}	14.5^{c}	18.6	18.5^{b}	12.4^{ab}	4.4
Bacteroidaceae and *Clostridiaceae*	10.8	13.8^{bc}	14.3^{c}	19.5	18.4^{b}	11.0^{b}	5.1
Pooled SEM	0.88	0.66	0.60	1.20	0.62	0.51	0.27
Jejunum	0d	1d	2d	3d	7d	16d	42d
Control	10.0^{b}	20.5	22.3^{b}	27.6	29.3	22.9	9.8^{a}
Bacteroidaceae	14.4^{a}	21.9	26.6^{a}	27.0	29.2	21.8	9.3^{a}
Clostridiaceae	9.8^{b}	19.4	22.1^{b}	29.5	31.1	22.4	8.0^{b}
Bacteroidaceae and *Clostridiaceae*	12.6^{ab}	20.0	22.7^{b}	25.6	28.3	20.2	8.9^{ab}
Pooled SEM	0.93	1.12	1.09	1.42	1.15	0.86	0.43
Ileum	0d	1d	2d	3d	7d	16d	42d
Control	7.81	14.30	17.14	19.93	19.67	15.78	8.05
Bacteroidaceae	11.72	13.72	17.65	20.18	20.34	16.91	7.27
Clostridiaceae	7.41	15.11	17.55	22.70	19.53	16.00	8.09
Bacteroidaceae and *Clostridiaceae*	8.83	14.21	17.72	22.43	19.54	15.00	7.31
Pooled SEM	1.20	0.76	0.84	1.07	0.76	0.58	0.45

[1]Means represent 3 pens per treatment, 4 randomly selected chicks per pen.
[2] Relative weight = g/kg of bodyweight.
[a-b] Means within a column and segment of small intestine with no common superscript differ significantly ($P < 0.05$).

Table 5.4. Effect of oral inoculation at 0 d of age with novel bacteria on the relative intestinal lengths of broilers

Treatments[1]	Relative Length[2] (cm/kg of BW)						
Duodenum	0d	1d	2d	3d	7d	16d	42d
Control	159	160	149ab	146	82	38	10
Bacteroidaceae	176	166	160a	133	79	37	10
Clostridiaceae	172	154	142b	144	80	34	10
Bacteroidaceae and *Clostridiaceae*	166	160	141b	142	79	33	10
Pooled SEM	5.85	6.38	4.59	5.85	2.78	1.46	0.52
Jejunum	0d	1d	2d	3d	7d	16d	42d
Control	318	321	303	309	181	89	27
Bacteroidaceae	375	342	316	306	188	83	28
Clostridiaceae	336	308	315	311	193	83	28
Bacteroidaceae and *Clostridiaceae*	339	322	321	293	179	84	27
Pooled SEM	17.05	14.04	11.22	11.55	6.42	2.98	1.32
Ileum	0d	1d	2d	3d	7d	16d	42d
Control	269	302	286	304	179	91	27
Bacteroidaceae	315	312	286	286	183	86	28
Clostridiaceae	294	321	309	314	193	87	29
Bacteroidaceae and *Clostridiaceae*	280	296	315	303	180	87	28
Pooled SEM	14.53	14.95	9.67	12.20	5.85	2.87	0.99

[1]Means represent 3 pens per treatment, 4 randomly selected chicks per pen.
[3] Relative length= cm/kg of bodyweight.

Table 5.5. Effect of oral inoculation at 0 d of age with novel bacteria on jejunum villi measurements of broilers

Treatments[1]	Height (μm)						
	0d	1d	2d	3d	7d	16d	42d
Control	149[b]	205	246[ab]	287[a]	409[b]	580[b]	692
Bacteroidaceae	136[b]	218	198[c]	242[b]	422[b]	654[a]	708
Clostridiaceae	124[b]	187	226[bc]	245[b]	441[ab]	645[a]	732
Bacteroidaceae and *Clostridiaceae*	218[a]	221	264[a]	272[ab]	467[a]	668[a]	694
Pooled SEM	12.3	9.4	15.2	10.9	13.6	23.2	23.2

	Height/Width Ratio (μm/μm)						
	0d	1d	2d	3d	7d	16d	42d
Control	3.6[b]	4.9[b]	5.9[a]	5.5[ab]	6.9[b]	8.0[c]	8.8[b]
Bacteroidaceae	3.5[b]	5.2[b]	4.3[b]	4.6[b]	6.4[b]	12.2[a]	9.8[b]
Clostridiaceae	4.8[b]	6.6[a]	5.1[ab]	4.4[b]	8.6[a]	11.2[ab]	8.4[b]
Bacteroidaceae and *Clostridiaceae*	6.7[a]	5.2[b]	5.0[ab]	6.1[a]	7.5[ab]	9.5[bc]	13.4[a]
Pooled SEM	0.22	0.38	0.40	0.37	0.42	0.73	0.88

[1]Means represent 3 pens per treatment, 4 randomly selected chicks per pen, 3 villi per chick.
[a-c] Means within a column and parameter with no common superscript differ significantly ($P < 0.05$).

Table 5.6. Effect of oral inoculation at 0 d of age with novel bacteria on ileum villi measurements of broilers

Treatments[1]	Height (μm)						
	0d	1d	2d	3d	7d	16d	42d
Control	115[a]	183[ab]	182	223	260[a]	314	379[c]
Bacteroidaceae	72[b]	180[ab]	187	214	238[b]	320	423[b]
Clostridiaceae	122[a]	168[b]	192	222	232[b]	304	529[a]
Bacteroidaceae and *Clostridiaceae*	139[a]	190[a]	207	222	239[b]	311	443[a]
Pooled SEM	8.9	6.2	19.8	10.3	6.5	16.9	14.2
	Height/Width Ratio (μm/ μm)						
	0d	1d	2d	3d	7d	16d	42d
Control	3.7[b]	5.6[a]	1.1[d]	4.2	4.1	4.6	5.4
Bacteroidaceae	1.9[c]	4.0[b]	2.0[c]	4.3	4.2	6.5	6.4
Clostridiaceae	4.6[b]	5.0[ab]	3.2[b]	4.5	3.7	4.5	7.1
Bacteroidaceae and *Clostridiaceae*	6.4[a]	5.5[a]	4.0[a]	4.4	3.9	5.4	7.4
Pooled SEM	0.45	0.38	0.25	0.31	0.26	0.60	0.52

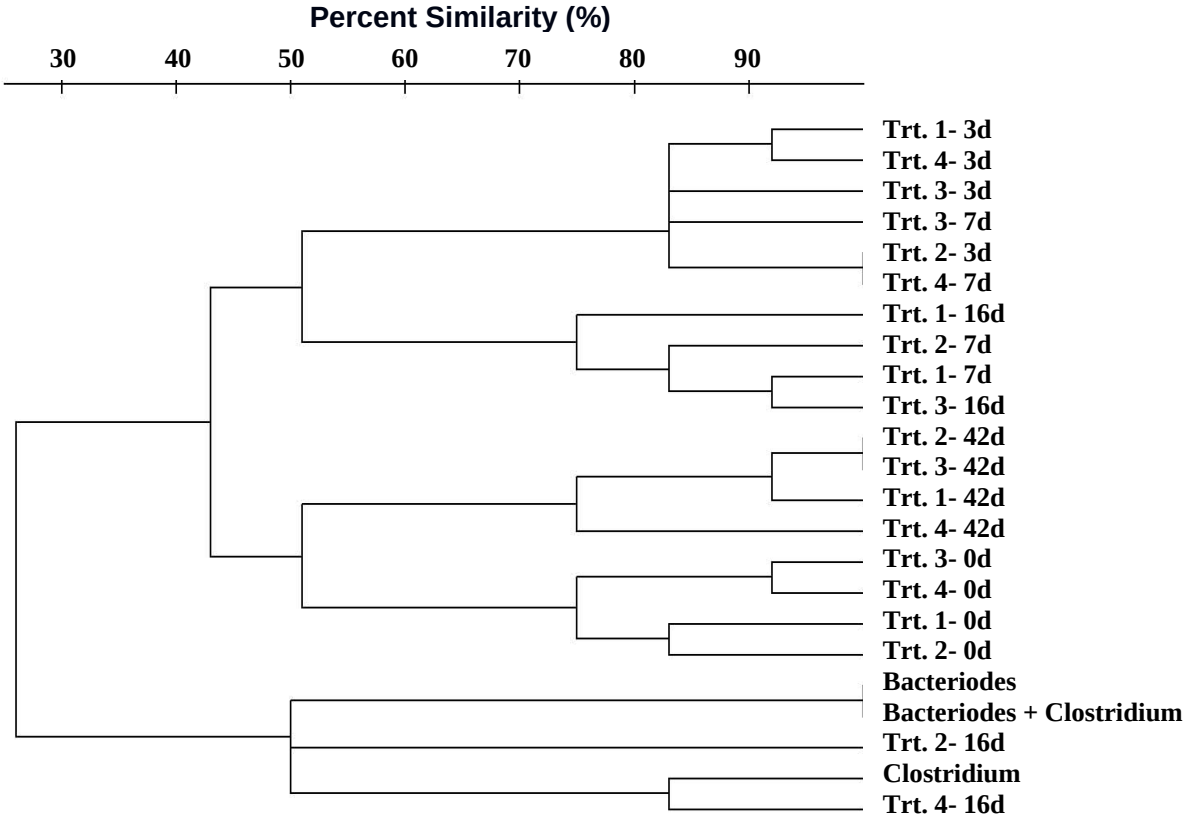
[1]Means represent 3 pens per treatment, 4 randomly selected chicks per pen.
[a-b] Means within a column and parameter with no common superscript differ significantly ($P < 0.05$).

Table 5.7. Effect of oral inoculation at 0 d of age with novel bacteria on goblet cell concentration of villi in the jejunum and ileum of broilers

Treatments[1]	----------(# of goblet cells/ villi)----------						
Jejunum	0d	1d	2d	3d	7d	16d	42d
Control	26[b]	26[ab]	26	26	42[ab]	47	156[a]
Bacteroidaceae	21[b]	21[b]	19	17	49[a]	59	126[ab]
Clostridiaceae	24[b]	36[a]	21	17	32[b]	51	130[ab]
Bacteroidaceae and *Clostridiaceae*	42[a]	28[ab]	27	17	40[ab]	61	100[b]
Pooled SEM	2.78	3.90	10.81	3.59	3.38	6.26	15.55
Ileum	0d	1d	2d	3d	7d	16d	42d
Control	22[b]	19	10[b]	18[b]	35	33	74[c]
Bacteroidaceae	15[c]	21	8[b]	25[a]	26	25	114[b]
Clostridiaceae	24[b]	18	14[b]	15[b]	35	31	179[a]
Bacteroidaceae and *Clostridiaceae*	34[a]	20	41[a]	21[ab]	33	28	127[b]
Pooled SEM	1.94	2.06	3.47	2.07	3.56	3.09	9.78

[1]Means represent 3 pens per treatment, 4 randomly selected chicks per pen.
[a-b] Means within a column and segment of small intestine with no common superscript differ significantly ($P < 0.05$).

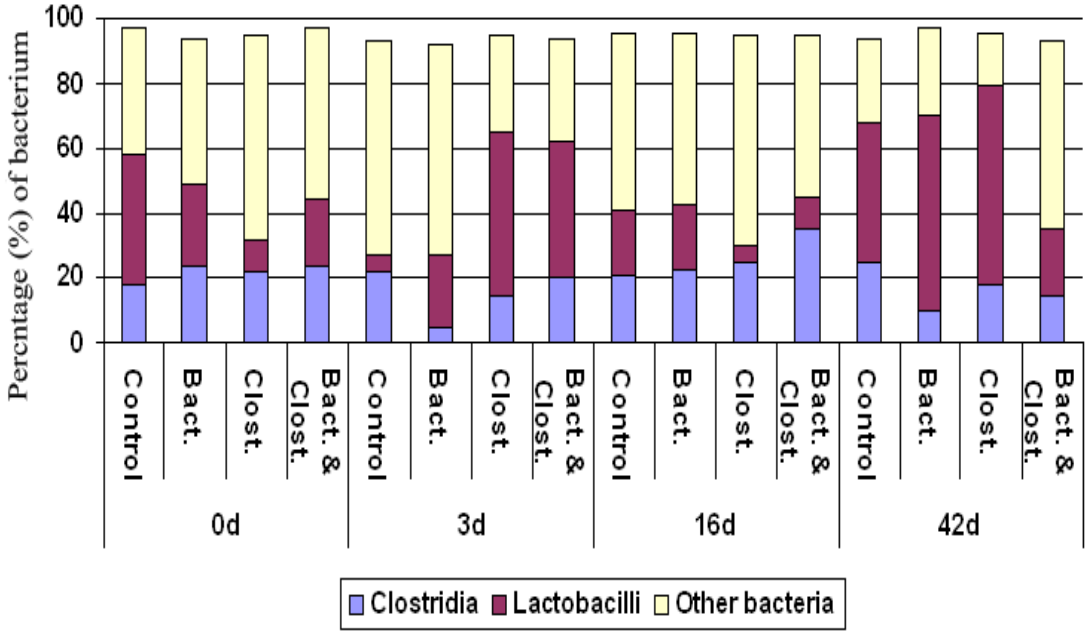
Figure 5.1. Dendogram showing the relatedness[1] of ileal bacterial community among broilers orally inoculated at 0 d of age with novel bacteria[2]



[1] Simple matching using clustering method of Treecon® to produce dendogram representing the relationship[1] between DGGE band patterns of V3-V4 region of 16s rDNA of the intestinal community DNA.

[2]Contents for bacterial analysis pooled from 12 birds per treatment per age period. Trt 1= control, Trt 2= Bacteriodes inoculation, Trt 3= Clostridium inoculation, and Trt 4= combination of Clostridium and Bacteriodes inoculation at 0 d of age.

Figure 5.2. Composition of ileal bacterial community of broilers orally inoculated at 0 d of age with novel bacteria, as determined by Terminal restriction fragment length polymorphism[1] analysis



[1]Contents for bacterial analysis pooled from 12 birds per treatment per age period. Trt 1= control, Bact.= Bacteriodes inoculation, Clost= Clostridium inoculation, and Bact. & Clost.= combination of Clostridium and Bacteriodes inoculation.

CONCLUSIONS

Since the 1700's, researchers have been aware that bacteria reside in the gastrointestinal tract (GIT) of all animals. The gastrointestinal tract provides a micro-ecosystem for intestinal bacteria, a situation which allows for a symbiotic relationship between the host and the residing bacterial community. It has been established that the intestinal bacterial community can have a significant role in poultry health and development. However, how various factors affect the GIT and the bacterial community is not fully understood. Therefore, preliminary studies, using modern molecular techniques, were conducted to gain a greater understanding of different factors can change the bacterial community and GIT development.

In the first experiments, feeding an alternative protein diet with fish and poultry by-product meal had very little impact on GIT development, compared to a standard corn-soybean meal diet. However, based on denaturing gradient gel electrophoresis (DGGE) analysis, the bacterial populations clustered together within treatment, indicating intestinal bacteria changes due to diet. Feeding a semi-purified diet to broilers negatively affected performance and GIT measurements compared to a standard corn-soybean meal diet. Similar to the separation in performance and GIT measurements, a separation of the bacterial community into 2 distinct bacterial clusters between the birds fed the semi-purified diet and those fed the corn-soybean meal diets manifested itself. In both studies, as the broiler aged and intestinal measurements changed, the bacterial populations also changed. A slight variation in feed ingredients can change the bacterial community without impacting the development of the GIT. However, a diet that affects the development of the GIT will greatly impact a change in the bacterial community.

Intestinal bacterial populations were clearly affected by changes in the diet. But when the diet remained the same, gender appeared to have a major effect on the bacterial community of broilers based on DGGE analysis from 0 to 21 d of age. The GIT measurements and body weight gain were similar between genders during the starter period, except for body weight gain at 21 d, when males started to develop at a pace different from that of the females. However, the clear separation in bacterial populations between the males and females indicates that another factor, possibly genetics, is involved in altering community diversity. As the broilers aged, changes in the residing bacterial community occurred in both genders.

Because a number of different genetic lines of broilers and laying hens are used throughout the poultry industry to reach particular production goals, producers need to know which methods of changing GIT dynamics produce the largest and healthiest birds. During the early stages of development (0 to 14 d of age), the multipurpose broilers had the fastest growth in weight gain and intestinal development, followed by the high-yielding broilers, and the 1957 Athens Canadian Random Bred. After 14 d of age, there was an increase in the growth rate of the high-yield broilers, resulting in performance and intestinal measurements similar to that of the multipurpose broilers. The Athens Canadian Random Bred birds had the lowest body weight gain and slowest rate of GIT measurement compared to either the multipurpose or high-yield broilers (modern genetic lines). Based on DGGE analysis, the intestinal bacterial populations of multipurpose and high-yield broilers grouped together with similarity at 21 d of age when body weight gain and GIT measurements were also similar. The bacterial community of the Athens Canadian Random Bred broilers clustered separately from the bacterial populations of the modern genetic lines. The modern genetic lines broilers had similar GIT development and changes in the bacterial community, while the Athens Canadian Random Bred had a different

rate of GIT development and intestinal bacterial community composition compared to modern genetic lines.

In evaluating a possible effect of a probiotic on bacterial community and GIT development, researcher introduced novel *Clostridia* and *Bacteriodes.* These were isolated from the gut of an older chicken and 10^8 CFU were orally inoculated at 0 d of age. The use of the *Clostridia* alone or in combination with *Bacteriodes* had a positive impact on body weight gain and intestinal development during the early stages of development (0 to 3 d of age). After the early stages of development, however, there was no effect due to the probiotic inoculation on performance or GIT measurements, and at 42 d of age no difference in carcass yield between treatments appeared. Furthermore, based on DGGE analysis, no difference in bacterial populations due to treatments occurred, and the main factor impacting the change in bacterial community was once again age. Based on genescan analysis, no definitive conclusion about the intestinal bacterial community could be drawn due to the lack of a consistent trend or effect of treatments. The rate of development was similar between treatments, and the intestinal bacterial community was also similar between treatments regardless of the novel bacterial inoculation.

In summary, a number of factors contribute to changes in the bacterial community: diet, gender, genetic line, and age. Many of the same factors that affect the bacterial community also impacted GIT measurements. Changes in GIT development greatly impact the intestinal bacteria, resulting in a community that is best suited for the niche created during a specific stage of development. Furthermore, changes in the bacterial community can be made through diet, giving great possibility to the use of prebiotics. The results from this research will hopefully provide important preliminary information of great interest to researchers and animal health companies in the future. It may help these producers understand the symbiotic relationship

between poultry and the intestinal bacterial community. Thus, they can increase the production of increasingly healthy poultry for a hungry world.