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〈Review〉

Antimicrobials, Gut Microbiota and Immunity in Chickens

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ABSTRACT The use of antimicrobials will be soon removed due to an increase of occurrence of antibiotic-resistant bacteria or ionophore-resistant *Eimeria* species in poultry farms and consumers' preference on drug-free chicken meats or eggs. Although dietary antimicrobials contributed to the growth and health of the chickens, we do not fully understand their interrelationship among antimicrobials, gut microbiota, and host immunity in poultry. In this review, we explored the current understanding on the effects of antimicrobials on gut microbiota and immune systems of chickens. Based on the published literatures, it is clear that antibiotics and antibiotic ionophores, when used singly or in combination could influence gut microbiota. However, antimicrobial effect on gut microbiota varied depending on the samples (e.g., gut locations, digesta vs. mucosa) used and among the experiments. It was noted that the digesta vs. the mucosa is the preferred sample with the results of no change, increase, or decrease in gut microbiota community. In future, the mucosa-associated bacteria should be targeted as they are known to closely interact with the host immune system and pathogen control. Although limited, dietary antimicrobials are known to modulate humoral and cell-mediated immunities. Ironically, the evidence is increasing that dietary antimicrobials may play an important role in triggering enteric disease such as gangrenous dermatitis, a devastating disease in poultry industry. Future work should be done to unravel our understanding on the complex interaction of host-pathogen-microbiota-antimicrobials in poultry.

(Key words : gut microbiota, immunity, antimicrobials, chicken)

INTRODUCTION

The human population is projected to grow to 9~10 billion by the year 2050 (Godfray et al., 2010). As a consequence of the population explosion, food animal production would confront a new array of challenges. Among these are global food security, climate change, emerging infectious diseases, regulatory ban on use of antimicrobials, high-density production conditions, and waste management (Grasty, 1999; Turnpenny et al., 2001; Bohannon, 2004). Approximately 71 million tons of poultry meats were produced worldwide in 2009 (USDA-FAS, 2009). In order to assure continuity in the supply of poultry food products, effective control measures against infectious diseases in the framework of environmental change are critical (Dekich, 1998). In the United States, *Clostridium*- related diseases, such as gangrenous dermatitis (GD) and necrotic enteritis (NE), and coccidiosis are among the most important infectious diseases in chickens and turkeys (Shane, 2004a, b; Smith and Helm, 2008).

Traditionally, antimicrobials, a combination of antibiotics and anticoccidial drugs are commonly practiced in food animal production. Recently, vaccination against coccidiosis plus antibiotics in diets has been increasingly implemented. Overall, these vaccination and medication practices are used as preventive measures on either pathogenic *Eimeria* spp. or growth depressing pathogenic and non-pathogenic bacteria, or both. In-feed antimicrobials are well known to affect gut microbiota due to their apparent *in vitro* antimicrobial properties. For example, Lu et al. (2006) observed that *Lactobacillus acidophilus* was the most abundant species in birds fed on a nonmedicated plain control diet, whereas *L. crispatus* represented the dominant *Lactobacillus* in birds fed a diet containing monensin at the concentration of 90 ppm using terminal restriction fragment length polymorphism (T-RFLP) analysis. Additionally, a higher relative abundance of gram-negative bacteria (e.g., *Proteobacteria* and *Bacteroides*) was observed in the monensin-fed chickens and especially Clostridia, i.e., *Clostridium irregularis* and *C. lituseburense* were particularly

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dominant. In their following study (Lu et al., 2008), gut microbiota of broiler chickens fed diet containing antibiotic growth promoters (bacitracin methylene disalicylate and virginiamycin; AGPs) was completely different from those seen in birds fed a non-medicated control diet, indicating that low levels of in-feed antimicrobials can substantially influence gut microbiota in broiler chickens.

In addition, the evidence is increasing that gut microbiota plays an important role in health, immunity and disease prevention (Dibner et al., 2008; Sekirov et al., 2008). The published data (Turnbaugh et al., 2006; Caesar et al., 2010; Wlodarska and Finlay, 2010) supported the view that altered microbiota is linked to obesity, inflammatory bowel disease (IBD), atherosclerosis and cancer. It has been proposed that there is close interaction between altered microbiota by in-feed antimicrobials and host susceptibility to pathogens (e.g., gangrenous dermatitis) in broiler chickens (Ritter 2006; Ritter et al., 2010). In this sense, commonly used antimicrobials in poultry production will have definite impact on intestinal microbiota and host immunity. Recent developments on microbiota analysis techniques broaden our understanding on interrelation between host immunity and microbiota, and their association with disease. Especially, there is increasing evidence that low-level inclusion of antimicrobials, e.g., antibiotics, antibiotic-like coccidiostats and chemicals may render the host susceptible to the enteric disease such as *Clostridium* spp. The present review will discuss the current understanding on the effects of antimicrobials on gut microbiota and immune systems of chickens.

GUT MICROBIOTA AND IMMUNE SYSTEM OF CHICKENS

At hatch, the alimentary tract and immune system of chicks is less well developed compared with mature birds (Lowenthal et al., 1994; Koenen et al., 2002). Thus, broiler chicks at early ages are very susceptible to pathogenic bacteria, which can otherwise be protected by a healthy gut microbiota (Nurmi and Rantala, 1973). It is well-known that a close relationship exists between the gastrointestinal tract (GIT) microflora and development and/or maintenance of a functional intestinal immune system (Salminen et al., 1998; Gabriel et al., 2006). For example, germ-free mammals have a higher susceptibility to intestinal infections (O'Hara and Shanahan, 2006) and are unable to mount an effective antibody response until re-establishment of their gut microflora (Rhee et al., 2004). Similarly, germ-free chicks failed to trigger intestinal immune response to heat inactivated *Escherichia coli* antigen. The immunological maturity of the germ- free chicks was delayed due to the lack of the stimulation by the gut microbiota on antibody producing B-cells (Parry et al., 1977).

At early ages, the gut microbiota of chicks is not well characterized compared with the adult birds. The adult GIT microflora is composed of 10^7 to 10^{11} bacteria per gram of gut contents (Apajalahti et al., 2004). From molecular studies, at least 640 species representing 140 genera are present in the intestinal cecum. Of these, 10% were identified as previously known bacteria based on 16S rRNA gene sequences, while the remaining sequences belonged to unidentified organisms (Apajalahti et al., 2004). In the modern intensive production system, multiple grow-out broiler flocks are commonly reared on a single batch of litter where day-old chicks are placed directly on litter (Volkova et al., 2009). Thus, litter types where the birds are raised determine their gut microbiota, developing immunity and birds' performance. According to Torok et al. (2009), when birds are raised on either various fresh litter materials (e.g., rice hull, softwood sawdust, pine shavings, hardwood sawdust, shredded paper, and chopped straw) or reused litter, significant litter effects on growth performance and cecal microbiota composition in broiler chickens were observed. Broiler chicks raised on the reused litter gained the least whereas those raised on the chopped straw the heaviest. Cecal microbiota profiles of chickens raised on the reused litter were significantly different from those raised on the fresh litters even though cecal microbiota was also differed depending on the fresh litters. Recently, Cressman et al. (2010) identified that *Lactobacillus* spp. dominated the ileal mucosal microbiota of fresh-litter chicks, while a group of bacteria unclassified within *Clostridiales* dominated the ileal mucosal microbiota in the reused-litter chicks. It is clear that litter materials where the birds are raised on are common source to significantly affect gut microbiota of the chicken.

Following hatching, chicken adaptive immunity requires at least three weeks for complete maturation and development

(Beal et al., 2006). In newly hatched chickens, some degree of immune protection is established by maternal antibodies, primarily IgY transmitted from hen yolk. However, antibodies are mainly effective against extracellular pathogens and generally do not protect against intracellular microbes, such as *Eimeria* and *Salmonella*. At hatch, birds also have natural defense mechanisms that function from hatch and quickly destroy any microbes. A group of effectors of this innate immune system are small positively charged molecules called antimicrobial peptides that are an evolutionary conserved component of innate immunity and found in plants, insects, mammals and birds (Wellman-Labadie et al., 2007). They are synthesized constitutively or induced, and display broad spectrum activity against bacteria, fungi and enveloped viruses. Additionally, $CD4^+$ and $CD8^+$ lymphocytes, the primary effectors of cell-mediated immunity, possess relatively naive phenotypes in germ-free animals, but following intestinal colonization, they acquire more typical activated phenotypes (Cebra, 1999).

EFFECT OF ANTIMICROBIALS ON GUT MICROBIOTA COMMUNITY

Recent development on molecular analytical tools on microbiota greatly enhanced our understanding on gut microbiota (Zoetendal et al., 2004). Although cultivation methods are still in use, culture-independent 16S rRNA gene-based fingerprinting techniques, i.e., denaturing gradient gel electrophoresis (DGGE) and terminal-restriction fragment length polymorphism (T-RFLP), or quantification of 16S rRNA using real-time PCR techniques have been developed to study microbiota community of intestine in broiler chickens (Oviedo-Rondón, 2009).

Among the antimicrobials, AGPs such as bacitracin, virginiamycin, and anticoccidial agents such as synthetic chemicals or antibiotic ionophores are frequently used to promote growth and/or prevent coccidiosis. Antibacterial effect of antibiotics or anticoccidials on pathogenic *C. perfringens* or soil bacteria has been reported (Watkins et al., 1997; Martel et al., 2004; Hansen et al., 2009). It is thus not surprising to expect the effect of in-feed antimicrobials on gut microbiota in chickens.

Lu et al. (2008) investigated the effect of AGP or ionophore monensin on the microbiota community using *Hae*III-digested T-RFLP of the 16S rRNA gene fragments in the ileal digesta of broiler chickens, and found significant differences in microbiota community of birds fed medicated diets compared with the non-medicated control birds. In addition, the number of T-RFLP phylotypes for the control, AGP- and monensin- treated groups were 5, 3, and 4, respectively. The bacterial community of monensin-fed chickens was rich in *Clostridia*, but low in *Lactobacillus* compared with those fed the control diet, whereas AGP-fed chicks had intermediate abundance of *Lactobacillus* and *Clostridia*. Either AGP- or ionophore-mediated shift in microbial communities has been well established in broiler chickens (Knarreborg et al., 2002; Pedroso et al., 2006; Hume et al., 2006; Johansen et al., 2007; Chee et al., 2010). Interestingly, Hume et al. (2006) demonstrated that challenge with *Eimeria acervulina*, *E. tenella*, and *E. maxima* also altered gut microbial community of broiler chickens, suggesting the close interaction of pathogen-microbiota-host immunity.

On the contrary, no significant effect of antimicrobials on gut microbiota was also reported. For example, Diarra et al. (2007) failed to observe any clear effect on the number of *C. perfringens* or *Enterococcus* spp. in cecal contents of broiler chickens fed diets containing bambermycin, penicillin, salinomycin, or bacitracin. Recently, Geier et al. (2009a) assessed ileal or cecal microbial communities of broiler chickens fed diets with or without AGP zinc bacitracin by *Msp*I-digested-T-RFLP. Feeding AGPs did not influence the microbial populations of the intestines. At this stage, the effect of antimicrobials on gut microbiota is clear, but the reported effects have been somewhat inconsistent. According to a series of studies by Geier et al. (2009a, b), they found antibiotic-mediated shifts in gut microbiota in one study, but not in the following study, although all the experimental settings between two experiments, i.e., birds, diet, type and concentration of antibiotics used, and management were identical. Authors postulated possible flock variation in susceptibility of the intestinal microbiota communities to antibiotics. Thus, a clear explanation on these conflicting results is not readily available. In any event, these conflicting results indicate the complexity of gut microbiota and warrant further researches into the gut microbiota.

In general, colonization of microbiota at the alimentary

tract of the chickens starts immediately after hatch. It has been reported that the composition of the ileal microbiota at early ages is transient and stabilized as the birds matured (Lu et al., 2003). Interestingly, the dominant *Lactobacillus* species in ileal digesta were shifted from *L. delbrueckii* at day 3 to *L. acidophilus* at days 7~21, *L. crispatus* at day 28 and *L. crispatus* and *L. salivarius* at day 49. Similarly, Amit-Romach et al. (2004) reported the age-dependent shift of microbiota in broiler chickens using the recent 16S rRNA-based molecular technique targeting 6 bacterial species i.e., *Lactobacillus*, *Bifidobacterium*, *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Clostridium*. It seems that newly hatched chicks vs. adult chickens have gut microbiota that is susceptible to dietary antimicrobials. Indeed, Gong et al. (2008) reported that the effect of dietary antibiotics on microbiota community was apparent when the chickens were young, while this effect became weaker when the birds were aged. Similarly, Knarreborg et al. (2002) observed the age-dependent shift in gut microbiota by in-feed antimicrobials. The number of ileal *Lactobacilli* was unchanged by antibiotic supplementation at days 7 and 35, but lowered at days 14 and 21 although antibiotic supplementation consistently lowered *C. perfringens* counts at all ages. Baurhoo et al. (2009) reported that feeding antibiotics lowered *Bifidobacteria* concentration at days 14 and 24, but not at 34 while it failed to affect *Lactobacilli* concentration at all ages (days 14, 24 and 34). On the contrary, Chee et al. (2010) reported that the number of *Lactobacilli* and *C. perfringens* in zinc bacitracin-fed chickens was not changed at day 7, but significantly reduced at day 21 compared with the non-medicated control birds. No antibiotic effect on cecal microflora at all ages (days 14, 28 and 42) was reported (Mountzouris et al., 2010). In line with the result by Mountzouris et al. (2010), Fairchild et al. (2005) studied the cecal microbiota community using T-RFLP analysis on 16S rRNA gene amplicons in 4-week-old broiler chickens that had been treated with oxytetracycline for 5 days. It was found that oral administration exhibited little effect on the cecal microbiota community at 2 days and 2 weeks after oxytetracycline treatment.

The bacteria can colonize both digesta in the lumen and the mucosa epithelium with its distinct microbiota. Gong et al. (2002) analyzed microbiota community on the mucosa and digesta of the ileum by T-RFLP with restriction enzymes such as *Alu*I, *Hha*I, and *Msp*I and found the heterogeneous bacterial population in the mucosa and digesta in the ileum. In many studies, the digesta vs. the mucosa is the preferred sample with the results of no change, increase, or decrease in gut microbiota community by dietary antimicrobials. The influence of dietary antimicrobials on the mucosa-associated bacteria is relatively limited. Recently, Chee et al. (2010) have reported that antibiotic treatment affected the number of lactobacilli in digesta of the ileum, but not on the mucosa. On the contrary, dietary antibiotics are known to alter the mucosa-associated bacteria by the DGGE analysis (Pedroso et al., 2006). Wise and Siragusa (2007) sampled the mixed ileal luminal and mucosal materials from chickens raised under drug-free or conventional antibiotic regimes and analyzed them using quantitative real time PCR with group-specific 16s rRNA primer sets. They noticed that total domain bacteria 16S rRNA on DNA isolated from the mixed digesta/mucosa-associated samples was not different in birds raised with or without AGP at days 7, 14 and 21. However, they found that populations of *Enterobacteriaceae* and *Bifidobacterium* spp. were significantly decreased in drug-fed chickens compared with those in drug-free chickens at days 7 and 21. On the contrary, the most abundant classes in ileal community, *Lactobacillus* group was not altered at all ages by medication. Given that the mucosa-associated microbiota has been known to play a role in pathogen control and immune modulation (Gong et al., 2002), future studies should be targeting on analyzing the mucosa-associated bacteria in relation to the responses to dietary antimicrobials.

EFFECT OF ANTIMICROBIALS ON IMMUNITY

Contrary to the many published reports on gut microbiota community, the effect of antimicrobials on developing immunity is not well studied. The fact that antimicrobials are not absorbed in the intestine would explain their paucity of studies to investigate the role of antimicrobials on immune organs. However, clinical studies with mammals have proved that gut microbiota play an important role in the development of the immunity (Hrncir et al., 2008; Maslowski and Mackay,

2011). This topic has been recently widely discussed at the symposium of Immunology, Nutrition, Genomics, and Gut Microbiota in the 2010 annual meeting of Animal Society of Animal Science at Denver in USA. The symposium presented recent developments in various fields of science to understand the latest progress in clinical medicine and to enhance our ability to apply integrated approaches using immunology and genomics to control pathogens in poultry as an attempt to reduce the use of antibiotics in poultry production (Lillehoj et al., 2010).

Although limited, the immunomodulatory effect of antimicrobials on immunity, especially humoral response has been reported. Brisbin et al. (2008) reported that adding virginiamycin into broilers' diet at the levels of 11 or 22 ppm enhanced antibody responses, at least systemically, to soluble antigens (keyhole limpet hemocyanin; KLH) in broiler chickens. Systemic IgG, and to a lesser extent IgM, antibody responses to KLH were greater in antibiotic-fed chicks compared with birds fed on antibiotic-free diet. Authors postulated that enhanced humoral immune response was associated with the use of antibiotics. On the other hand, no clear effect of antimicrobials on antibody concentration was reported. Mountzouris et al. (2010) measured chicken-specific plasma immunoglobulins (IgA, IgM, and IgG) in broiler chicken fed diets with or without antibiotic avilamycin (2.5 mg/kg of diet) and found no difference in the concentration of IgA, IgM, IgG between the antibiotic-fed birds and birds fed with antibiotic-free diet at 14 and 42 day of age.

Interestingly, several researchers investigated the effects of antimicrobials on antibody titers following vaccination against Newcastle disease (ND). Murwani and Murtini (2009) found that chlortetracycline-treated broiler chicks produced high antibody titers against ND when measured at day 18, but not at days 21 and 25, compared with the non-medicated control group. This finding indicates that chlortetracycline is beneficial in augmenting humoral response to ND vaccination at early age of chicks, but once immunity developed especially when birds aged, its effect on humoral immunity is less likely. In contrast, suppressive effect of antimicrobials on antibody titers against ND was also reported. Khalifeh et al. (2009) reported that antibiotics, especially florfenicol administered during ND vaccination in laying hens reduced humoral immune response measured by ELISA IgG concentration compared with the ND-vaccinated, non-medicated control group, indicating the down-regulating effect of antibiotics on humoral immunity. However, all ND-vaccinated groups had above the protective titers. On the other hand, no clear effect on the vaccine-induced antibody titers was reported as well. Kwon et al. (2008) measured serum antibody titers against ND and infectious bronchitis virus (IBV) in broiler chickens fed diets containing with or without chlortetracycline. All chicks were intramuscularly vaccinated against both ND and IBV at week 2 and boosted at week 4. At one week post the second immunization, sera were obtained to measure antibody titers by hemagglutination inhibition (HI) test. Authors did not find any difference in antibody titers against ND or IBV in broiler chickens fed diets with or without antibiotics. Nonetheless, all HI titers observed in that study seemed to reach to the protective titers.

Munir et al. (2007) investigated whether vaccination in combination with antimicrobials (salinomycin and monensin) would protect broiler chickens following experimental challenge with ND and Angara Disease (AD) virus. Measurements included antibody titers post vaccination and post challenge, body to lymphoid organ weight ratios, and survival rates as protective indices. It was shown that antimicrobial treatment increased antibody titers against ND and AD in broiler chickens, but did not affect the body to lymphoid organ weight ratios. No mortality occurred in the vaccinated broiler chickens fed diet containing salinomycin or monensin following the challenge with AD or ND. Thus, Munir et al. (2007) concluded that dietary antimicrobials could control coccidiosis and augment protective immunity of vaccines against ND and AD, supporting their intended use for the growth and health promoters in poultry industry.

Following the observation that salinomycin enhanced humoral response of ND-vaccinated broiler chickens following challenge with ND (Munir et al., 2007), Munir et al. (2009) further studied whether dietary salinomycin would effect on protective cell-mediated immunity in broiler chickens vaccinated with ND and hydropericardium syndrome (HPS) following challenge with virulent ND virus and HPS virus. Especially, skin contact sensitivity and phytohemagglutinin-stimulated lymphocyte proliferation assay were used to determine the

effect of salinomycin on the cell-mediated response in broiler chickens vaccinated with HPS and ND. For skin contact sensitivity, birds from salinomycin or untreated group were sensitized with 2,4-dinitrochlorobenzene (DNCB) at 28 d of age and were challenged DNCB (1.5 mg/mL) after 14 days, and the contact sensitivity was assessed at zero (defined as immediately after DNCB challenge), 24, 48, 72, and 96 h post-DNCB challenge. It was found that the salinomycin-fed vs. non-medicated control birds had significantly higher proliferation at days 21, 28, 35, and 42, but not at day 49 although the mean skin thickness was not different at all times. This study indicates the beneficial effect of salinomycin on cell-mediated immunity of broiler chickens vaccinated and challenged with HPS and ND.

Similarly, the enhanced cell-mediated immunity by antibiotic treatment was reported with laying hens. Khalifeh et al. (2009) reported the antibiotic effect on cell-mediated immunity response by measuring chicken interferon gamma (IFNγ) produced in splenocytes stimulated with concanavalin A (Con A). The birds were either non-vaccinated/antibiotic treated or ND vaccinated/antibiotic treated and measured IFN-γ in Con-A-stimulated splenocytes using commercial ELISA. When the birds were treated with antibiotics without vaccination regime, no stimulatory effect of antibiotics on IFNγ production were observed. However, with ND vaccination regime, IFN-γ production in Con-A-stimulated splenocytes was significantly elevated in antibiotic-treated chickens compared with the non-medicated control group. These two studies (Munir et al., 2007; Khalifeh et al., 2009) support the view that antimicrobials can enhance cell-mediated immunity in broiler chickens.

Intraepithelial lymphocytes (IELs) constitute the primary immune effector cells in the gut and play a critical role in eliciting protective immunity to enteric pathogens (Lillehoj et al., 2004; Lillehoj and Trout, 1996). Stimulation or suppression of specific IEL subsets by in-feed antimicrobials will likely contribute to increased or decreased host resistance to enteric pathogens that would cause clinical disease. Given the earlier observations that gut microbiota can influence the development of immune system, Arias and Koutsos (2006) investigated whether antibiotics would affect lymphocyte populations in broiler chickens raised in re-used or fresh litters. Lymphocytes in lamina propria and IELs were counted on hematoxylin and eosin stained sections from duodenum, jejunum and ileum. Unfortunately, they did not use the sensitive flow cytometer assay targeting specific lymphocyte population. Duodenal, but not jejunal and ileal, lamina propria lymphocytes were significantly low in antibiotic-fed chicks compared with the non-medicated control group when they were raised on the reused litter. For IELs, antibiotic treatment did not affect IEL numbers when the birds were raised on the reused litter. However, antibiotics increased duodenal IELs and decreased ileal IELs in broiler chickens raised on the fresh litter. This study (Arias and Koutsos, 2006) indicates that antibiotics can influence intestinal lymphocyte population, but the effect was different in high or low microbial environment, indicating complex interaction between antibiotics, gut microbiota and development of gut immunity.

At this stage, the study on the effect of antimicrobials on cytokine expression patterns is limited although dietary antimicrobials would modulate the expression patterns of various cytokines via alteration in gut microbiota. Especially, Chichlowski et al. (2007) investigated the immune response (e.g., cytokines [interleukin (IL)1β, IL6, IL10]) in ileum of broiler chickens fed diet with or without antibiotic ionophore salinomycin (50 ppm). No clear differences in cytokine expression patterns between the salinomycin-fed chicks and those fed non-medicated diet were seen in this experiment. However, *in vitro* studies with human monocytes or polymorphonuclear neutrophils, antimicrobials have been known to stimulate or inhibit cytokine mRNA levels (Morikawa et al., 1996; Reato et al., 2004). Recently, Takahashi et al. (2011) monitored cytokine expression levels in small and large intestines, and Bursa of the cage-raised broiler chicks that provided with or without salinomycin and enramycin. IFNγ was highly expressed by antimicrobial treatment in small intestine at days 5 and 8, but not at day 15. In contrast to small intestine, transcript levels of IFNγ at large intestine was not affected at days 5 and 8, but significantly repressed in antimicrobial-fed group compared with the non-medicated control group. In Bursa, no treatment effect on levels of transcripts encoding cytokines was observed. Tentatively, it seems that the effect of antimicrobials on cytokine expression differs depending on type of antimicrobials, tissues assayed and age of birds. Further studies are warranted to see whether various antimicrobials

currently used in poultry industry would indeed influence or modulate cytokine production at the expression levels in chickens.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Dietary antimicrobials influence gut microbial community, and the development and regulation of the host immune systems. Therefore, any non-drug alternative disease intervention strategies (e.g., nutrients, environment, antimicrobials, and feed additives, etc.,) that may alter gut microbiota could affect the protective immune responses to enteric pathogens including *Eimeria* spp., *Salmonella* spp. and *Clostridium* spp. In commercial poultry settings where the use of certain ionophoric antimicrobials in diets led to the disturbance of gut microbiota, increased outbreaks of clostridial infections such as GD have been reported. The interaction of gut microbiota and host immunity is closely linked to the outcome of host-pathogen interaction and further studies to investigate the role of dietary antimicrobials on the complex interaction of host-pathogen-microbiota-antimicrobials in poultry will be necessary. Especially, positive or negative correlation between the presence of certain gut microbiota and host immunity in a quantitative and qualitative way is hardly known that needs to be addressed. Although the immunopotentiating effect of the selected antimicrobials on vaccination has been reported, most studies were done at the laboratory settings and the results may not have much relevance to the field situations. The commercial settings, e.g., the frequent reuse of litters and use of the antimicrobials may raise somewhat different results compared with those seen in the laboratory settings.

Although not discussed in this review, the use of AGPs and anticoccidials in the future poultry industry will be phased out in many countries due to emergence of drug-resistance strains and consumers' preference for drug-free meats. Since the EU's ban on AGPs in 2006, this trend will expect to spread to the rest of the world. For example, Korean Authority decided to remove AGPs from animal feeds, and only 9 anticoccidials (salinomycin, monensin, lasalocid, narasin, maduramicin, semduramicin, clopidol, fenbendazole, and diclazuril) will be used. This Act (Control of Livestock and Fish Feed Act) will be effective from July 2011. In USA, AGPs and anticoccidials are widely used in poultry feeds. However, U.S. Food and Drug Administration has been trying to persuade pharmaceutical companies to voluntarily stop providing antibiotics to promote livestock growth. In any event, without AGPs, it can be readily expected that NE, the most important infectious diseases in the current poultry production system globally with estimated annual economic loss of more than $ 2 billion, will be re-emerged as a significant problem. Thus, it is certain that we will continue to face the challenges in identifying alternatives that can effectively replace AGPs. Although the searching for the alternatives to AGPs is not the scope of the current review, the integrated research system involving diverse scientists in the field of immunology, genomics, molecular biology, proteomics, parasitology, and nutrition should be applied to gain better knowledge in developing novel alternatives to AGPs.

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