BROILER PERFORMANCE AND INTESTINAL ALTERATIONS WHEN FED DRUG-FREE DIETS

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(ABSTRACT)

A study was carried out to investigate the effects of a drug-free feeding program on broiler performances. A total of 2,496 Cobb 500 chicks were randomly assigned to one of four dietary treatments with each group replicated 13 times. The four diets evaluated were: 1) negative control (NC): basal diet without growth promoter or coccidiostat; 2) positive control (PC): diet 1 + Lincomycin; 3) Program 1 (PG1): diet 1 + Bio-Mos®, Vegpro®, MTB-100®, Acid Pak 4-Way®, and All-Lac XCL®; 4) Program 2 (PG2): diet 1 + Bio-Mos® and All-Lac XCL®. Additives were used at commercially recommended rates. All chicks were vaccinated with a live oocyst coccidia vaccine on d 0 at the hatchery. Four phases of feeding were used during the trial with changes occurring at d 14, 28, and 35. Performance values measured were body weight, feed intake, yield, and mortality, while body weight gain and feed conversation rate (FCR) were calculated. Chicks were challenged with coccidia at d 14 to evaluate the protective effect of the feeding programs and coccidia vaccination. Segments of duodenum, ileum, and ceca were removed to measure intestinal morphology. Final body weight gains of birds on PC (2.736 kg) were greater ($P < 0.05$) compared to NC (2.650 kg), while birds on PG1 (2.681 kg) and PG2 (2.710 kg) were similar to positive and negative control. Overall, feed intake was similar across the treatments with the exception of period 2 (15 to 28 days) when birds consumed more ($P < 0.05$) of PC and PG1 compared to NC. Cumulative FCR at d 35 and 49 was improved ($P < 0.05$) in birds consuming PC and PG2 when compared to NC. Overall, birds consuming NC had greater mortality ($P < 0.05$; 12%) compared to PC (7.6 %), PG1 (4.6%) and PG2 (6.7 %) with most of the mortality occurring from d 0 to d 28. Mortality for birds consuming PG1 was also lower ($P < 0.05$) compared to the PC. There were no dietary effects on lesion scores or yields of processed products at d 42 (females) or d 49 (males). Interaction of dietary treatments with age and days of age alone showed effects ($P < 0.0001$) on the morphology of duodenum, ileum, and ceca. Lamina propria in ceca was thicker ($P < 0.008$) in birds

consuming NC compared to PG1 and PG2. This study indicated that feeding birds without growth promoters resulted in greater mortality and decreased performance compared to using an antibiotic, while Bio-Mos® in combination with All-Lac XCL® helped to reduce the negative effects.

Keywords: Broiler, Bio-Mos®, Antibiotic, Mortality, Performance.

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Introduction

Antibiotics are frequently used therapeutically and prophylactically for the treatment of disease in poultry. Antibiotics have also been a common feed additive in poultry feed as growth promoters to improve performance for many years. It was until 1946 when Moore et al. reported the first research exploring the positive effects of antibiotics on chicken growth. Nowadays, a total of 32 antimicrobial compounds are approved for use in broiler feeds in the U.S. without a veterinary prescription (Jones et al. 2003). Eleven of these antibiotics, such as bacitracin, chlortetracycline, erythromycin, lincomycin, novobiocin, oxytetracycline, and penicillin, are used in feed as growth promoters. The growth promoters increase broiler's weight gain, improve feed conversion rate and mortality through decreasing microbial load in the intestinal tract, ultimately resulting in more nutrients available for broilers.

However, increasing pressures from consumer concerns make it necessary to reduce the use of antibiotics in feed due to the negative human health issues by antibiotic resistance. In 1994, Bates et al. first reported that vancomycin-resistant enterococci (VRE) could be traced to farm animals in Great Britain and they suggested that farm animals could be a reservoir for the VRE infection. They isolated 62 of VRE from non-human sources, of which 22 were from farm animals and 5 from uncooked chicken. Roy and coworkers (2002) also reported that 91 of the 92 *Salmonella* samples isolated from poultry products, live poultry, and poultry environment were resistant to erythromycin, lincomycin, and penicillin.

Many measurements have been developed to reduce the use of antibiotics as growth promoters. Enhanced biosecurity of poultry farm (Tablante et al. 2002), genetic selection of poultry resistant to disease (Gross et al., 2002), and vaccination to pathogenic microbes (Williams 2002) have successfully protected poultry production from disease loss. Competitive exclusion (Nurmi et al. 1973) is also a popular strategy for preventing poultry from intestinal infectious disease due to the effective inhibition of pathogenic bacteria (La et al. 2003) among hundreds of microbial population (Vaughan et al. 2000) in the gut. Probiotic (Fuller, 1989) with defined bacteria and prebiotic with ability to aid

growth of beneficial bacteria have been reported to enhance poultry growth (Jernigan et al., 1985; Fernandez et al., 2002). Organic acids, enzymes, and mycotoxin binding agents also have positive effects on poultry growth (Chaveerach et al. 2004; Raju et al., 2000).

In this literature review, basic information of gastrointestinal microbial population is introduced, followed by recent issues of antibiotic resistance, strategies to reduce antibiotic use, and intestinal morphology by diets.

Microbial community in gastrointestinal tract:

General information

It is well established that the gastrointestinal normal microflora plays an important role in the health and well-being of poultry. Various pathogenic microbes, such as *Escherichia coli,* have been implicated to reduce the growth of poultry. Possible mechanisms for this reduction of growth are: toxin production, utilization of nutrients essential to the host, and suppression of microbes that synthesize vitamins or other host growth factors. Avians possess the same basic structures for nutrient extraction as other vertebrate, a tubular intestine, but specific variation within the avian gastrointestinal tract (GIT) includes a crop for storage of feed, proventriculus (simple stomach), gizzard, and paired ceca (Duke, 1986). The pH values of specific sections of the chicken GIT are: crop 4.5, proventriculus 4.4, gizzard 2.6, duodenum 5.7 to 6.0, jejunum 5.8, ileum 6.3, colon 6.3, ceca 5.7, and bile 5.9 (Farner, 1942). These pH values in specific areas of the avian GIT selectively allow establishment of a specific microbial population in birds.

Animal microbial populations, which grow anaerobically, can be differentiated into two groups: autochthonous (normal) and allochthonous (transient) microbes (Dubos et al., 1965). Peristalsis of GIT drives unidirectional flow of materials through the lumen of the upper and middle GIT and prevents microbial communities from developing unless they attach to underlying epithelial structures (Savage, 1983). Some microbes adhere to the epithelial cells of GIT, but others may colonize in secretions of intestinal line, principally mucins. Some gram-negative bacteria, such as *E. coli,* grow to express the type 1 fimbria and adhere efficiently to the crop epithelium, lamina propria, and apical surfaces of intestinal villi. The adhesion can be inhibited by manna oligosaccharide (Edelman et al., 2003).

Within a few hours of feeding, the pH of the crop falls to about 5.0 due to the microbial production of lactic acid. Few types of bacteria are present in the crop, with *Lactobacilli* being the predominant microbes, especially *Lactobacillus salivarius* (Sarra et al., 1985), which produces lactic acid and subsequently reduces pH value. *Lactobacilli* attach to the epithelial surface of the crop, forming an almost complete layer with two to three cells thick and remaining there throughout the bird's lifetime. The ability of these microbes to adhere to the crop epithelium is restricted to avian strains; those from other species fail to attach (Fuller, 1973). *Lactobacilli* are capable of controlling populations of *E. coli* in the crop, and their effects are both bacteriostatic (depressing growth of other bacteria by their secretions) and bacteriocidal (killing other bacteria by secretions) (Fuller, 1977). Strict anaerobes, such as *Bacteroides* spp., do not appear in the crop due to its unfavorable oxidation-reduction (redox) potential (essential for bacteria to oxidize nutrients), the predominance of *Lactobacilli*, and possible absence of a suitable growth substrate such as glucose. But the relatively aerotolerant anaerobe, *Clostridium perfringens*, is sometimes found in the crop, as are micrococci, staphylococci, and yeasts (Mead, 1997). Feed can be retained in the crop for as long as 20 hours, but if the organ fails to empty, microbe activity continues and can lead to a condition known as "sour crop". Microbial populations in the crop are also susceptible to other dietary factors. For example, when turkeys are given a high-glucose diet, yeasts become predominant and produce abundant gas, which cause the crop to become large and pendulous (Jayne-Williams et al., 1971).

The proventriculus and gizzard of the chicken are harsh habitats for most microbes, with pH values of 1 to 4. However, Smith (1965) reported the presence of *Lactobacilli* (10^8/g content) with low numbers of *E. coli*, enterococci/streptococci, and yeasts.

Rapid food passage rate in the duodenum makes it an unfavorable area of the GIT for microbes to attach and colonize. However, Salanitro et al. (1978) reported that aerobic and anaerobic counts from the duodenum and ileum of the chicken were similar, with *Streptococcus (enterococcus)*, *Staphylococcus*, *Lactobacilli*, and E. *coli* being the predominant bacteria present. However, obligate anaerobes were also present in both the duodenum and ileum, including anaerobic cocci, *Eubacterium*, *Propionibacterium*, *Clostridium*, *Gemmiger* and *Fusobacterium* spp., comprising 9% to 39% of the total

number of strains isolated with the greatest diversity being evident in the duodenum. 70% of 16S rRNA (species specific) sequences from the ileum were related to *Lactobacillus*, with the majority of the rest 11% *Clostridiaceae*, 6.5% *Streptococcus*, and 6.5% *Enterococcus* (Lu et al., 2003).

The ceca in poultry provide a relatively stable environment for microbes. Bacteria are the predominant microbes, specifically obligate anaerobes, which are found in the lumen at up to 10^{11} / g wet weight, while yeasts, molds, and protozoa are not normally present in significant numbers. Lu et al. (2003) observed that in the ceca *Clostridiaceae*-related consisted of 65% with the remainder as *Fusobacterium* (4%), *Lactobacillus* (8%) and *Bacteriodes* (5%).

Microbe-microbe interaction

Beneficial or competitive interactions exist among different microbial populations. Dofing et al. (1988) reported beneficial interactions among microbial communities with interspecies acetate transfer as an electron carrier within an anaerobic environment, where these microbes cooperatively play respective roles on oxidizing substrates. When available nutrients become limiting, competition for the carbon and energy sources develops among various members of the microbial community (Veldkamp et al., 1986). Competition among microbial communities may be influenced by environmental factors such as concentrations of carbon and energy substrates, oxygen, nitrite, sulfate, sodium chloride, antibiotics, temperature, osmotic strength, and pH (Dofing et al., 1997). Indirect and direct antagonisms are utilized by microbes to inhibit other microbial growth. Indirect antagonisms include deconjugation of bile acids (Bricknell et al., 1969), induction of host immunologic processes (Freter, 1974), and stimulation of peristalsis in the GIT (Drasar et al., 1974). Direct antagonisms between microbes include depletion of essential substrates, competition for receptor sites, creation of a restrictive physiologic environment, and secretion of antibiotic-like substances. Wilson et al. (1988) observed that an unidentified organism(s) more efficiently competed for monomeric glucose, N-acetylglucosamine, and sialic acid compared to *C. difficile* in a continuous flow culture model of mouse cecal bacteria. Some by-products of bacterial metabolism such as hydrogen ion concentration, oxidation-reduction potential, hydrogen sulfide, and volatile

fatty acids (VFAs), create inhibitory physiologic environments. Low pH may be the major mechanism by which lactic acid bacteria (primarily *Lactobacillus*, *Bifidobacterium* and *Streptococcus*) inhibit growth of various facultative and anaerobic bacteria *in vivo* and *in vitro* (Tannock, 1984). Low rodox potential in the GIT is critical for protection against enteric infection by pathogens whose growth requires more oxygen (Meynell, 1963). Byrne et al. (1979) reported that short chain VFAs which are fermented by gastrointestinal microbes, inhibited the growth of other gastrointestinal microbes with the undissociated form of VFAs in lower pH. Inside the cell, VFAs inhibit bacterial growth by uncoupling oxidative-phosphorylation and inhibiting adenosine triphosphate – inorganic phosphate exchange. Bacteriocins, which are also produced by some bacteria to inhibit the growth of others, play a role in regulating the microbial population of the GIT (Rusch, 1980).

Microbe-host interaction

Chicks are immunologically naive and prone to rapid and persistent colonization by beneficial and pathogenic bacteria, with anaerobic bacteria dominating the first 3 to 4 weeks of life (Barrow et al., 1988). The beneficial gastrointestinal microbes present in the GIT are important in protecting the host against the invasion of pathogens. Most components of the gastrointestinal microbes are poor inducers of immunoglobulin (Foo et al., 1974), because of the close antigenic similarities between intestinal microorganisms and tissues of the animal host. Proteins, which pathogens may secrete into the medium, include enzymes (proteases, nucleases, lipases, and carbohydrases), toxins, and hemolytic and cytotoxic proteins (Lory, 1992). Pathogenic microbes also secret enterotoxic proteins to maintain their survival and to cause disease to the host. ToxR is a transcription regulatory protein produced by *Vibrio cholerae* which causes cholera. Skorupski et al. (1997) observed higher expression of the ToxR regulon at pH 6.5 and 30 ºC but reduced expression at pH 8.5 and 37 ºC. *In vitro*, toxin production in a pure culture is increased under osmolar condition similar to that in mucus, and in the presence of amino acids likely to be present in mucosal secretions. These results suggested that environmental factors, including pH, temperature, osmolarity, and certain amino acids, could increase the activity of ToxR and result in more virulent bacteria.

Coccidiosis

Commercial chicken flocks free from coccidia are extremely rare. Several common species of coccidia infect broiler with *Eimeria acervulina* at duodenum, *E. maxima* at midgut, and *E. tenella* at ceca. The oocysts which contain a single cell (diploid) are passed in the feces. In the presence of oxygen and room temperature, for two days this cell undergoes reduction division and divides into four sporoblasts, each of which develops into a sporocyst containing two sporozoites (Levine 1982). If the mature oocysts are eaten by a chicken, the sporozoites emerge from them and enter the wall of the intestine when exposed to bile and trypsin. Concentrations of bile and trypsin also showed effects on coccidia development. Hammond et al. (1969) found that *in vitro* cattle *E. bovis* merozoites do not enter cells if pre-incubated for 10 min in a medium containing 0.2% trypsin and 5% bile. However, a medium containing 0.5% trypsin and 2% bovine bile increases the motility of intra- and extracellular *E. callospermophili* merozoites and increases the number of merozoites leaving a schizont (Speer et al., 1970). Coccidial infections generally consist of three types: clinical coccidiosis characterized by mortality, morbidity, diarrhea or bloody feces, as well as adverse effects on economic performance; subclinical coccidiosis, defined as not clinically obvious but causing reductions in weight gain and feed conversion efficiency of the host; a mild infection causing no adverse effects on the host (Williams, 1999). Coccidiosis is responsible for greater than $600 million in annual loss to U.S. poultry industry due to weight loss and poor feed utilization associated with *Eimeria* infection. Poultry farmers also spend greater than $50 million per year on coccidiostats (Jenkins et al., 2000). Nowadays, coccidia vaccines preventing *Eimeria* from infecting broilers have been developed to address the inefficiency of chemotherapy against coccidia and human concerns of the antibiotic resistance (Williams, 2002). Coccivac®-B/D and Immucox®C_1 are some of these coccidia vaccines. More information about coccidia vaccination will be addressed below in part of vaccination.

Antibiotic resistance:

Antibiotic use

First antibiotic was found in the early 1900's, when chemical Salvarson was discovered to cure human disease (syphilis) while prontosil rubrum protected mice and rabbits from infection by streptococci and staphylococci (Jones et al., 2003). However, it wasn't until 1946 when Moore et al. first reported their research indicating the positive effects of antibiotics on chicken growth.

Meanwhile, broiler production in the US increased dramatically after World War II from approximately 5 billion pounds in 1962 to nearly 48 billion pounds in 2002 (USDA-NASS, 2003). Broilers today are raised with greater rearing density (10,000- 20,000 chicks per house) and scale of production so that the danger and frequency of infectious disease outbreaks within flocks has increased dramatically. Many problematic infectious diseases are controlled with antibiotics (NASC, 1999). A total of 32 antimicrobial compounds are approved for use in broiler feeds in the U.S. without a veterinary prescription (Jones et al., 2003). Eleven of these antibiotics, including bacitracin, chlortetracycline, erythromycin, lincomycin, novobiocin, oxytetracycline, and penicillin, are used for growth promotion to increase weight gain, improve feed conversion rate, and decrease mortality by reducing microbial load in the intestinal tract, ultimately resulting in more nutrients available for the host. Stutz et al. (1984) reported that broilers fed 4.4 ppm lincomycin had improved weight gain and reduced *Clostridium perfringens* in the ileum environment. FDA evaluates antibiotic use in animals on the basis of safety for humans consuming the products, animal safety, efficacy, and effect on production. According to a survey of the members of the Animal Health Institute in 1999, 20.4 million pounds of antimicrobials were used in animal production in 1999 with 17.6 million pounds (86%) for prevention and treatment of disease and 2.8 million pounds (14%) for growth promotion (Jones et al., 2003).

Antibiotic resistance

Resistance to antibiotic agents has existed in nature before they were utilized in human and veterinary medicine. However, the long-term presence of antibiotics in the microbial population has made antibiotic-resistant stains by gene mutation more efficient in survival compared to normal microbes (Murray et al., 1978). The introduction of

streptomycin for the treatment of tuberculosis in 1974 led to the selection of resistant strains due to gene mutations in ribosome (Zhang et al., 1994).

Antibiotic resistance across many animal species has been observed. McEwen et al. (2002) indicated that antibiotic use in animals caused resistance in commensals (*Salmonella, Campylobacter*, some stains of *E. coli*) and in zoonotic enteropathogens (enterococci, most generic *E. coli*). The practice of using antibiotics as growth promoters in animal production is under scrutiny because it has been implicated as the major cause for the rise in antimicrobial resistance. Additionally they are not critical to animal health as disease treatment or prophylaxis. Bates et al. (1994) first reported that vancomycin-resistant enterococci (VRE) could be isolated in farm animals in Great Britain, suggesting farm animals possibly as reservoir for the VRE infection. In the study, they isolated 62 VRE from non-human sources, of which 22 were from farm animals (sheep, swine, poultry, and cattle) and 5 from uncooked chicken. In another research, 91 of the 92 *Salmonella* samples isolated from poultry products, poultry, and the poultry rearing environment were resistant to erythromycin, lincomycin, and penicillin (Roy et al., 2002).

Once antimicrobial pressure has been introduced into an environment, resistance can quickly develop and spread by horizontal transfer of plasmids (Salyers et al., 1997). Many factors contribute to the spreading of antibiotic resistance, for example, the movement of carrier animals between herds or flocks and between countries, the assembly of susceptible animals in close confinement, and the movement of resistance determinants throughout the ecosystem by means of vectors such as rodents, insects, and birds. The fecal waste from broilers reared under intensive conditions often is spread as fertilizer (McEwen et al., 2002). Groundwater, streams, and other waterways contaminated with these wastes may also facilitate the spread of bacterial resistance. The emergence of bacteria strains resistant to antibiotics may be due to either long-time exposure of bacteria to antibiotic or multi-functional gene activation (Salyers et al., 1997). However, other studies show that bacteria which do not contact with antibiotics possess antibiotic resistance, suggesting resistance genes are stably maintained in the absence of antibiotic pressure.

Alternatives to antibiotics:

Improved biosecurity and management

Biosecurity refers to all of the measures that should be taken to prevent viruses, bacteria, fungi, protozoa, parasites, insects, rodents, and wild birds from entering and infecting the wellbeing of poultry. Biosecurity measures can be applied at many points: source of birds, barriers and access to the flock, disinfection station, worker disinfection practices, visitor precautions, disposal of dead birds, disposal of waste, clean-up and disinfection between flocks and contact with other animals. The outbreak of *Mycoplasma gallisepticum* (MG) in North Carolina in 1999 was found to be due to contact with MG-positive farm (Vaillancourt et al., 2000). After conducting a survey of biosecurity practices, Tablante and coworkers, (2002) reported significantly better broiler flock performance in enhanced biosecurity farms compared to other farms.

Vaccination

The founding father of vaccination is Edward Jenner, who discovered that cowpox pustules obtained from an infected milkmaid protected an 8-year-old boy, James Phipps, against smallpox related virus with cowpox in 1796. One hundred years later, Pasteur accidentally developed the first attenuated bacteria culture against fowl cholera. The culture was called *Pasteurella multocida* 'vaccine', in honor of Jenner (Payette et al., 2001). Vaccination is a highly effective tool to protect poultry against viral infections (Davison, 2003). Many commercial products are available, including vaccines for prevention of Newcastle Disease (ND), Infectious Bursal Disease (IBD), and Infectious Bronchitis (IB).

Recently, coccidia vaccines against *Eimeria* infection have been developed, such as Coccivac®-B/D and Immucox®C_1, to address the inefficiency of chemotherapy against coccidia and human concerns of the antibiotic resistance (Williams, 2002). There are many acceptable methods of administration of coccidia vaccines, including intra-ocular, hatchery spray, edible gel, or spraying on feed. Among them, the hatchery spray is applied by aerosolizing the vaccines over the trays of chicks in the hatchery along with a dye to monitor the coverage or application efficiency. Chicks ingest the oocysts partly by the direct oral and ocular routes but mainly by self-preening and pecking drops of diluted vaccine off surrounding chicks (Schetters et al., 1999). Such ingestion behavior is

encouraged by including the coloring agent with the vaccine spray. Hatchery spraying also has an incidental benefit in providing a first source of water, which might help to delay dehydration of chicks during their transport to the farm. The level of preening activity can be improved by increasing sound intensity, decreasing temperature, or increasing light intensity (Caldwell et al., 2001).

Several species of coccidia are included in vaccines for broilers with *E. acervulina*, *E. maxima,* and *E. tenella* essential, because younger birds rarely encounter all coccidia species (Chapman, 2000). Following vaccination, immunity is initially stimulated by vaccine oocysts, and is subsequently boosted and maintained by multiple reinfections initiated by cycling of oocysts in the litter, which originated from the vaccine and from adventitious infections by local wild-type strains (Chapman et al., 1997). This cycling of oocysts is crucial for the development of fully protective immunity, because vaccinated birds reared in cages remain susceptible to challenge, while birds reared on the floor become immune.

Although Williams (1999) concluded no clear understanding was yet attainable, he discussed the possible relationships between live oocyst coccidia vaccines and outbreaks of necrotic enteritis, which is a serious problem in broilers worldwide and is typically caused by toxin produced by *C. perfringens* (Kohler, 2000). The types of cereals included in poultry diets have different effects on the susceptibility of birds to intestinal disease. Wheat based diets exacerbate both necrotic enteritis and coccidiosis when compared with corn based diets (Riddell et al., 1992; Williams, 1992).

Considering the three major criteria of evaluating broiler performance are daily weight gains, adjusted feed conversion rate and percent mortality, utilizing coccidia vaccines produced better performance broilers 32 out of 43 occasions when compared with groups treated with anticoccidial drugs (Williams, 2002). However, among the numerical differences, only 10 out of 43 of them were statistically significant. Williams suggested that there is unlikely to be a consistent improvement of weight gain or FCR by anticoccidial vaccines unless drug resistance has previously been a problem on a farm.

Vaccines for preventing pathogenic bacteria in the GIT are not as prevalent as compared to vaccines for controlling common viruses and coccidia possibly because of the hundreds of bacteria present in the GIT. One bacteria type has many strains with

different immunogenecity; and some bacteria pathogenicities occur only under specific conditions (Oshop et al., 2002). The avian immune system may be compromised when reducing *S. enteritidis* by vaccinating at day one, and d 28 may be the optimum vaccination time for *S. enteritidis* (Holt et al., 1999).

Genetic selection

Increasing poultry immunoresponsiveness and resistance to pathogens by genetic selection is expected to reduce disease-related losses. Success for selecting for high (H) or low (L) antibody responses to sheep red blood cells (SRBC) or selected diseases has been accomplished. Body weights of H white leghorn at 6 wk of age were 85% of those of L chickens (Gross et al., 2002), while antibody titers of the H chickens were 181% of L line, indicating that antibody response may be resource expensive. Broiler chicks from an H (response to *E. coli*) line exhibited significantly higher antibody titers than L chicks in response to whole inactive IBDV (Pitcovski et al., 2001). It was suggested that selecting for high or low antibody response to *E. coli* at an early age resulted in similar divergence in the response to IBDV antigens, providing another parameter for genetic selection of disease resistance strains. Al-Murrani (2002) reported that the heterophil to lymphocyte ratio could be used as a criterion for selection for resistance to *S. typhimurium* in chicks.

Competitive exclusion (CE)

A complex community of microorganisms inhabits the GIT from the beak to the cloacae. The main function of these microorganisms, from the host's side view, is to prevent colonization by potentially pathogenic microorganisms (Tuohy et al., 2003). Gut microorganisms compete with invading pathogens for ecological positions and metabolic substrates. They also serve as an important source of energy for the gut wall, providing up to 50% of the daily energy requirements of colonocytes in humans by fermentation of carbohydrates to organic acids, mainly butyrate. But some pathogens, antibiotics, or chronic diseases can occasionally disrupt the defense mechanisms of natural microflora. Milner et al. (1952) discovered that suppression of *Salmonella* infection in young birds corresponded with bird age, which was called 'competitive exclusion' (CE) by Nurmi et

al. in 1973. The goal of CE is to maintain beneficial microflora for the host through suppressing the growth of pathogenic bacteria but encouraging beneficial bacteria. Competition exclusion products include probiotics, prebiotics, acidifying agents, and others.

Healthy adult chickens raised in a free-range environment are resistant to intestinal colonization by the *Salmonella spp*. (Nurmi et al., 1973), but modern hatchery practices prevent newly hatched chicks from having contact with the protective intestinal microflora of adult chickens. By orally providing chicks and poults at hatch with microflora obtained from healthy adults, colonization of birds by *Salmonella* can be prevented (Snoeyebos, et al., 1978). Protection from *Salmonella* was accomplished by administering anaerobic broth cultures of intestinal microflora from selected donor birds. Protection was sustained for 63 days the longest period tested, although it could be overcome by severe challenge. Because an undefined CE product ultimately consists of the GIT microflora of healthy birds, *E. coli* is expected to be present in this product since it is a constituent of the bird's normal microflora (Nurmi et al., 1973). Maurer et al. (2002) found that *E. coli* in CE products are not likely to cause a serous systemic infection in humans because most of *E. coli* isolated (78%) were sensitive to killing by 12.5% human sera. The microorganisms are also less likely to cause systemic disease or airsacculitis in poultry than pathogenic strains commonly isolated from diseased birds because they lack the gene *tsh*, which encodes bacteria adhesion.

The intestinal microflora of warm-blood animals consists of at least 400 species of bacteria, but only 30 species account for the bulk of the intestinal population (Vaughan et al., 2000). Undefined CE products have the possibility of violating biosecurity if used in the hatchery. Therefore, CE products of defined bacteria population have been explored, which are also called probiotics. Probiotics have been defined as "live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance" (Fuller, 1989). Eastern Europeans and Asians have for centuries used natural remedies invariably containing lactic acid bacteria (*Lactobacilli* and *Bifidobacteria*) delivered in the form of yogurt and drink. They are the most studied probiotics in human and animal nutrition. Probiotics have been used in human therapy for lactose maldigestion, diarrhea, inflammatory bowel disease, colorectal cancer, and

irritable bowel syndrome (Tuohy et al., 2003). Mechanisms of probiotic responses involve the production of directly inhibitory compounds, reduction of luminal pH through short chain VFAs production, competition for nutrients and adhesion sites on the gut wall, modulation of the immune response, and regulation of colonocyte gene expression (Fooks et al., 2002; Steer et al., 2000; Mack et al., 1999). Van der Wielen and coworkers (2002) reported growth inhibition of *S. enterica in vitro* by a mix culture of *L. crispatus* and *C. lactatifermentans* in a sequencing fed-batch reactor mimicking the cecal ecophysiology of broiler chickens. La et al. (2001) recognized *Bacillus subtilis* PY79^{hr} as a CE agent that prevents the colonization of *E. coli* in liver, spleen, and ceca in chicks. The shedding of *E. coli* detected by cloacal swabbing was reduced significantly for 35 days. *B. sutilis* PY79^{hr} as a CE agent also successfully suppressed the colonization and persistence of *S. enteritidis* and *C. perfringens* in chicks (La et al., 2003). Fecal shedding of *S. enteritidis* was significantly reduced for up to 36 days. All-Lac XCL® is a source of beneficial lactic acid bacteria (*Lactobacilli* and *Bifidobacteria*) and should help colonization of beneficial bacteria in broilers when used immediately posthatch with coccidia vaccine. Ehrmann et al. (2002) reported that *in vitro*, several *Lactobacilli* strains had inhibiting effects on fecal indicator strains (*E. coli* CTC 1028, *S. enteritidis* CTC 1039, and *S. typhimurium* CTC 1037) and had strong adhesion to epithelial cells in the duck crops. Avian pathogenic *E. coli* strain O789 shares similar adhesion sites in chicken intestinal tissue as does indigenous *L. crispatus*. *L. crispatus* has been reported to efficiently prevent *E. coli* adherence *in vitro* (Edelman et al., 2003). Fukata et al. (1991) reported that use of *L. acidophilus* or *Streptococcus faecalis* reduced the pathogenicity of *C. perfringens*, which is the causative agent of necrotic enteritis. However, it is increasing evident that some group of microbes, such as *bacteroidaceae* and *bifidobacteria*, are relatively host specific (Mead, 1997).

Prebiotics have been defined as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health (Gibson et al., 1995). Oligosaccharides, which predominantly escape digestion in the upper gastrointestinal tract, are important sources of energy for bacteria in the ceca-colon which express enzymes such as β-fructosidase, β-galactosidase, xylanase or any other hydrolases to

enhance nutrient utilization by bacteria (Delzenne, 2003). Intestinal immunity is also enhanced by dietary oligosaccharides. Synthetic fructans at a dose of 50-80 g/kg diet increase Peyer's patches in mice, and at a dose of 100 g/kg promote cecal and colonic macrophages in rats (Schley et al., 2002). The mechanism of such an effect could be attributed to the prebiotic effect of oligosaccharides on lactic acid bacteria. *Bifidobacteria* are able to stimulate production of cytokine and reactive molecules (NO, H_2O_2) by macrophages. The fermentation of oligosaccharides (fructo-oligosaccharides, galacto-oligosaccharides) reaching the cecal-colon contributes to increased cation solubility through decreased pH (Greger, 1999). This effect may facilitate the dissociation of bivalent cation-phytate complexes, thus counteracting the 'anti-nutrient' effect of dietary components. In humans, prebiotics commercially available are fructooligosaccharides (FOS), inulin, lactulose, and galactooligosaccharides. Mechanisms of prebiotics involve an increasing numbers of *bifidobacteria*. Some pathogenic gram-negative bacteria such as *E. coli* and *Salmonella* grow to express the type 1 fimbria and adhere efficiently to the crop epithelium, lamina propria, and apical surfaces of intestinal villi. This adhesion was inhibited by α-methyl-D-mannoside (Edelman et al., 2003). Mannose oligosaccharides (MOS) are non-digestible for monogastric animals but can be utilized by lactic acid bacteria as an energy source (Delzenne, 2003). They also have the characteristic of mannose-specific binding with fimbriae of pathogenic gram-negative bacteria such as *E. coli* and *Salmonella* (Ofek et al., 1977) so that the bacteria are subsequently washed out of the small intestine with the flow of intestinal contents. Spring et al. (2000) reported that MOS agglutinated *S. typhimurium* 29E *in vitro* and reduced cecal *S. typhimurium* concentrations, while there was no effect on cecal concentrations of *Lactobacilli*, *enterococci*, anaerobic bacteria, lactate, volatile fatty acid, or cecal pH. Diets supplemented with MOS or palm kernel meal significantly impact the chicken's intestinal microflora by increasing the *Bifidobacterium* spp. and *Lactobacillus* spp. and decreasing the *Enterobacteriaceae*, while reducing susceptibility to S. *enteritidis* colonization in young chickens (Fernandez et al., 2002). Dietary Bio-Mos® (commercial MOS), derived from the yeast *Saccharomyces cerevisiae* (Iji et al., 2001), has been reported to improve body weight gain of poults, especially birds with *E. coli* challenge (Fairchild et al., 2001). The protein/RNA and RNA/DNA ratios in ileum were also significantly influenced by

the presence of Bio-Mos®, indicating higher activities of gene transcription and translation (Iji et al., 2001). The activities of maltase and leucine aminopeptidase, and alkaline phosphatase in the jejunum were also greater in birds fed with Bio-Mos®, suggesting increased digestibility and absorption ability.

Enzymes

Enzymes are naturally occurring and are produced by all living organisms for catalyzing chemical reactions. Enzymes were discovered in the latter part of the 19th century and have been used in industry and food processes since the early 1900s. The majority of enzymes products have been derived from fermentation products of alkaliphilic microorganisms (Clarkson et al., 2001). In animals, enzymes are used to break down feedstuff from polymers to simple structures for easily absorption by animals, for example, protease breaks protein into peptides and amino acids. The benefits of exogenous enzymes in feed applications include: balancing deficiencies in the endogenous enzyme system of the animal; eliminating of anti-nutritional factors; increase utilization of the feed (Bedford et al., 1998). The majority of protein ingredients incorporated into chicken feed are supplied by vegetable proteins, such as oilseeds. But the availability of nutrients in feed is sometimes limited by anti-nutritional factors. Huo et al. (1993) reported that protease could inactive trypsin inhibitors and lectin in raw soybean and low-temperature extruded soybean *in vitro.* Reduced viscosity of ileal contents and increased fat digestibility were observed when wheat based diets were supplemented with enzyme (Preston et al. 2001). Schang et al. (1997) observed that birds fed corn/soybean meal based diets supplemented with Vegpro® had no improvement in high nutrient density formulations, while improved body weight gain was found in low nutrient density fed group.

Acidify agent

Organic acids have usually been used as feed additives for fungistats (Paster, 1979). Formic and propionic acids and various combinations have been explored for potential bactericidal activity in feed contaminated with food borne pathogens, particularly *Salmonella* spp. (Khan et al., 1969; Mchan et al., 1992). It is assumed that undissociated

forms of organic acids can easily penetrate the lipid membrane of bacterial cells and dissociate into anions and protons after entering the neutral pH of the cell cytoplasm (Eklund, 1985). Inside the cell, organic acids inhibit bacterial growth by breaking oxidative-phosphorylation and inhibiting the exchange of adenosine triphosphate-inorganic phosphate (Byrne et al., 1979). Export of excess protons by the ATPase pump may spend bacterial energy and inhibit their growth (Ricke, 2003). Chaveerach et al. (2004) reported birds drinking organic acid water of pH 4 had decreased *Campylobacter* infection with intact intestinal epithelial cells. However, many microorganisms possess an adaptive stress response that gives them the ability to survive exposure to extremely acidic environments. This acid-tolerance response (ATR) has been studied in *E. coli* and *S. typhimurium* and demonstrated that exposure to sublethal pH induces the expression of numerous acid-shock proteins that promote bacterial survival in subsequent extreme acid environments (Merrell et al., 2002). For *Vibrio cholerae,* acidic environment stimulates ATR by activating ToxR gene that is the major virulence gene regulating expression of cholera toxin (CT) and other components essential for colonization (Merrell et al., 2000). *S. enteritidis* with enhanced acid tolerance were also demonstrated to be more virulent in mice and more invasive in chickens (Humphrey et al., 1996).

Mycotoxin binding

Various feed ingredients are frequently contaminated by mycotoxins, which are a group of secondary fungal metabolites, such as aflatoxin, ochratoxin, zearalenone, T-2 toxin, vomitoxin, and fumonisin (Jelinek et al., 1989). Many strategies to detoxify mycotoxins have been explored and the most popular method is the use of absorbents such as clay. Compared to the ability of clay to bind limited mycotoxins, esterified glucomannan (E-GM) was found to bind multiple mycotoxins in broiler feed (Raju et al., 2000). Aravind et al (2002) reported that including E-GM in feed reduced the growth depression due to a diet naturally contaminated by mycotoxins. MTB-100 is a commercial product of E-GM produced by Alltech, US. Significant improvements in body weight and feed intake were observed in broilers fed diets supplemented with MTB-100 (Raju et al., 2000).

Intestinal morphology alterations by diets

The intestinal villus and crypt morphology in chickens has been associated with intestine function and chicken growth. Yamauchi and coworkers (1991) discovered larger villi in broiler chickens than those of White Leghorns, an egg laying strain with lighter body weight. Chickens fed wet diets have increased villi and less depth of crypts in duodenum, jejunum, ileum, ceca and colon compared to birds given dry diets (Yasar et al., 1999), suggesting the effect of dietary treatment on villus morphology. Tarachai et al. (2000) discovered that Leghorn chickens at 142 d with a five-day feed withdrawal had reduced villus height in duodenum, while other birds hold feed for three days then refed for two days restored their villus height in the duodenum. Chicks fed a crystalline amino acid diet from 7 to 21 days of age had depressed jejunal villus height and crypt depth compared to corn-soybean meal (Batal et al., 2002). Samanya et al. (2002) reported that villus height of duodenum and ileum in 28 day old chicks fed with *B. subtilis* significantly increased, while villus height of jejunum only numerically improved. Villi in broilers of the same breed grew larger in high protein and low energy diets compared to broilers fed low protein and high energy diet (Yamauchi et al., 1993). Iji and coworkers (2001) found increased jejunal villus height at 28 days of age in broilers fed 5g Bio-Mos/kg from 7 to 28 days, while villus surface area and crypt depth in jejunum and ileum and villus height in ileum were not significantly affected.

In birds, there exists a separate mucosal immune system that exhibits a number of specific features such as antigen-presenting cells, immunoregulatory cells, and effector cell types, which are distinct from their counterparts in the systemic immune system (Lillehoj et al., 1996). The mucosal immune system consists of two compartments: mucosal inductive sites, which consist of the nasal-associated and gut-associated lymphoid tissues located where they encounter environment antigens, and mucosal effector sites which include the lamina propria of the intestine and the upper respiratory tract (McGhee et al., 1990). The thickness of ceca lamina propria is an indication of recent infection of the ceca wall by a pathogen. Tellez et al. (1994) reported that increased lamina propria thickness in ceca was related to organ infectivity based on morphometric analysis, suggesting probably a marked infiltration of inflammatory cells in the cecal mucosa.

INTRODUCTION

Antibiotics have been a common feed additive in poultry feed as a growth promoter to improve performance by reducing the burden of pathogens for many years. Antibiotics can and are frequently used therapeutically and prophylactically for the treatment of disease in poultry. In the early 1900's, the antibiotic pioneers first discovered the chemical substance Salvarson could cure a human disease (syphilis), while prontosil rubrum (a red dye) was discovered to protect mice and rabbits from infection by streptococci and staphylococci (Jones et al., 2003). It wasn't until 1946 when the first recorded research indicated the positive effects of antibiotics (sulfasuxidine, streptothricin, and streptomycin) on chicken growth (Moore et al., 1946) which was originally believed to be related to the discovery of vitamin B_{12} (Jones et al., 2003). However, increasing pressure to reduce or eliminate the use of antibiotics in feed is occurring due to the negative human health issue of antibiotic resistance. In 1994, it was first shown that vancomycin-resistant enterococci (VRE) could be isolated from farm animals in Great Britain, and it was suggested that farm animals could be a reservoir for the VRE infection (Bates et al., 1994). In the study, 62 samples of VRE were isolated from non-human sources of which 22 were from farm animals and 5 from uncooked chicken. Roy and coworkers (2002) also isolated *Salmonella* from poultry products, live poultry, and poultry rearing environments and found that 91 of the 92 samples tested were resistant to erythromycin, lincomycin, and penicillin.

A number of strategies to reduce the use of antibiotics in feed have been explored, including improved biosecurity, vaccination, genetic selection, and competitive exclusion (CE). Vaccination can be effective, but the avian immune system may be compromised when vaccinating to reduce *S. enteritidis* at day 1, and it was subsequently determined that the optimum vaccination time for *S. enteritidis* was at d 28 (Holt et al., 1999). Chicks are immunologically naive and prone to rapid and persistent colonization by beneficial and pathogenic bacteria in the gastrointestinal tract during the first 3 to 4 weeks of life (Barrow et al., 1988), indicating that CE may be a beneficial approach. One CE approach utilizes either a complex mixture of bacteria derived from the gut of healthy birds or defined beneficial bacteria and subsequently orally administered to day old birds to

establish a beneficial microflora. This microflora in turn prevents the colonization of pathogenic microorganisms such as *Salmonella* and *E. coli*. Probiotics have been defined as "live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance" (Fuller, 1989). A number of researchers have reported that the addition of probiotics to the diet of broilers and layers leads to improved performance (Jernigan et al., 1985; Barrow, 1992; Jin et al., 1997). All-Lac XCL® is a source of beneficial lactic acid bacteria (*lactobacilli* and *bifidobacteria*) and has been used posthatch to help develop beneficial microflora in the gut of broilers.

Another CE product uses the characteristic of mannose-specific binding with fimbria of pathogenic gram-negative bacteria, such as *E. coli* and *Salmonella.* These bacteria grow to express the type 1 fimbria and adhere efficiently to the crop epithelium, lamina propria, and apical surfaces of intestinal villi. The adhesion was inhibited by α-methyl-D-mannoside (Edelman et al., 2003). Mannan oligosaccharides (MOS), derived from yeast cell wall, are non-digestible for monogastric animals and can be utilized by lactic acid bacteria. MOS also bind the fimbria of pathogenic bacteria to prevent them from attaching and colonizing on the small intestine mucosa (Ofek et al., 1977). Adhered bacteria are subsequently washed out of the small intestine with the flow of intestinal contents. Diets supplemented with MOS significantly impact the chicken's intestinal microflora and reduce susceptibility to S. *Enteritidis* colonization (Fernandez et al., 2002). Dietary Bio-Mos® (commercial MOS) improved the body weights and gains of poults, especially birds with *E. coli* challenge (Fairchild et al., 2001). Ideally birds treated with CE will have decreased pathogenic bacteria loads in intestine and subsequently increased villus height and decreased lamina propria thickness.

Feed enzymes are used to increase digestibility of feed ingredients to enhance the growth of broilers. Additionally, the use of more digestible feedstuffs reduces the risk of increased intestinal viscosity associated with high non-starch polysaccharide containing products decreasing feed passage through the gastrointestinal tract (Preston et al. 2001), subsequently decreasing pathogenic bacteria. Dietary addition of Vegpro®, a commercial enzyme, improved the broiler performances in energy utilization, protein digestibility, live weight gain, and feed conversion rate (Schutte, J. B. et al., 1998). Many feed ingredients, such as corn, are often contaminated by mycotoxins, which are a group of

secondary fungal metabolites. MTB-100 is a commercial product of esterified glucomannan, which can bind and detoxify mycotoxin. Raju and coworkers (2000) reported significant improvements in body weight and feed intake in broilers fed mycotoxin-contaminated diets supplemented with MTB-100.

Organic acids suppress pathogenic bacteria in intestine by providing an unfavorable acidic environment for pathogen bacteria but favorable for beneficial bacteria. Decreased *Campylobacter* at broiler cecal was observed when the broilers administrated organic acids by water (Chaveerach et al., 2004), indicating a possible role in reducing pathogentic bacteria in the gut of poultry. All of the aforementioned products have been observed to improve bird performance by the manipulation of the microflora present in the gut of poultry, the combination of such products has not been attempted to evaluate possible additive or synergistic affects on bird performance without the use of antibiotics.

OBJECTIVE

The objective of the present trial was to explore the possibility of supplementing Bio-Mos® associated with All-Lac XCL® and/or other feed additives in broiler feed as an alternative drug-free approach to antibiotics. The effects of Bio-Mos®, All-Lac XCL®, Acid Pak 4-Way®, MTB-100® and Vegpro® on improving broiler body weight gain, feed efficiency, and intestinal morphology were determined.

MATERIALS AND METHODS

Animals and diets

A total of 2,496 Cobb 500 straight run broiler chicks were sprayed with Coccivac® vaccine against coccidia at the hatchery. Additionally, half of the chicks (n=1,248 birds) were administered All-Lac XCL® at the hatchery (5 g/2,000 birds) by mixing it with the coccidia vaccine solution. After transport to the Virginia Tech Turkey Research Farm, birds were randomly divided into 52 pens (n=26 pens for birds treated with All-Lac

XCL®; 48 birds/pen) with a stocking density of 14.4 chicks /m^2 at d 0, and were assigned one of four dietary treatments with each treatment being replicated 13 times. The four dietary treatments consisted of: 1) negative control (NC), only basal diet without growth promoter or coccidiostats; 2) positive control (PC), basal diet + Lincomycin (2 g/ton starter and 4 g/ton grower); 3) Program 1 (PG1), basal diet + Acid Pak 4-Way® ($^1/_2$ g/L water, 0-5days then 1 d per week there after), VegPro® (0.91 kg/ton), MTB-100® (0.45 kg/ton), Bio-Mos® (1.81 kg/ton starter, 0.91 kg/ton grower, 0.45 kg/ton finisher and withdrawal), and All-Lac XCL® at hatchery; 4) Program 2 (PG2), basal diet + Bio-Mos® (same inclusion as PG1) and All-Lac XCL® (at hatchery). Four phases of feeding (starter, grower, finisher, and withdrawal) were used during the trial with feed changes occurring at d 14, 28, and 35. The basal diets consisted of mainly corn, soybean meal, bakery meal and animal protein and had nutrient levels comparable to commercial operations (Table 1). After 29 days of age, birds in NC and PC were fed the same basal diets until the end of trial. All feed was in mash form and fed *ad libitum* along with water (via nipple drinkers). The pH value in water containing dissolved Acid Pak 4-Way® was measured by Accunet® AB 15 pH meter[1]. pH value of Acid Pak 4-Way® water was 3.22, while pH of tap water was 7.4.

Performance record

Feed consumption and average body weight was obtained by pen on d 0, 14, 28, 35, and 49, while body weight gain and feed conversion rate (FCR) was calculated. Mortality, house temperature, and humidity were recorded daily. At d 41 (females) and 48 (males) four birds per pen (total n = 52/trt/sampling) were randomly selected, banded, weighed and put in separate pens without feed. Ten hours later, the birds were moved to the processing room at Virginia Tech Turkey Research Farm, weighed, stunned and killed. After 3 min suspended for bleeding, birds were scalded, defeathered, eviscerated, and cut off necks and feet. Resulting warm carcass were weighed and then chilled in ice water. Four hours later, the cold carcasses were weighed and the fat pad was removed from the abdominal cavity. Removal of wings, thighs, drums, tenders, and fillets were accomplished on stationary deboning cones and the individual products were weighed.

[1] Fisher Scientific, NH

Processed product percentage was calculated in relation to cold carcass weight. An estimate of total live weight gain/100 birds started at d 0 was calculated based on the following equation: total live weight gain = average body weight gain × livability × 100 at d 49. The gross income per 100 initial chicks was calculated base on gross income = (live bird weight price per lb * average live weight * 100 birds * livability) - feed cost.

Coccidia challenge

Three birds per pen were randomly selected, weighed and banded, and birds from replicates in the same dietary treatment were combined into one pen in a separate building and given a mixed coccidia challenge (4×10^4 *Eimeria acervulina,* 2×10^4 *Eimeria maxima, and* 1.5×10^4 *Eimeria tenella*) by *per os* gavage on d 14. The coccidia challenge strains were donated by Dr. H. D. Danforth (USDA/ARS/LPSI/PBEL, Beltsville, MD). Body weight and lesion scores (Johson et al., 1970) of duodenum, ileum, and ceca were evaluated on d 20 (six days post-challenge).

Intestinal morphology

After weighing birds on d 14, 28, 35 and 49, one bird per pen was euthanized by cervical dislocation. Four-centimeter segments of duodenum (from the top of one side loop to distal), ileum (from Mickel diverticulum to distal) and ceca (from ileo-cecal junction to distal) were removed, rinsed, cut into five equal pieces and placed into buffed formalin until further processing. For each sampling, ten of the thirteen tissues were cut into 5 mm sections and put into tissue cassettes. The tissues were processed, embedded in paraffin, and subsequently cut and placed onto slide with 5μm thickness. The tissues were stained with 0.02% toluidine blue for light microscope measurement of villus height and crypt depth in duodenum (magnification 20x) and ileum (magnification 40x) and cecal lamina propria (magnification 100x). Three of five pieces on each slide were measured with four measurements per piece (n = 12 measurements/bird; 10 birds/treatment). Pictures of villus height, crypt depth, and cecal lamina propria were obtained by Olympus

Polaroid DMC-IE camera[2] with measurements made using the software of SigmaScan Pro 5[3].

Statistics analysis

Most data was analyzed by the MIXED procedure of SAS (SAS Institute, Cary, NC, 1999) for an RCBD experimental design with row location of pens as block to minimize the influence of ventilation differences on performance with pen being experimental unit. The statistic model is $y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$, with y_{ij} = observed dependent variable, μ = grand mean, $\alpha_i = i^{th}$ dietary treatment effect, $\beta_j = j^{th}$ random block effect, ε_{ij} = error for treatment i of block j ~ N (0, σ_ε). Treatment means were separated by the least squares means procedure of SAS software with the level of significance $P < 0.05$ unless otherwise stated. Processing yield percentage data were transformed with arcsine to analyze. Mortality data was evaluated by using FREQ procedure of SAS. Data of villus height, crypt depth, and cecal lamina propria were analyzed by using MIXED procedure of two-factorial design with days of age and diet factors. The model is $y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$, with y_{ijk} = observed dependent variable, μ = grand mean, α_i = days of age treatment effect for level A_i, β_j = dietary treatment effect for level B_j, $(\alpha\beta)_{ij}$ = interactions between levels A_i and B_j, ε_{ijk} = error for k^{th} replicate of (A_i ,B_j) ~ N (0, σ_ε). To further estimate the effects of density and intestinal morphology on the performance, linear models were built by REG stepwise selection procedure of SAS with $\alpha = 0.05$.

RESULTS

Growth performance

Overall, broiler chicks consuming the negative control diet (NC) had lowest body weight gains through the four growth periods when compared to birds fed other diets (Table 2). This response was most noted at d 28 and 35 with cumulative daily body weight gains of NC birds being lower ($P < 0.05$) compared to those of PG2 birds (42.7 vs. 44.5 and 49.0 vs. 50.3 g/d, respectively). At d 35 and 49, cumulative daily body

[2] Polaroid Corporation, MA
[3] SPSS Inc., IL

weight gain of PC birds improved ($P < 0.05$) compared to birds fed NC diet (49.0 vs. 50.4 and 54.1 vs. 55.8 g/d). Cumulative daily body weight gains of PG1 birds were numerically improved compared to those of NC. Final body weight gains of birds on PC (2.736 kg) were greater ($P < 0.05$) compared to NC (2.650 kg), while birds on PG1 (2.681 kg) and PG2 (2.710 kg) were similar to positive and negative control.

Feed consumed by NC birds (101.2 g/bird/d) was less ($P < 0.05$) compared to PC (104.0 g/d) and PG1 (104.0 g/d) birds from 15 to 28 days of age (Table 3). At d 49, the total feed consumed by NC, PC, PG1, and PG2 were 5.29 kg, 5.37 kg, 5.31 kg, and 5.29 kg, respectively. Cumulative feed conversion rate (CFCR) was numerically higher ($p = 0.06$) in NC birds (1.63) compared to PG2 (1.59) at d 28 (Figure 3). At days 35 and 49, CFCR was significantly ($P < 0.05$) improved in PC and PG2 birds compared to NC birds (1.76 vs. 1.73, 1.73; 2.00 vs. 1.96, 1.95, respectively). At day 49, birds fed NC diet had numerically higher CFCR ($P= 0.27$) compared to PG1 birds (2.00 vs. 1.98).

Product yields of wing, fat pad, tender, thigh, drum, fillet, and sum of all-products for female birds at d 42 were not significantly ($P > 0.05$) affected by dietary treatments (Table 4) during the trial. The fillet weight in relation to cold carcass from birds fed PC and PG2 diets were numerically improved compared to those consuming NC diet (16.12% and 16.13% vs. 15.8%, respectively).

Product yields from male birds at d 49 were also not altered ($P > 0.05$) by dietary treatments (Table 5). Fillet percentage from male birds fed PC, PG1, or PG2 diets were numerically improved compared to those consuming NC feed (15.41, 15.40, or 15.40 vs. 15.21 %, respectively).

Overall, birds consuming NC diet had greater ($P < 0.05$) mortality (12%) compared to PG1 (4.6%), PG2 (6.7 %) and PC (7.6 %) with most of the mortality occurring from d 0 to 28 (Figure 4). Mortality for birds consuming PG1 was also significantly ($P < 0.05$) improved compared to PC birds (4.6% vs. 7.6%). Birds fed PG1 diet had less ($P < 0.05$) mortality compared to NC from d 0 to 14 and d 15 to 28 (1.3% vs. 3.0% ; 1.6% vs. 7.6%, respectively). Less birds died when consuming PG1 diet ($P < 0.05$) compared to birds consuming PG2 from d 0 to 14 (1.3% vs. 3.0%). Mortality of birds in groups PC (3.1%) and PG2 (2.5%) was also significantly ($P < 0.05$) reduced compared to NC (7.6%) from 15 to 28 d.

Changes in stocking density (Table 6) during the trial were evaluated and indicated the group fed PG1 was more density stocked ($P < 0.05$) compared to the group fed NC after 28 days of age due to the improvement in livability. The relative stocking density at d 49 in PG1 group was 11%, 5%, and 5% higher compared raised on NC, PC, and PG2 feeding programs, respectively.

An estimate of total live weight gain/100 birds started at d 0 was calculated (average body weight× livability ×100 at end of trial) and indicated a significant increase ($P < 0.05$) for PG1 (259.79 kg), PG2 (256.87 kg), and PC (256.96 kg) compared to NC (237.47 kg). The gross income per 100 initial chicks (birds sold on board minus feed cost) was \$145.27, \$155.28, \$153.91, and \$156.44 in NC, PC, PG1, PG2, respectively.

Challenge performance

During the challenge study, the lesion score of challenge birds was not statistically ($P > 0.05$) affected by different diet treatments (Table 7). The lesion score of *E. acervulina* in duodenum in PG1 birds (1.51) was numerically higher compared to other treatment groups. The lesion scores of *E. maxima* and *E. tenella* in the ileum (1.26) and ceca (0.38) in NC birds were numerically higher compared to PC (1.04, 0.19), PG1 (0.96, 0.21) and PG2 (1.02, 0.25) birds. The body weight gain in PG2 birds was improved ($P < 0.05$) compared to NC birds (0.27 vs. 0.23 kg).

Intestinal morphology

Morphologic measurements in the duodenum displayed a significant ($P < 0.02$) interaction of days of age vs. diets in regards to villus height (Table 8). Duodenum villus height was increased from d 14 to d 35, and then decreased in birds consuming all treatments except PG2 which remained at the same level from d 36 to 49 (Figure 5). Especially at d 49, villus height in PG2 birds was significantly ($P < 0.01$) longer when compared to NC, PC, or PG1 (2.39 vs. 1.97, 1.96, or 2.05 mm, respectively). Days of age had a significant ($P < 0.001$) effect on the duodenum villus height, crypt depth and the ratio of villus height to crypt depth (V/C), while dietary treatments had no significant effect.

In the ileum, interaction of days of age and diets played a significant ($P < 0.01$) role in villus height, crypt depth and V/C ratio. Development pattern in regards to ileum villus height increased to a plateau at d 35 and decreased afterward except increase in PG2 and flat in PG1 (Figure 6). At d 49, villus height in PG2 was longer ($P < 0.05$) compared to NC, PC, and PG1 (1.24 vs. 1.00, 0.92, and 1.11 mm, respectively), while that of PG1 was greater ($P < 0.003$) than PC; crypt depth in PG2 was deeper ($P < 0.003$) compared to NC, PC, and PG1 (0.123 vs. 0.082, 0.075, and 0.090 mm, respectively) (Figure 7); At d 35, V/C ratio in birds fed PG2 was greater ($P< 0.02$) compared to NC birds (10.94 vs. 8.95), while those consuming PC (9.98) and PG1 (10.59) were numerically improved. At d 49, V /C ratio in PG2 was smaller ($P < 0.006$) compared to NC, PC, and PG1 (10.17 vs. 12.68, 12.67, and 12.57) (Figure 8).

In ceca, days of age had a significant ($P < 0.0001$) effect on the thickness of lamina propria (LP). Cecal LP thickness was significantly ($P < 0.003$) increased through four periods (16.9, 20.6, 23.7, 26.5 μm at d 14, 28, 35, 49, respectively). Dietary treatment also ($P < 0.02$) influenced the thickness of cecal LP. Cecal LP thickness in PG1 and PG2 birds was ($P < 0.008$) lower compared to NC birds (20.9 and 21.3 vs. 23.5 μm, respectively), while LP in PC birds was numerically thicker than NC.

DISCUSSION

PC bird growth and performance in terms of livability, BW gain and FCR were improved compared to NC birds. The growth performance responses to antibiotics are similar to that previously reported by Dafwang and coworkers (1984) although the response difference was not dramatic. This may indicate a difference in bird genetics from 20 years ago, possibly bacterial resistance to today's antibiotics, differences in dietary nutrient levels, different microbial populations, or different experimental conditions. Based on previous research by Stutz and coworkers (1984), the improvement in weight gain and FCR may have resulted from improved gut health (reduced *Clostridium. perfringens* counts). The result of a numerical reduction in cecal lamina propria thickness in PC birds provides an indirect indication of reduced pathogen

infection in the ceca. Tellez et al. (1994) reported a positive relationship between pathogen infection and cecal lamina propria thickness. During a pathogenic bacteria infection, lymphocytes will accumulate to kill the pathogens and cause inflammation which in turn increases lamina propria thickness.

PG2 birds were treated with All-Lac XCL® at the hatchery supplemented and with Bio-Mos® in the feed. Growth and performance in regards to livability, BW and FCR in PG2 birds was greater compared to NC birds. All-Lac XCL® is a mixture of beneficial lactic acid bacteria (*lactobacilli* and *bifidobacteria*), and *in vitro* indicated that *lactobacilli* efficiently inhibited the adhesion of *E. coli* on the chicken intestinal wall (Sanna et al., 2002) thus reducing the pathogen load in the gut. Van der Wielen and coworkers (2002) reported an inhibition of growth of *Salmonella enterica in vitro* by a mix culture of *L. crispatus* and *C. lactatifermentans*. Cecal lamina propria thickness in PG2 birds was thinner compared to NC, suggesting lower pathogenic bacterial load in ceca in PG2 birds. In addition, pathogenic bacteria in the intestinal tract are bound by Bio-Mos® (Ofek et al., 1977) and are subsequently washed out of the intestine with other non-digested feedstuffs. Both may have improved gut health of birds fed PG2 and contributed to reduced mortality and increased performance without the use of antibiotics compared to NC group. In contrast, it has been reported that supplementing Bio-Mos® alone had no effect on broiler performance (weight gain, feed efficiency and nutrient utilization) or immune response to IB, IBD and ND vaccines (Shafey et. al. 2001) indicating that Bio-Mos® may only work in the gut.

In addition to All-Lac XCL® and Bio-Mos® (PG2 treatment), PG1 birds were also supplemented with Vegpro®, MTB-100® and Acid Pak 4-Way®. Livability in PG1 was greatest compared to the other three dietary treatments, suggesting beneficial effects contributed by these additional additives. Like PG2 birds, cecal lamina propria in PG1 birds was thinner compared to NC birds, suggesting lower bacteria load in PG1 bird ceca. From 15 to 29 days of age (peak mortality), mortality was suspected to be the result of necrotic enteritis (Figure 9, 10) by *C. perfringens*. PG1 had best livability when birds were affected by *C. perfringens*, suggesting greatest protection effect by PG1. However, growth and performance as measured by BW gain and FCR in PG1 birds was only numerically improved compared to NC birds but was not as good as PG2 birds, seemly

suggesting an adverse effect of these additives. However, the higher stocking density present in the PG1 group may have had a negative effect on performance compared to PG2 as indicted by the numerical reduction in body weight gain for PG1 birds. Feddes and coworkers (2002) reported a positive affect of reduce stocking density on body weight gain. Although organic acids suppress bacteria growth (Eklund, 1983), some pathogenic bacteria can adapt to an acidic environment and become more virulent (Humphrey et al. 1996). Humphrey and coworkers also observed that certain isolates of *S. enteritidis* with enhanced acid tolerance were demonstrated to be more virulent in mice and more invasive in chickens. Acid Pak 4-Way® might also play negative impact on performance in PG1 birds.

Product yields were not significantly affected by dietary treatments, although there were numerical improvements in fillet percent in birds fed PC and PG2 compared to NC fed birds. In contrast, Kalavathy et al. (2003) reported that birds fed *Lactobacilli* had reduced abdominal fat deposition after 28 d of age. The birds in this trial were raised to 49 days of age with high temperature (summertime) so it's possible that the stocking density may have played a negative role on yield due to the inability of the ventilation system to maintain optimal conditions in the research house. Feddes et al. (2002) reported that stocking density had no effect on mortality, breast yield, and carcass grading when raised under optimal conditions.

The lesion scores of challenge birds were not affected by different dietary treatments, which may mean the coccidia vaccine provided sufficient protection for all birds from the infection of mixed *Eimeria* challenge. The cecal lamina propria thickness in NC group was thicker compared to other dietary treatments. It is suggested that dietary treatments may contribute to higher lesion scores in NC due to the inability to inhibit pathogenic bacteria growth by antibiotics, probiotics or prebiotics. The weight gain in PG2 birds was higher compared to NC birds, which was similar with the rest of the birds raised under normal feeding conditions in the separate building.

Significant interactions between days of age and diets existed for the duodenum and ileum villus height, ileal crypt depth, and ileal V/C ratio. Iji et al. (2001) reported similar data with villus height of birds fed from d 1 to d 21 increasing in the duodenum, jejunum, and ileum with age, while crypt depth increased in duodenum and jejunum. Although

Samanya et al. (2002) reported that villus height in duodenum and ileum significantly increased in 28 day old chicks fed *Bacillus subtilis*. Few significant effects on villus height by diets were observed in this trial in regards to all treatments with the exception of birds fed PG2, which had a significantly increased villus height in duodenum and ileum at d 49 compared to NC birds. Crypt depth increased with age, with the exception of the decrease in PG1 at d 28 and PG2 at d 49. Significantly shorter crypt depth existed in PG2 at d 49. Yasar et al. (1999) observed that chickens fed wet diets had increased villus height and lower depth of crypts in duodenum, jejunum, ileum, ceca and colon compared to birds given dry diets, indicating dietary effects on intestinal morphology. Tarachai et al. (2000) discovered that Leghorn chickens at 142 d with withdrawal of feed for five days had reduced villus height in the duodenum while birds starved for three days then refed for two days restored their villus height in duodenum, suggesting dietary effects on villus morphology is fast and easily recovered in short term. The following two cited manuscripts suggested differences in macronutrient level will affect gut structure development. Report from Yamauchi et al. (1993) indicated villus was larger in broilers fed high protein and low energy diets compared to broilers fed low protein and high energy diets. Also reported was chicks fed crystalline amino acid diet from 7 to 21 days of age had depressed jejunal villus and crypt depth compared to corn-soybean meal (Batal et al., 2002). Iji and coworker (2001) reported micronutrients also influenced the morphology of intestine. They observed an increased jejunal villus height in broilers at 28 days of age fed 5g Bio-Mos®/kg from 7 to 28 days while villus surface area and crypt depth in jejunum and ileum and villus height in ileum were not significantly affected. In this study, at d 49, significant effects of dietary treatment on intestine morphology were observed, indicating possibly effects of micronutrient factors on intestinal morphology easily seen in this long-term trial. In summary, days of age play an important role in changes in intestinal morphology while micronutrients, such as Bio-Mos®, may have an effect on intestinal structure development after 35 days of age.

To predict the mixed effects of many different factors in the performance of birds, multiple linear models were used to estimate the responses. Bird surface area (SA) is estimated based on the equation of $SA = (BW)^{0.75}$. Bird surface area over pen area (SAPA) is calculated by SA/pen area. Regressors are SAPA, duodenum villus height

(DV), duodenum crypt depth (DC), ileum villus height (IV), ileum crypt (IC). Responses are cumulative feed intake (CFI), cumulative body weight gain (CWG), and cumulative feed conversion rate (CFCR). The linear regression equations are: CFCR = 1.1598 - 0.05894 DV – 0.35284 DC + 0.12487 IV + 0.03759SAPA. CWG = 13.61084 +3.52105 IV +1.75047 SAPA. CFI had no significantly linear regression. These results indicate that bird stocking density, duodenum villus height, ileum villus height, and ileum crypt depth could have effects on the growth and performance of broilers, specifically CFCR and CWG.

When evaluating the different feeding programs based on live weight gain and gross income, NC birds had the lowest live weight gain and gross income when compared to other dietary treatments. PG1 birds had the greatest live weight gain while PG2 had the numerically highest gross income, due to the decrease in feed cost. Based on the data observed in the present study, Bio-Mos® in combination with All-Lac XCL® can be an efficient feeding program to overcome the negative impacts of utilizing drug-free diets.

SUMMARY AND CONCLUSIONS

Cumulative weight gain and feed conversion rate were greater in PC and PG2 when compared to NC, while PG1 had numerical improvements. Cumulative feed intake was lowest in NC compared to other three dietary treatments.

Overall mortality in NC was greatest while PG1 had the least mortality. No difference existed in lesion scores in the *Eimeria* challenge trial or processed products at trial end. Days of age had greater impact on gastrointestinal villus height and crypt depth, while dietary treatments imposed their influence more in cecal lamina propria thickness.

Overall, there is no doubt that feeding broilers a drug-free diet can significantly impair livability, body weight gain and cumulative FCR. When looking at the 49 day data, there is about a 22.49 kg or 9.5% weight improvement per 100 birds started when using PG1 compared to the NC birds and 3 kg or 1.2% improvement compared to PC birds. Cecal lamina propria thickness indirectly proves the protection capacity from disease by using PG1 or PG2. There was no affect on carcass yields, but the improvement in livability improved total product to market and increased gross income was obtained in

PG1 and PG2 compared to negative control. Based on the data obtained in this trial, PG1 or PG2 seems to be solid programs for feeding a drug-free feed to broiler birds. Further research is necessary to identify changes in intestinal protein/RNA, nutrition absorption, and microflora with the addition of multiple feed additives to further enhance performance without antibiotics. In addition, the negative affects of using the PG1 program observed after 28 days needs to be evaluated to determine if it truly was simply a result of increased stocking density or interactions between the products used.

Table 1: Composition and nutrient content of basal diets **(%)**[1]

Ingredients	Starter	Grower	Finisher	Withdrawal
	----------%----------			
Corn	58.81	63.09	72.02	71.57
Soybean Meal	29.98	23.7	19.17	18.78
Bakery FD Doswel	5.1	5.07	3.05	5.12
Valley Protein	2.55	5.07	2.54	1.32
Poultry Fat	1.02	1.27	1.27	1.28
Phosphate, Deflr	0.93	0.32	0.43	0.41
Limestone	0.7	0.56	0.71	0.78
salt	0.26	0.3	0.22	0.22
S-Carb	-----	-----	0.16	0.16
Alimet	0.21	0.22	0.12	0.11
Liq. Lysine 50%	0.16	0.16	0.14	0.13
Choline Chlor-70	0.1	0.08	0.05	-----
Copper Sulfate	0.08	0.08	0.07	0.08
Trace Mineral	0.06	0.05	0.03	0.02
Vitamin	0.03	0.02	0.01	0.01
NP5000	0.01	0.01	0.01	0.01
Calculated Composition				
Energy ME (Kcal/Kg)	3068	3155	3204	3229
	----------%----------			
Dry Matter	87.64	87.71	87.34	87.43
Protein	22.30	21.10	18.02	17.35
Fat	4.34	4.94	4.67	4.75
Lysine	1.11	1.01	0.83	0.80
Methionine	0.52	0.52	0.39	0.37
Met+Cys	0.87	0.86	0.69	0.66
Calcium	0.90	0.80	0.73	0.67
AV. Phos	0.43	0.37	0.33	0.31

[1]Starter, grower, finisher, or withdrawal diets were used during d 0 to 14, 15 to 28, 29 to 35, and 36 to 49, respectively.

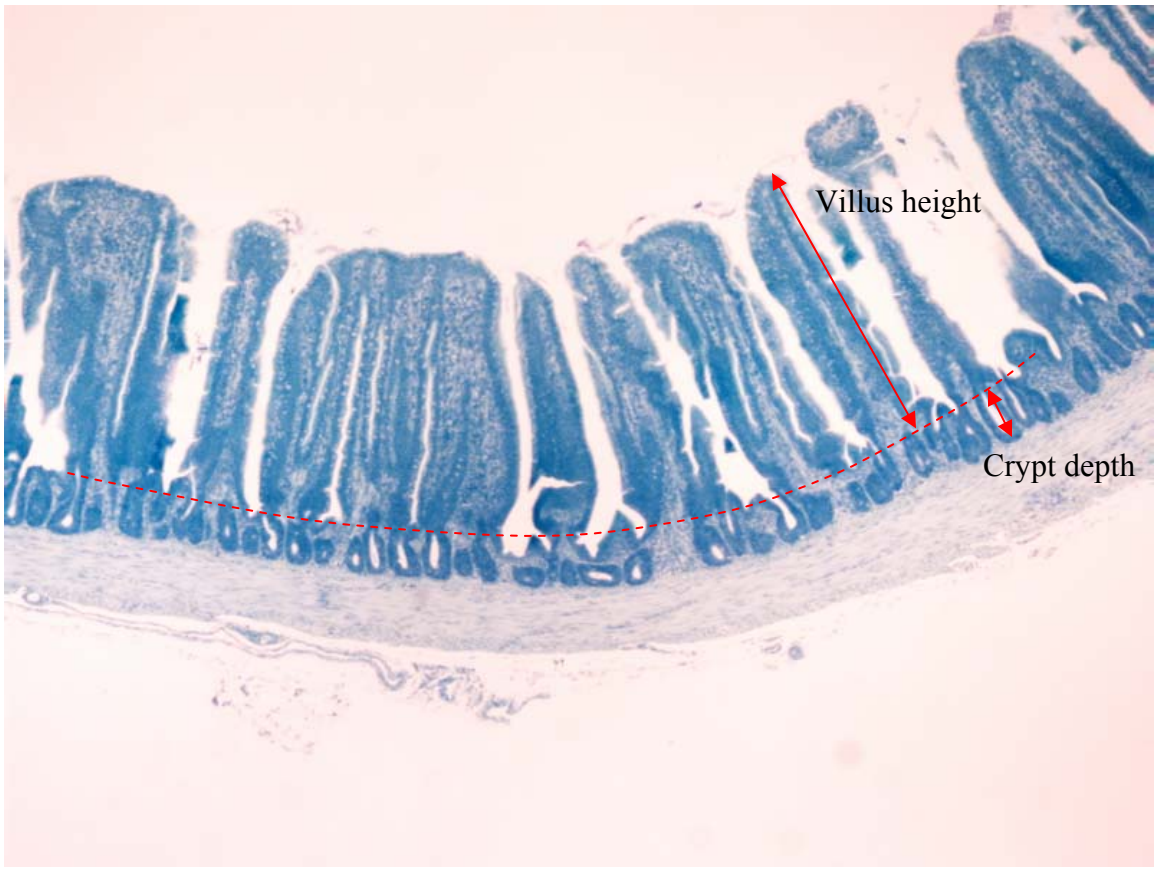

Figure 1. Measurement of ileum villus height and crypt depth with 40X magnification. The longer red arrow line indicates villus height and the shorter one represent crypt depth.

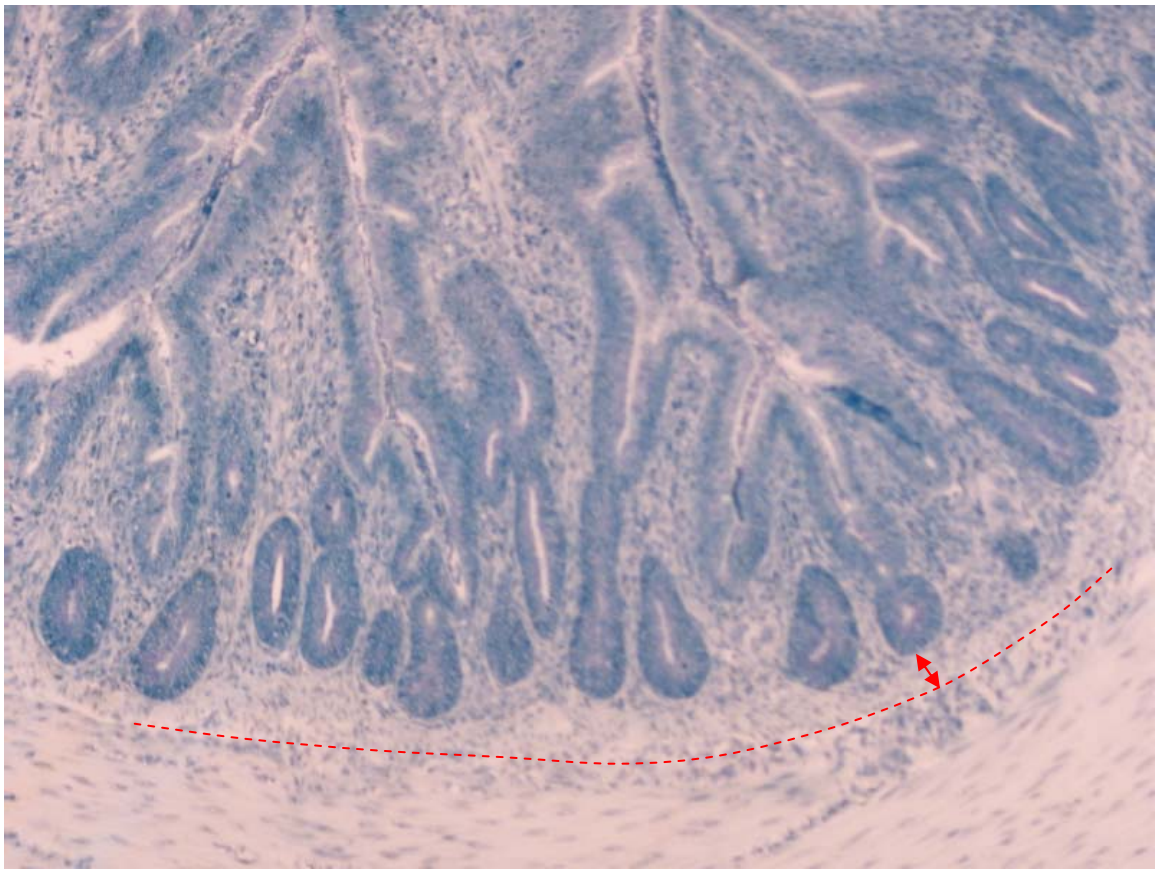

Figure 2. Measurement of cecal lamina propria with 100X magnification. The red arrow line represents cecal lamina propria.

Table 2. Effect of drug-free feeding programs on cumulative BW gain (g/bird/d)[1]

	Days of age			
Diet	14	28	35	49
Negative control[2]	27.7	42.7[b]	49.0[b]	54.1[b]
Positive control[3]	28.1	44.0[ab]	50.4[a]	55.8[a]
Program 1[4]	28.4	44.3[ab]	50.0[ab]	54.7[ab]
Program 2[5]	28.4	44.5[a]	50.3[a]	55.3[ab]
Pooled SEM (n = 13)	0.4	0.6	0.4	0.5
Main Effects	0.54	0.11	0.09	0.13

[1 a, b] Means within a column without common superscript are significantly different at $P < 0.05$

[2] Basal diet (no growth promoter or coccidiostat)

[3] Basal diet with lincomycin

[4] Basal diet with Bio-Mos® associated with All-Lac XCL®, Veg pro®, MTB-100®, and Acid Pak 4-Way®

[5] Basal diet with Bio-Mos® associated with All-Lac XCL®

Table 3. Effect of drug-free feeding programs on broiler period feed intake (g/bird/d)

	Days of age[1]			
Diet	0 to 14	15 to 28	29 to 35	36-49
Negative control[2]	38.0	101.2[b]	151.9	162.5
Positive control[3]	38.1	104.0[a]	151.3	165.5
Program 1[4]	38.4	104.0[a]	149.9	161.9
Program 2[5]	38.5	102.9[ab]	152.1	160.5
Pooled SEM (n = 13)	0.4	0.9	2.3	2.3
Main Effects	0.34	0.04	0.53	0.13

[1 a, b] Means within a column without common superscript are significantly different at $P < 0.05$

[2] Basal diet (no growth promoter or coccidiostat)

[3] Basal diet with lincomycin

[4] Basal diet with Bio-Mos® associated with All-Lac XCL®, Veg pro®, MTB-100®, and Acid Pak 4-Way®

[5] Basal diet with Bio-Mos® associated with All-Lac XCL®

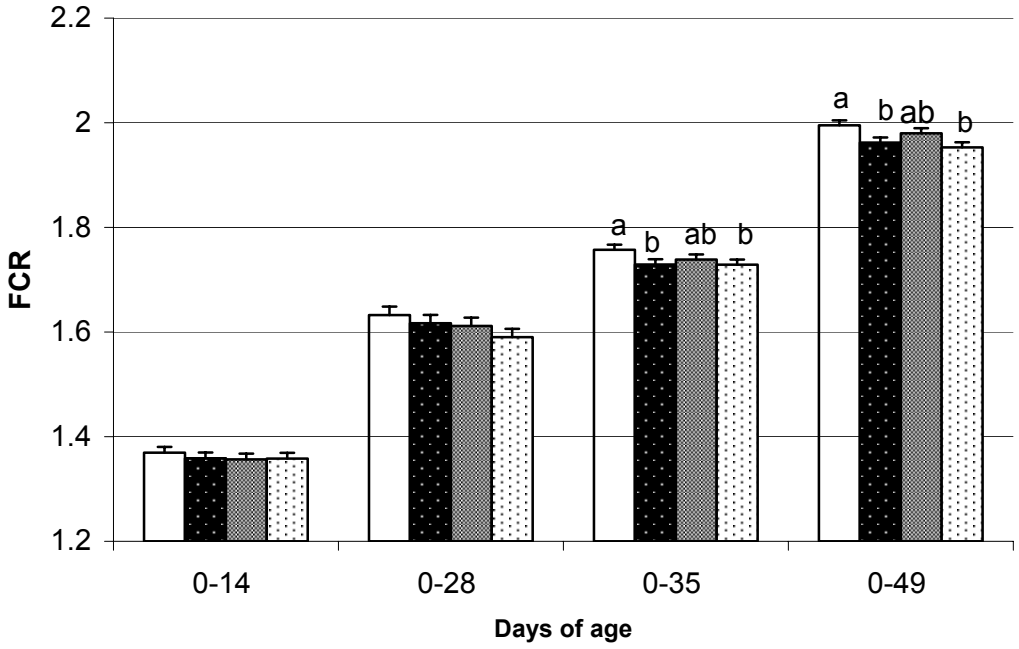

Figure 3. Effect of drug-free feeding programs on cumulative FCR of broilers. Negative control: only basal diet (□); positive control: basal diet + lincomycin (■); Program 1: basal diet + Bio-Mos® + All-Lac XCL®, Vegpro®, MTB-100® and Acid Pak 4-Way® (▨); Program 2: basal diet + Bio-Mos® + All-Lac XCL® (⊡). [a, b] Means columns with no common superscript differ significantly ($P < 0.05$) within a period of tiem, n = 13.

Table 4. Effect of drug-free feeding programs on ratio (%) of processed products to cold carcasses for female birds at d 42

Diet	Wing	Fatpad	Thigh	Drum	Tender	Fillet	All products
NC[1]	11.14	2.42	16.88	13.05	4.77	15.79	64.07
PC[2]	11.25	2.33	16.85	12.87	4.70	16.12	64.12
PG1[3]	11.18	2.30	16.97	12.96	4.82	15.60	63.85
PG2[4]	11.37	2.33	16.54	12.94	4.61	16.13	63.92
Pool SEM (n = 13)	0.14	0.12	0.24	0.12	0.10	0.35	0.47
Main effects	0.69	0.92	0.61	0.77	0.45	0.64	0.97

[1] Negative control or Basal diet (no growth promoter or coccidiostat)

[2] Positive control - Basal diet with lincomycin

[3] Program 1 - Basal diet with Bio-Mos® associated with All-Lac XCL®, Veg pro®, MTB-100®, and Acid Pak 4-Way®

[4] Program 2 - Basal diet with Bio-Mos® associated with All-Lac XCL®

Table 5. Effect of drug-free feeding programs on ratio (%) of processed products to cold carcasses for male birds at d 49

Diet	Wing	Fatpat	Thigh	Drum	Tender	Fillet	All products
NC[1]	10.99	2.38	17.51	13.52	4.25	15.21	63.86
PC[2]	10.87	2.42	16.92	13.45	4.28	15.41	63.37
PG1[3]	10.99	2.41	17.27	13.62	4.41	15.40	64.10
PG2[4]	10.95	2.38	17.28	13.43	4.36	15.40	63.81
Pool SEM (n = 13)	0.09	0.08	0.28	0.14	0.08	0.22	0.38
Main effects	0.76	0.99	0.54	0.79	0.46	0.90	0.60

[1] Negative control or Basal diet (no growth promoter or coccidiostat)

[2] Basal diet with lincomycin

[3] Basal diet with Bio-Mos® associated with All-Lac XCL®, Veg pro®, MTB-100®, and Acid Pak 4-Way®

[4] Basal diet with Bio-Mos® associated with All-Lac XCL®

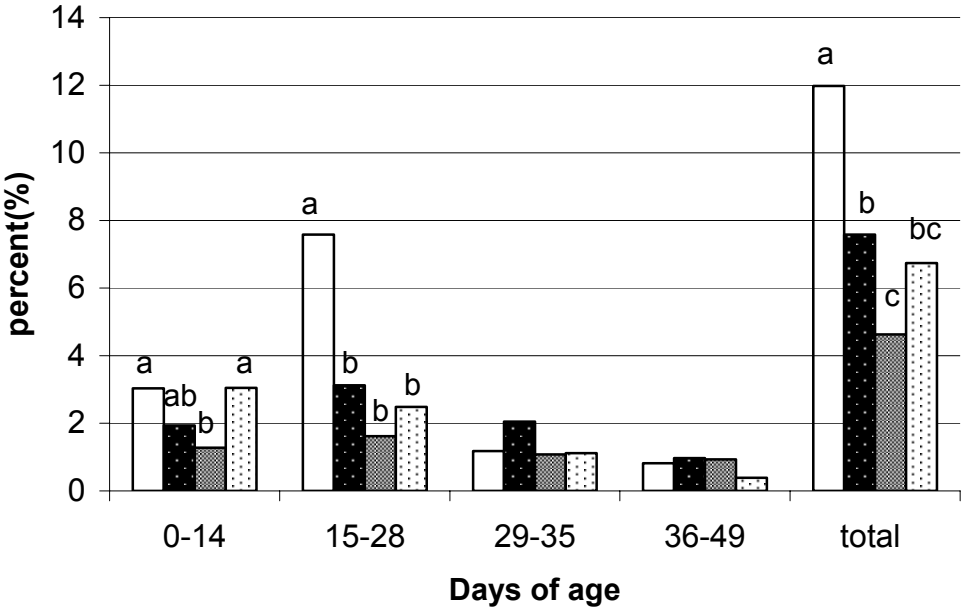

Figure 4. Effects of drug-free feeding programs on broiler mortality. Negative control: only basal diet (□); positive control: basal diet + lincomycin (■); Program 1: basal diet + Bio-Mos® + All-Lac XCL®, Vegpro®, MTB-100® and Acid Pak 4-Way® (▨); Program 2: basal diet + Bio-Mos® + All-Lac XCL® (⊡). [a, b] Means columns with no common superscript differ significantly ($P < 0.05$) within a period of time.

Table 6. Effects of drug-free feeding programs on the chick rearing density (birds/m^2)[1]

	Days of age			
Diet	14	28	35	49
Negative control[2]	13.9	11.6[a]	11.0[a]	9.6[a]
Positive control[3]	14.0	12.3[ab]	11.5[ab]	10.1[ab]
Program 1[4]	14.2	12.8[b]	12.0[b]	10.6[b]
Program 2[5]	13.9	12.3[ab]	11.6[ab]	10.2[ab]
Pooled SEM	0.14	0.23	0.23	0.23
Main Effects	0.39	0.02	0.02	0.04

[1 a, b] Means within a column without common superscript are significantly different at $P < 0.05$

[2] Basal diet (no growth promoter or coccidiostat)

[3] Basal diet with lincomycin

[4] Basal diet with Bio-Mos® associated with All-Lac XCL®, Veg pro®, MTB-100®, and Acid Pak 4-Way®

[5] Basal diet with Bio-Mos® associated with All-Lac XCL®

Table 7. Effects of drug-free feeding programs on lesion scores and BW gain from post-challenge with mixed *Eimeria*[1]

	Lesion scores [2]and BW gain of 6 days post-challenge (PC)			
Diet	*Duodenum*	*Ileum*	*Ceca*	*BW gain (d14-20 PC)*, kg
Negative control[3]	1.32 ± 0.100	1.26 ± 0.091	0.38 ± 0.069	0.23^{b} ± 0.011
Positive control[4]	1.34 ± 0.100	1.04 ± 0.091	0.19 ± 0.069	0.26^{ab} ± 0.011
Program 1[5]	1.51 ± 0.100	0.96 ± 0.091	0.21 ± 0.069	0.24^{ab} ± 0.011
Program 2[6]	1.38 ± 0.099	1.02 ± 0.090	0.25 ± 0.068	0.27^{a} ± 0.010

[1] [a,b] Values expressed as mean ± SEM, means within a column without common superscript are significantly different at $P < 0.05$, n = 38 for NC, PC, PG1, n = 39 for PG2

[2] 0 = no lesions, 1 = mild lesions, 2 = medium lesions, 3 = severe lesions, 4 = very severe lesions

[3] Basal diet (no growth promoter or coccidiostat)

[4] Basal diet with lincomycin

[5] Basal diet with Bio-Mos® associated with All-Lac XCL®, Veg pro®, MTB-100®, and Acid Pak 4-Way®

[6] Basal diet with Bio-Mos® associated with All-Lac XCL®

Table 8. Effects of drug-free feeding programs on broiler intestinal morphology [1]

	Days of age						Diets						
	14	28	35	49	SEM[8]	P	NC[2]	PC[3]	PG1[4]	PG2[5]	SEM	P	Interaction
Duodenum:													
Villus (mm)	1.99	2.33	2.42	2.09	0.05	< 0.001	2.18	2.16	2.19	2.28	0.46	0.26	0.02
Crypt (mm)	0.14	0.19	0.23	0.13	0.01	< 0.001	0.17	0.17	0.17	0.18	0.01	0.38	0.15
Villus/Crypt[7]	14.96	12.50	11.14	16.10	0.39	< 0.001	13.99	13.62	13.39	13.69	0.39	0.76	0.39
Ileum:													
Villus (mm)	0.700	1.05	1.07	1.07	0.02	< 0.001	0.94	0.96	0.98	1.01	0.02	0.14	0.001
Crypt (mm)	0.08	0.12	0.11	0.09	0.004	< 0.001	0.10	0.10	0.10	0.11	0.004	0.39	0.001
Villus/Crypt	8.79	8.81	10.11	12.02	0.30	< 0.001	9.48	10.09	10.28	9.89	0.30	0.27	0.014
Ceca:													
LP[7] (µm)	16.9	20.6	23.7	26.5		< 0.001	23.5	22.00	20.9	21.3		0.011	0.08
SEM	0.53	0.53	0.53	0.75			0.59	0.59	0.59	0.59			

[1] Significantly different when $P < 0.05$; [2] Negative control or Basal diet (no growth promoter or coccidiostat); [3] Basal diet with lincomycin; [4] Basal diet with Bio-Mos® associated with All-Lac XCL®, Veg pro®, MTB-100®, and Acid Pak 4-Way®; [5] Basal diet with Bio-Mos® associated with All-Lac XCL®; [6] Villus height/crypt depth ratio; [7] Lamina propria thickness; [8] For most SEM n = 10 except LP at d 49 n = 5

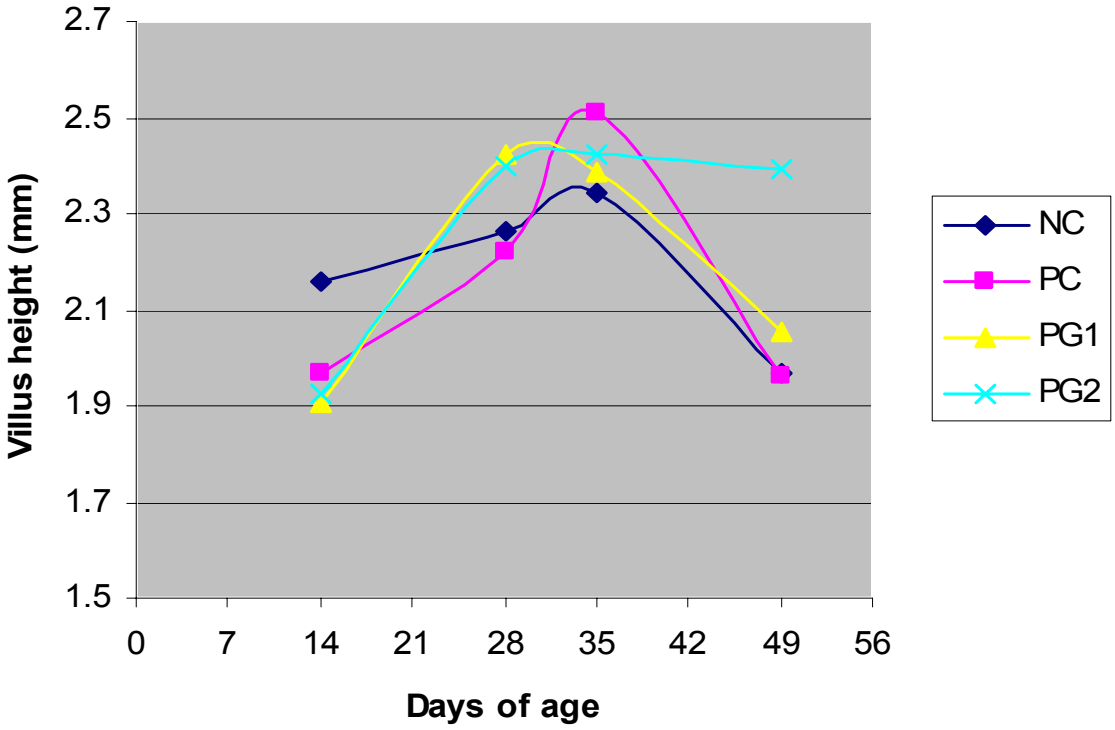

Figure 5. Interaction of age and diet on duodenum villus height. Negative control (NC), only basal diet, positive control (PC): basal diet + lincomycin; Program 1 PG1): basal diet + Bio-Mos® + All-Lac XCL®, Vegpro®, MTB-100® and Acid Pak 4-Way®; Program 2: basal diet + Bio-Mos® + All-Lac XCL®.

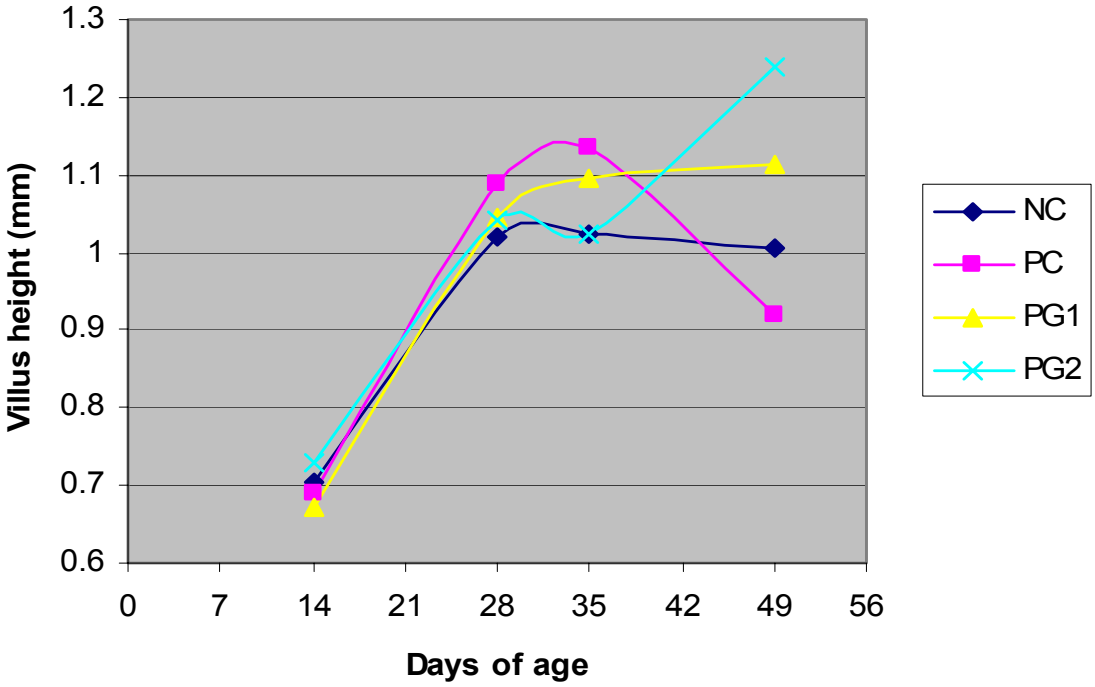

Figure 6. Interaction of age and diet on ileum villus height. Negative control (NC), only basal diet, positive control (PC): basal diet + lincomycin; Program 1 (PG1): basal diet + Bio-Mos® + All-Lac XCL®, Vegpro®, MTB-100® and Acid Pak 4-Way®; Program 2: basal diet + Bio-Mos® + All-Lac XCL®.

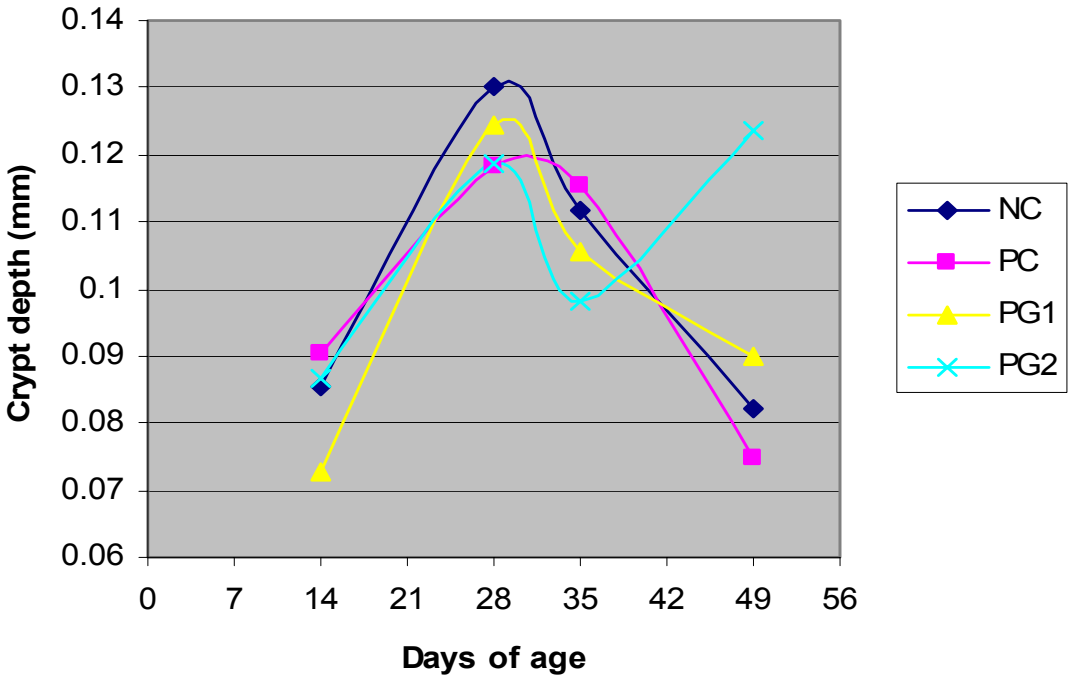

Figure 7. Interaction of age and diet on ileum crypt depth. Negative control (NC), only basal diet, positive control (PC): basal diet + lincomycin; Program 1 (PG1): basal diet + Bio-Mos® + All-Lac XCL®, Vegpro®, MTB-100® and Acid Pak 4-Way®; Program 2: basal diet + Bio-Mos® + All-Lac XCL®.

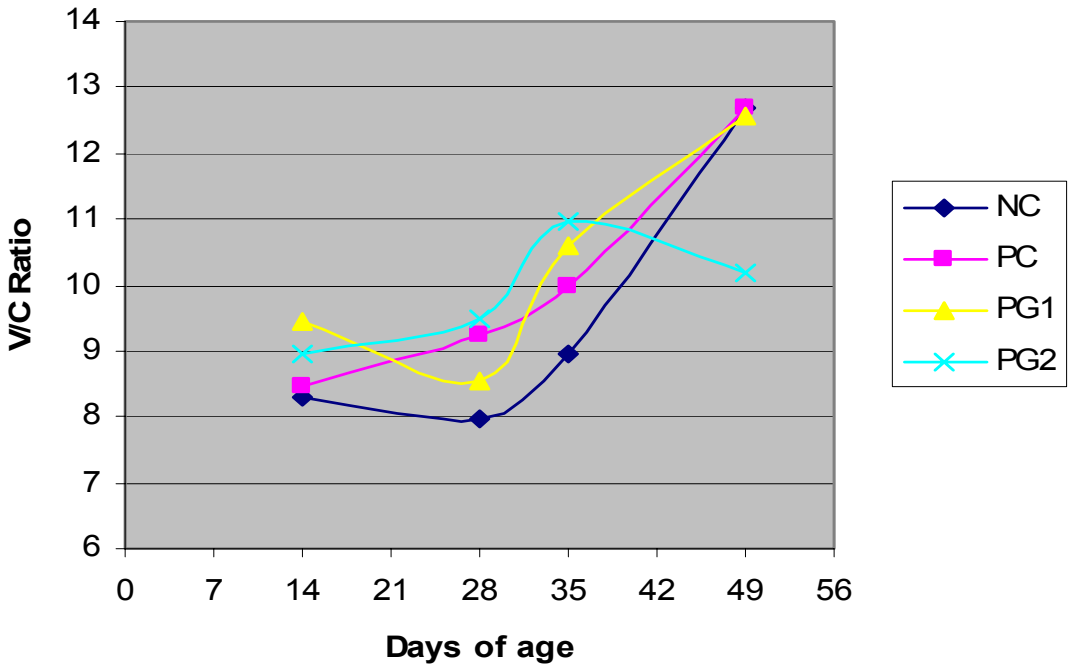

Figure 8. Interaction of age and diet on ratio of ileum villus height to crypt depth. Negative control (NC), only basal diet, positive control (PC): basal diet + lincomycin; Program 1 (PG1): basal diet + Bio-Mos® + All-Lac XCL®, Vegpro®, MTB-100® and Acid Pak 4-Way®; Program 2: basal diet + Bio-Mos® + All-Lac XCL®.

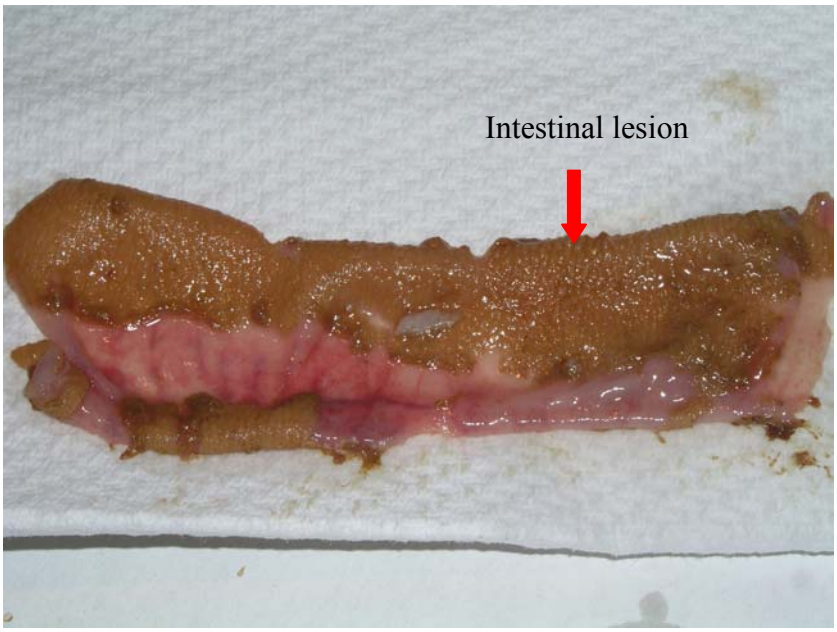

Figure 9. Intestinal lesion in morbid bird at d 23 due to infection by *Clostridium perfringens* (from diet 1 – Negative control).

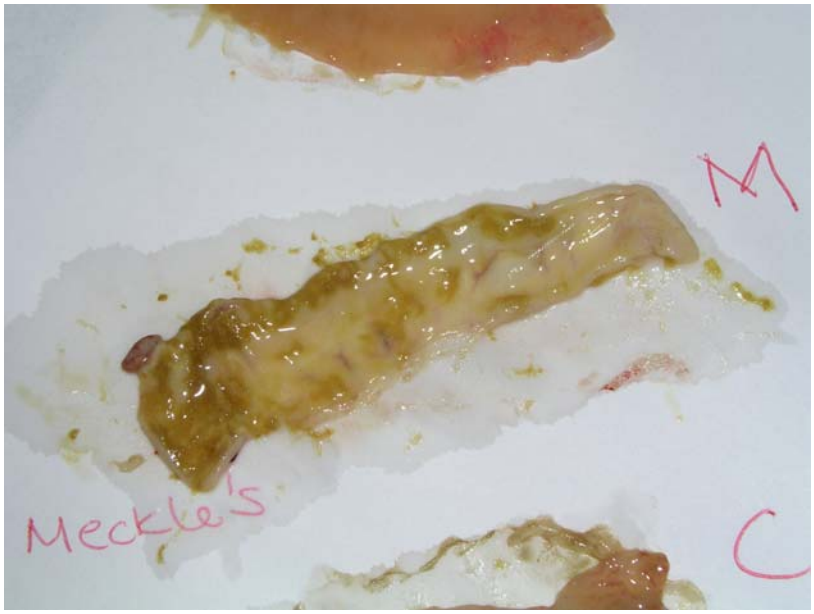

Figure 10. Mid gut at 35 days of age from a broiler consuming Program 2 feed.

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VITA

Xiaolun Sun, husband of Qun Luo and father of Zhihong Sun, was born on July 20, 1967, in Feixi, Anhui, China. Elementary, middle school, high school education were complete in Feixi, China. After his high school graduation in 1986, he attended South China Agricultural University in Guangzhou, a thousand miles away from his hometown. In March, 1990, he granted Bachelor of Agricultural Science ahead of a semester in Poultry Science in Guangzhou, Guandong, China.

In December, 1990, he was hired in Anhui feed mill as a technician and became supervisor after one year. In September, 1992, he entered Hefei ChaTai Co Ltd. after Anhui feed mill was merged as joint-ventured. He experienced from feed and chick salesman, service technician, supervisor, to assistant manage in live division in charge of operation of broiler integrity. In 2000, he went to Shanghai DCH JiangNangFeng Co Ltd in Shanghai as manager of live division in charge of operation broiler breed and broiler integrity. In 2001, he went to Hefei Zhengwan Live Co. Ltd in Hefei as manager of live division in charge of operation of broiler breed production. In 2002, he started to study at Virginia Tech. His major area is poultry nutrition and research focuses on poultry production substitution of antibiotics.

Xiaolun Sun is a member of Poultry Science Association. He received Pratt Fellowship for supporting his two-year master study.