

Effects of Various Field Coccidiosis Control Programs on Host Innate and Adaptive Immunity in Commercial Broiler Chickens

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ABSTRACT Coccidiosis control programs such as vaccines or in-feed anticoccidials are commonly practiced in the poultry industry to improve growth performance and health of commercial broiler chickens. In this study, we assessed the effects of various coccidiosis control programs (e.g., *in ovo* vaccination, synthetic chemicals, and antibiotic ionophores) on immune status of broiler chickens vaccinated against infectious bronchitis virus and Newcastle disease virus (ND) and raised on an *Eimeria*-contaminated used litter. In general, the levels of α -1-acid glycoprotein, an acute phase protein, were altered by the treatments when measured at 34 days of age. Splenocyte subpopulations and serum antibody titers against ND were altered by various coccidiosis control programs. *In-ovo*-vaccinated chickens exhibited highest mitogenic response when their spleen cells were stimulated with concanavalin A (Con A) at 7 days of age. It is clear from this study that the type of coccidiosis control program influenced various aspects of innate and adaptive immune parameters of broiler chickens. Further studies will be necessary to delineate the underlying relationship between the type of coccidiosis control program and host immune system and to understand the role of other external environmental factors such as gut microbiota on host-pathogen interaction in various disease control programs.

(Key words : infectious bronchitis virus, Newcastle disease virus, coccidiosis control program, immune status, broiler chickens)

INTRODUCTION

Avian coccidiosis is major parasitic disease of high economic concern in the poultry industry worldwide and is caused by at least seven distinct species of *Eimeria* apicomplexan protozoa that infect the various areas of intestinal mucosa. The economic loss for avian coccidiosis is estimated to be more than \$3 billion worldwide (Williams, 1999) and this cost includes in-feed medication, mortality, impaired growth rate, inefficient feed utilization and reduction in egg production. According to the previous reports, the *Eimeria* spp. prevalence has been estimated in more than 50% of the flock level around the world (Williams et al., 1996; Al-Natour et al., 2002; Haug et al., 2008; Nematollahi et al., 2009; Sun et al., 2009; Lee et al., 2010a). In general, the majority of *Eimeria*-infected chickens are afflicted by multiple species of *Eimeria* with the most prevalent species including *E. tenella*, *E. praecox*, *E. acervulina*, and *E. maxima*.

It has been a common practice to implement vaccination against coccidiosis or to add anti-coccidial feed additives

either in the form of synthetic chemicals or antibiotic ionophores to control coccidiosis by the poultry industry (Williams, 2005; Lee et al., 2009). Although coccidiosis control programs have been widely used in poultry production worldwide, their impact on the developing host immune system and on host-pathogen interaction has not been studied so far. Because there are scientific data that show the influence of gut microbiota, drugs, and dietary feed-additives (e.g., probiotics and phytonutrients) on developing host immune system (Lee et al., 2010b; Yin et al., 2010; Crhanova et al., 2011; Lee and Lillehoj, 2011; Lee et al., 2011a), it is important to gain better understanding of how these factors affect host ability to elicit immune response to field pathogens.

Recent studies document significant immunoregulatory influence of the gut commensal bacteria on host immune status. For example, *Bifidobacteria infantis* or *Faecalibacterium prausnitzii* induced Foxp3⁺ T regulatory cells and increased the expression of the anti-inflammatory cytokine, interleukin (IL)-10, whereas segmented filamentous bacteria (*Candidatus arthromitus*) induced T-helper (Th)17 cell development (Lee

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and Mazmanian, 2010; Atarashi et al., 2011). In addition, potential interaction of various disease control programs with gut microbiota community, populations of *Eimeria* spp., and host immunity was recently demonstrated (Lee and Lillehoj, 2011; Lillehoj and Trout, 1996). Infection with *Eimeria* spp. induces antigen-specific humoral and cell-mediated immune responses (Lillehoj and Trout, 1996) and affects gut microbiota communities (Hume et al., 2006) in broiler chickens. Moreover, broiler chickens that are naturally exposed to *Eimeria*-contaminated litter developed different patterns of *Eimeria*-specific antibody response depending on the coccidiosis control programs used (Lee et al., 2011b). Finally, gut microbiota plays an important role in health, immunity, and disease prevention of chickens (Dibner et al., 2008). Altered microbiota is linked to obesity, inflammatory bowel disease, atherosclerosis, and cancer (Wlodarska and Finlay, 2010). Collectively, these aforementioned studies raise a possibility that coccidiosis control programs would affect gut microbiome-immune balance in broiler chickens. Unfortunately, very limited information is available on the effect of different kinds of coccidiosis control programs on the host immune status and its responses in broiler chickens. Thus, the present study was aimed to explore whether various coccidiosis control programs involving *in-ovo* vaccine, synthetic chemicals, and ionophores would affect the immune responses (e.g., innate and adaptive immunity) in commercial meat-type chickens. In this study, we evaluated various immune parameters including serum acute phase protein concentration, subpopulations of spleen lymphocytes, and serum antibody responses following vaccination against infectious bronchitis virus (IBV) and Newcastle disease (ND), two important respiratory viral diseases of poultry.

MATERIALS AND METHODS

1. Birds, Diets and Experimental Design

Two-hundred eighty, one-day-old broiler chickens (Ross) were randomly placed in eight pens ($n = 35/\text{pen}$) with used litter at the University of Delaware Lasher Poultry Diagnostic Laboratory Research Farm. Coccidiosis control programs included *in ovo* vaccination, six straight programs with either chemicals or ionophores, or both, and one shuttle program

(NCB/SAL) with nicarbazin at 0~18 days and salinomycin at 19~35 days. The straight programs contained the following chemicals or ionophores: diclazuril (CLIN), decoquinate (DECX), monensin (COBN), nicarbazin + narasin (MAXI), and salinomycin (SAL), respectively. In addition to the chemicals or ionophores, both straight and shuttle program treatments contained bacitracin methylene disalicylate (BMD) and roxarsone (3-nitro) during the experimental period (0 to 35 days of age). For the vaccination group (CVAC), 18-day-old fertilized and developing eggs were *in ovo* vaccinated with Inovocox[®] (Embrex, Pfizer, Durham, NC) and the hatched chicks were provided diets with virginiamycin at 0 to 18 days, and BMD and 3-nitro at 19 to 35 days. The experimental design and the antimicrobial concentrations in diets used are shown in Table 1. The control group (NONE) was provided with non-medicated base diet throughout the whole experimental period. The base diet was a mash type consisting of corn, soybean meal, poultry and animal by-products, and distiller's dried grains soluble. The experimental diets were formulated by mixing the base diet with the indicated antimicrobials (Table 1). All broiler chicks used in this study had been vaccinated against ND and IBV via coarse spray after hatch. The population density was set at 0.06 m²/bird at 1 week, 0.07 m²/bird at 2 week, 0.09 m²/bird at 3 week, 0.11 m²/bird at 4 week, and 0.15 m²/bird at 5 or more weeks. All protocols were approved by the Beltsville Animal Care and Use Committees of the Beltsville Agricultural Research Center and the University of Delaware.

2. Used Litter

Used litter was obtained from a commercial broiler house with endemic gangrenous dermatitis, transported to the research facility for the current study, and evenly distributed into eight pens after homogenization. Care was taken to ensure that all pens were given homogenized litter to minimize the potential pen effect. Presence of *Eimeria* spp. oocysts in litters was as low as 107 (SD 1.8) per g of litter without inter-group differences (Lee et al., 2011b). With *Eimeria*-specific PCR assay, we also confirmed the presence of *E. acervulina*, *E. maxima*, *E. mitis*, *E. praecox*, and *E. tenella*, but not *E. brunetti* or *E. necatrix*, in the litter samples.

Table 1. Experimental designs

	Coccidiosis control programs ^{1,2}							
	NONE	CVAC	CLIN	COBN	DECX	MAXI	NCB/SAL ³	SAL
Vaccination	-	+	-	-	-	-	-	-
0~18 d								
Cocciostat	0	0	1	100	27	82	113	60
Bacitracin methylene disalicylate	0	0	50	50	50	50	50	50
3-Nitro	0	0	45	45	45	45	45	45
Virginiamycin	0	20	0	0	0	0	0	0
19~35 d								
Cocciostat	0	0	1	100	27	82	60	60
Bacitracin methylene disalicylate	0	50	50	50	50	50	50	50
3-Nitro	0	45	45	45	45	45	45	45

¹NONE, non-medicated control; CVAC, Pfizer inovocox *in-ovo* vaccination; CLIN, diclazuril as ClinacoxTM; COBN, monensin as Coban[®]; DECX, decoquinate as Deccox[®]; MAXI, nicarbazin + narasin as Maxiban[®]; NCB/SAL; nicarbazin/salinomycin; SAL, salinomycin.

²Values are g/ton.

³NCB/SAL indicates addition of nicarbazin during 0 to 18 d and switch to salinomycin during 19 to 35 d.

3. Sampling

At 7 days of age, 5 birds per group were randomly selected for blood after euthanasia. Immediately after blood sampling, spleen was also sampled and pooled by treatment group to obtain splenocytes (Lee et al., 2010b) for analysis. At 14, 25, and 34 days of age, five or six birds per group were randomly selected and blood was sampled by heart puncture immediately after euthanasia. Serum was obtained by gentle centrifugation and stored at -20°C until use.

4. Chicken α -1-Acid Glycoprotein ELISA

Levels of chicken α -1-acid glycoprotein (α -1-AGP) in serum samples obtained at 34 days of age were measured by ELISA (Life Diagnostics, Inc., West Chester, PA) according to the manufacturer's instructions.

5. Splenocyte Proliferation

At 7 days of age, pooled splenocytes per group were seeded at 5.0×10^5 /well in 96-well microtiter plates and incubated with medium alone (control) or 2.0 μ g/mL of concanavalin A (ConA, Sigma, St. Louis, MO) in a humidified incubator at 41°C with 5% CO₂ for 48 hrs. Cell proliferation

was measured using 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (Cell Counting Kit-8, Dojindo Molecular Technologies, Gaithersburg, MD) by measuring optical density (OD) at 450 nm (OD₄₅₀) using a microplate spectrophotometer (BioRad, Hercules, CA) as described (Lee et al., 2010b). Proliferation was expressed as the stimulation index (SI), calculated as the ratio of the mean OD₄₅₀ value of ConA-stimulated cells divided by the mean OD₄₅₀ value of medium alone-stimulated cells. Each analysis was performed in quadruplicate.

6. Analysis of Lymphocyte Subpopulations in Spleen

Splenocytes were sampled at 7 days and pooled by the treatment groups. They were > 95% viable by trypan blue exclusion. The cells were stained with monoclonal antibodies (mAb) against chicken CD4 (T helper cells) (Lillehoj et al., 1988), CD8 (cytotoxic T cells) (Lillehoj et al., 1988), TCR1 ($\gamma\delta$ T cell receptor) (Chen et al., 1986), or TCR2 ($\alpha\beta$ TCR) (Chen et al., 1986) and analyzed with a FACSCaliburTM flow cytometer (BD Biosciences, San Jose, CA). As a positive control, the cells were stained with mAb K55 (chicken pan

lymphocyte) (Chung et al., 1991). As a negative control, the cells were stained with mAb HB2 (human T cell; American Type Culture Collection, Manassas, VA). Data were obtained from a total of 10^4 viable cells. Each analysis was performed in quintuplicate.

7. Measurement of IBV- and ND-reactive Antibody Responses

Viral antibodies against IBV and ND in sera sampled at 7, 14, 25, and 34 days of age were determined using commercial ELISA kits (IDEXX Laboratories, Westbrook, MA) according to the manufacturer's instructions. The results were expressed as antibody titer that was calculated using the formula provided by IDEXX.

8. Statistical Analysis

All data obtained in this study were compared among the different groups by the Duncan's Multiple Range test following ANOVA using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). Statistical significance was pre-set at $P < 0.05$.

RESULTS

The levels of α -1-AGP at 34 days of age were significantly changed depending on the coccidiosis control programs (Fig. 1). In general, the concentration of α -1-AGP ranged as low as $3.0 \mu\text{g/mL}$ in COBN-treated chickens and as high as $282 \mu\text{g/mL}$ in CLIN-treated chickens. All treatment groups except the CLIN-treated chickens showed significantly low levels of α -1-AGP compared with the non-medicated controls.

At 7 days of age, *in ovo*-vaccinated broiler chickens showed the highest splenic mitogenic response to ConA compared with the non-medicated control chickens or the groups treated with anticoccidials (Fig. 2).

Composition of splenocyte subpopulations was also altered by the type of coccidiosis control programs at 7 days of age (Fig. 3). In general, the percentage of CD4^+ cells were high ($P < 0.05$) in MAXI-treated chickens compared with the non-medicated control chickens. Most groups, not including NCB/SAL, SAL, and CLIN groups, showed lower percentages of CD8^+ cells compared with the NONE group. Increase in the percentages of TCR1^+ cells of COBN- and MAXI-treated

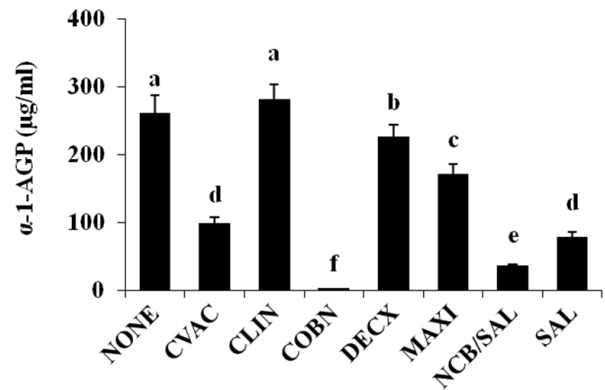


Fig. 1. Effect of coccidiosis control programs on acute phase protein concentrations in broiler chickens. Sera were sampled at 35 days of age and analyzed for the levels of alpha-1-acid glycoprotein (α -1-AGP) by commercially available ELISA. Each bar represents the mean \pm SD ($n = 5$). Bars with different letters differ significantly at $P < 0.05$.

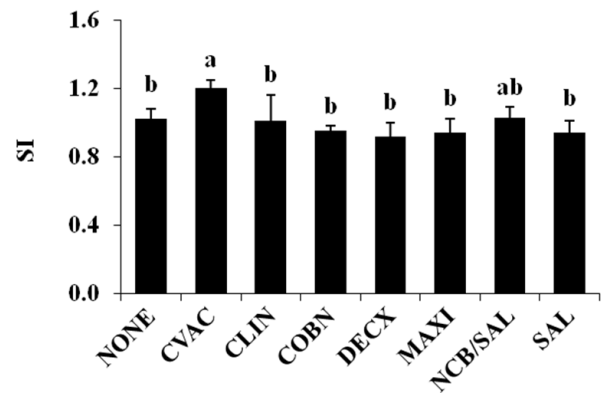


Fig. 2. Effect of coccidiosis control programs on concanavalin A (ConA)-stimulated splenocyte proliferation. Splenocytes sampled at 7 days of age were stimulated with ConA and the stimulation index (SI) for each treatment was calculated as described in the Materials and Methods. Each bar represents the mean \pm SD value ($n = 4$). Bars with different letters differ significantly at $P < 0.05$.

chickens, and decrease in TCR2^+ cells in DECX-treated chickens were also noted compared with the non-medicated control chickens. In all groups, the percentage of cells stained by the positive control K55 mAb was consistently $> 99\%$, and the percentage of cells stained by the negative control HB2 mAb was consistently $< 2\%$ (data not shown).

Serological monitoring of serum antibody titers against IBV and ND is shown in Table 2 and Table 3. In general, IBV-

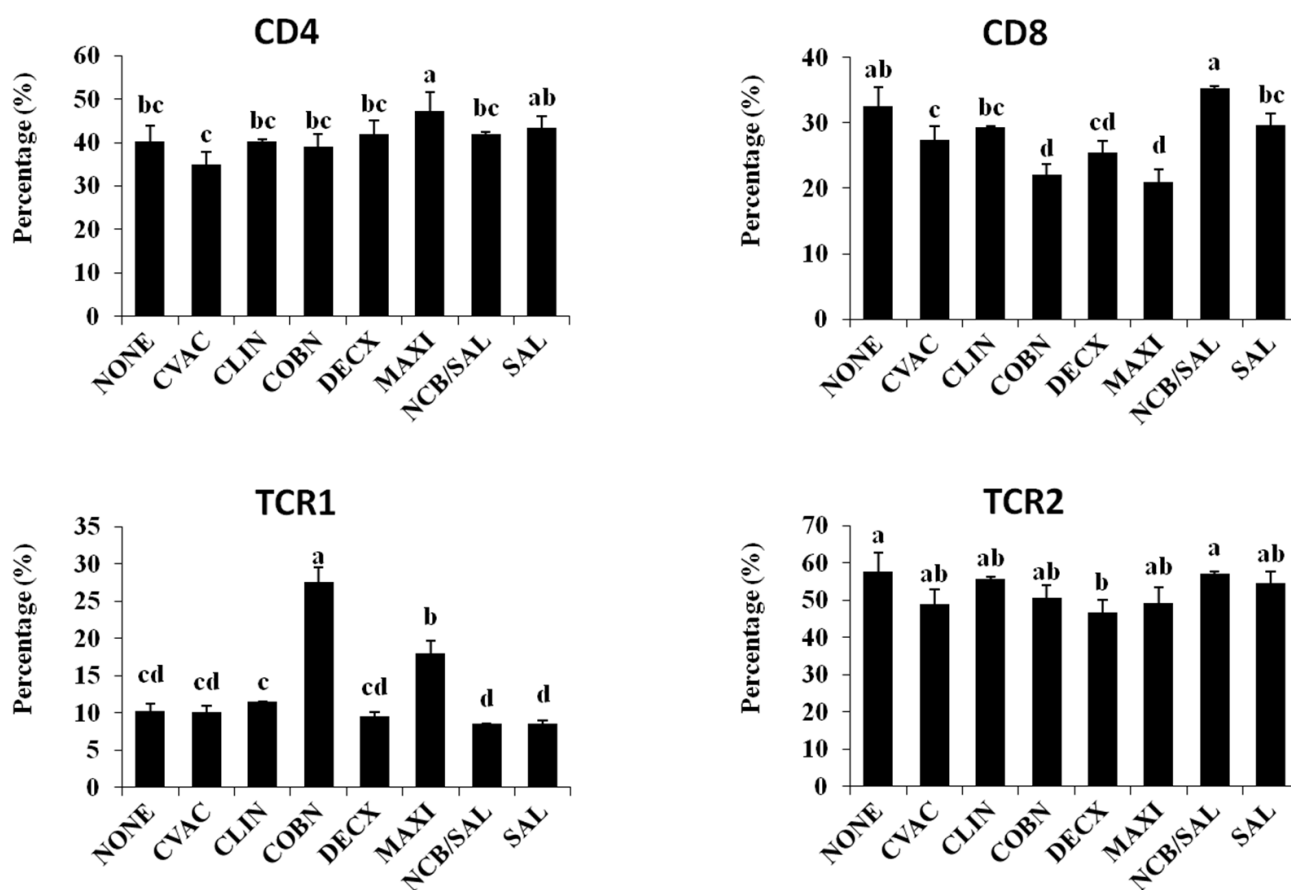


Fig. 3. Effect of coccidiosis control programs on splenocyte subpopulations. Splenocytes sampled at 7 days of age were stained with the indicated mAbs and analyzed by flow cytometry. Each bar represents the mean \pm SD value ($n=5$). Bars with different letters differ significantly at $P<0.05$.

specific antibody titers decreased with age and then increased at 34 days of age (Table 2). There were no statistical differences in IBV antibody titers among the different treatment groups at all ages. In contrast, ND-reactive antibody titers were changed by treatment groups (Table 3). Especially, at 14 days of age, non-medicated control chickens exhibited significantly lower ND-reactive antibody titers compared with all treatment groups except for DECX which remained intermediate. At 25 days of age, ND antibody titers were significantly low in the *in-ovo*-vaccinated chickens compared with those of the non-medicated controls or the anticoccidial-treated chickens.

DISCUSSION

The experiment was conducted to monitor immune status of broiler chickens raised on used litter and subjected to

various coccidiosis control programs including *in ovo* vaccination or anticoccidial drugs (e.g., chemicals and ionophores) that are commonly used in poultry production. In summary, the levels of α -1-AGP at 34 days of age were significantly lowered ($P<0.05$) by most of the treatments compared with the non-medicated controls. The *in ovo*-vaccinated chickens exhibited the highest ConA-stimulated splenocyte mitogenic response at 7 days of age. Different types of coccidiosis control programs changed T-cell subsets in the spleen. Finally, non-medicated control chickens at 14 days of age and *in-ovo* vaccinated chickens at 25 days of age had the lowest levels of serum antibody titers against ND. It is clear from this study that the coccidiosis control programs commonly used in poultry production influenced various immune parameters of chickens.

α -1-AGP which has been used as an indicator of innate

Table 2. Effect of coccidiosis control programs on IDEXX ELISA titers (log10) against infectious bronchitis virus in broiler chickens

Treatments ^{1,2}	Day 7	Day 14	Day 25	Day 34
NONE	2.69 ± 0.37	1.69 ± 0.88	1.43 ± 1.31	2.93 ± 0.36
CVAC	2.88 ± 0.16	2.01 ± 0.55	1.61 ± 1.09	2.92 ± 0.43
CLIN	1.99 ± 1.34	1.44 ± 0.85	1.22 ± 1.36	2.40 ± 0.45
COBN	2.43 ± 0.56	1.02 ± 1.27	1.40 ± 1.41	2.20 ± 0.48
DECX	2.82 ± 0.37	1.20 ± 0.95	2.18 ± 0.73	2.43 ± 0.77
MAXI	2.67 ± 0.44	1.83 ± 0.42	1.92 ± 1.26	2.93 ± 0.58
NCB/SAL	2.88 ± 0.58	1.57 ± 0.84	1.62 ± 0.27	2.83 ± 0.30
SAL	2.62 ± 0.17	0.93 ± 1.24	2.53 ± 1.02	2.29 ± 1.51

¹Values are mean ± SD.

²NONE, non-medicated control; CVAC, Pfizer inovocox *in-ovo* vaccination; CLIN, diclazuril as Clinacox™; COBN, monensin as Coban®; DECX, decoquinate as Deccox®; MAXI, nicarbazin + narasin as Maxiban®; NCB/SAL; nicarbazin/salinomycin; SAL, salinomycin.

Table 3. Effect of coccidiosis control programs on IDEXX ELISA titers (log10) against Newcastle disease virus in broiler chickens

Treatments ¹	Day 7	Day 14	Day 25	Day 34
NONE	1.23 ± 0.83	0.36 ± 0.62 ^b	2.60 ± 0.69 ^a	2.41 ± 0.62
CVAC	2.29 ± 1.34	1.44 ± 0.37 ^a	0.34 ± 0.75 ^b	1.27 ± 1.22
CLIN	1.63 ± 1.56	1.25 ± 0.80 ^a	1.87 ± 1.29 ^a	2.49 ± 0.62
COBN	1.37 ± 1.26	1.57 ± 0.84 ^a	2.40 ± 0.78 ^a	2.01 ± 1.14
DECX	1.71 ± 0.37	1.11 ± 0.81 ^{ab}	1.63 ± 0.41 ^a	2.40 ± 0.76
MAXI	0.79 ± 1.09	1.45 ± 0.86 ^a	1.76 ± 0.87 ^a	2.36 ± 0.65
NCB/SAL	1.77 ± 0.67	1.86 ± 0.78 ^a	2.37 ± 1.00 ^a	2.67 ± 0.82
SAL	1.83 ± 0.58	1.67 ± 0.50 ^a	2.10 ± 0.52 ^a	1.50 ± 1.39

¹NONE, non-medicated control; CVAC, Pfizer inovocox *in-ovo* vaccination; CLIN, diclazuril as Clinacox™; COBN, monensin as Coban®; DECX, decoquinate as Deccox®; MAXI, nicarbazin + narasin as Maxiban®; NCB/SAL; nicarbazin/salinomycin; SAL, salinomycin.

^{a,b}Values (mean ± SD) within the same column not sharing a common superscript differ significantly at $P < 0.05$.

immunity is an acute phase protein (APP) that contributes to restoring homeostasis and restricting microbial growth in an antibody-independent manner following infection, inflammation, or stress (Murata et al., 2004). In broiler chickens treated with *Salmonella* Typhimurium-derived lipopolysaccharide (Kefali and Toker, 2006), a 4.0-fold increase in the APP levels was observed. In this study, the levels of α -1-AGP were significantly lowered by most of the anti-coccidial treatments. This result supports a notion that different coccidiosis control programs could reduce factors inducing systemic inflammation or infection in broiler chickens. In addition, since the

experimental diets contained antibiotic growth promoters (AGPs) such as virginiamycin or BMD, which are commonly accepted drug programs in poultry industry, the coccidiosis control programs or AGPs, working singly or preferentially in combination, may reduce pathogen loads in the intestines, thereby further decreasing inflammation or infection.

As a measure of general systemic lymphocyte immune function, we stimulated splenocytes with T cell mitogen, ConA. It is well known that cell proliferation is an accurate indicator of general cell-mediated immune function. In this study, CVAC-treated chickens exhibited significantly increased ConA-

stimulated splenocyte proliferation compared with untreated controls or anticoccidials-treated chickens. This result is not surprising given that vaccination against coccidiosis can induce robust humoral and cell-mediated immunities in broiler chickens (Jang et al., 2011). However, we failed to see anticoccidials-altered splenocyte proliferation, which is contrary to the previous studies (Baba et al., 1998; Munir et al., 2009) in which antibiotic or anticoccidial treatment exhibited increased mitogen-stimulated proliferation compared with the controls. It cannot be excluded the possibility that chickens treated with anticoccidials for 7 days may not be enough to see the effects on splenocyte proliferation. Unfortunately, we did not assay splenocyte proliferation from chickens sampled at 14, 25, and 34 days. Thus, further studies are warranted to address the concern raised in this study.

Splenocyte subpopulations were changed by the coccidiosis control programs, but the effect was dependent on the types of programs used. Specifically, most T-cell subsets (i.e., CD4, CD8, TCR1, and TCR2) were unaffected or decreased by the treatments compared with the controls. In contrast, CD4⁺ splenocytes in MAXI-treated chickens, and CD8⁺ splenocytes in COBN- or MAXI-treated chickens were significantly higher compared with those of the non-medicated controls. In agreement with our result, earlier studies reported discordant results on the lymphocyte subpopulations. For example, the absence of an effect of antibiotic treatment on the percentages of B cells, CD4⁺, and CD8⁺ T cells in spleen or peripheral blood has been noted (Baba et al., 1998). In a study by Arias and Koutsos (2006), they reported that antibiotic-fed chickens raised on fresh litter had higher duodenal lymphocytes, but lower ileal lymphocytes compared with non-medicated control chickens. In contrast, antibiotics-fed chickens raised on the used litter had unaltered duodenal or ileal lymphocytes compared with the non-medicated control chickens (Arias and Koutsos, 2006). Our study is the first report showing the effect of various field coccidiosis control programs on host innate and adaptive immunity.

There have been several attempts to modulate the humoral response, especially in viral- or *Eimeria*-vaccinated chickens by adding natural alternatives to antibiotics such as probiotics or medicinal plants into broilers' diets (Talebi et al., 2008; Khaligh et al., 2011; Lee et al., 2011a). Although the mecha-

nisms underlying the diet-induced immunomodulation are not well understood, altered gut microbiota which is known to affect host immunity may play an important role (Lee and Lillehoj, 2011). Thus, any measures (e.g., diet or environment) that influence gut microbiota could affect host immunity. Indeed, several reports including our current study showed the influence of antimicrobials on antibody titers in ND-vaccinated chickens. For example, it was found that chlortetracycline- or salinomycin-treated broiler chickens produced high antibody titers against ND compared with the control chickens (Munir et al., 2007; Murwani and Murtini, 2009). In contrast, the depressive effect of antimicrobials on antibody titers against ND was also reported (Khalifeh et al., 2009). Finally, no clear antibiotic effect on the antibody levels in broiler chickens following vaccination against ND and IBV was observed (Kwon et al., 2008). The discordant results on antimicrobial-induced humoral responses between experiments may be due to several factors including the age of birds, types of chickens (fast growing meat-type chicks vs. slow growing layer-type chick), housing, or diet composition, which might influence the gut microbiota or development of immune systems.

In conclusion, the present study showed that the type of coccidiosis control programs commonly used in field poultry production modulate various systemic immune parameters such as acute phase protein concentration, ConA-stimulated splenocyte proliferation, splenocyte subpopulations, and antibody titers against IBV and ND in broiler chickens. The underlying immunomodulatory mechanism(s) seen in this study may be in part related to altered host immune capability and gut microbiota composition by various coccidiosis control programs. Thus, further studies are required to assess the effects of the coccidiosis control program on microbial community profile and host immune response and their association in disease pathogenesis in broiler chickens.

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LITERATURE CITED

- Al-Natour MQ, Suleiman MM, Abo-Shehada MN 2002. Flock-level prevalence of *Eimeria* species among broiler chicks in northern Jordan. *Prev Vet Med* 53:305-310.
- Arias VJ, Koutsos EA 2006 Effects of copper source and level on intestinal physiology and growth of broiler chickens. *Poultry Sci* 85:999-1007.
- Atarashi K, Umesaki Y, Honda K. 2011. Microbiota influence on T cell subset development. *Semin Immunol* 23:146-153.
- Baba T, Yamashita N, Kodama H, Mukamoto M, Asada M, Nakamoto K, Nose Y, McGruder ED 1998 Effect of tylosin tartrate on humoral immune responses in chickens. *J Vet Med B* 45:279-286.
- Chen CL, Ager LL, Gartland GL, Cooper MD 1986 Identification of a T3/T cell receptor complex in chickens. *J Exp Med* 164:375-386.
- Chung KS, Lillehoj HS, Jenkins MC 1991 Avian leucocyte common antigens: Molecular weight determination and flow cytometric analysis using new monoclonal antibodies. *Vet Immunol Immunopathol* 28:259-273.
- Crhanova M, Hradecka H, Faldynova M, Matulova M, Havlickova H, Sisak F, Rychlik I 2011 Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica* serovar Enteritidis infection. *Infect Immun* 79:2755-2763.
- Dibner JJ, Richards JD, Knight CD 2008 Microbial imprinting in gut development and health. *J Appl Poult Res* 17:174-188.
- Haug A, Gjevre A, Thebo P, Mattsson JG, Kaldhusdal M 2008 Coccidial infections in commercial broilers: epidemiological aspects and comparison of *Eimeria* species identification by morphometric and polymerase chain reaction techniques. *Avian Pathol* 37:161-170.
- Hume ME, Clemente-Hernandez S, Oviedo-Rondon EO 2006 Effects of feed additives and mixed *Eimeria* species infection on intestinal microbial ecology of broilers. *Poultry Sci* 85:2106-2111.
- Jang SI, Lillehoj HS, Lee SH, Lee KW, Lillehoj EP, Bertrand F, Dupuis L, Deville S 2011 Mucosal immunity against *Eimeria acervulina* infection in broiler chickens following oral immunization with profilin in Montanide™ adjuvants. *Exp Parasitol* 129:36-41.
- Kefali S, Toker NY 2006 Effects of probiotics on some acute phase proteins in broilers exposed to *Salmonella typhimurium* lipopolysaccharides. *Arch Geflügelk* 70:270-277.
- Khalifeh MS, Amawi MM, Abu-Basha EA, Bani Yonis I 2009 Assessment of humoral and cellular-mediated response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. *Poultry Sci* 88:2118-2124.
- Khaligh F, Sadeghi G, Karimi A, Vaziry A 2011 Evaluation of different medicinal plants blends in diets for broiler chickens. *J Med Plants Res* 5:1971-1977.
- Kwon HS, Kim JY, Kim JS, Lee BK, Lee SY, Lee WS, Ahn BK, Kim EJ, Kang CW 2008. Effects of dietary supplementation of domestic skullcap (*Scutellaria baicalensis*) extracts on performance, immune response and intestinal microflora in broiler chickens. *Korean J Poult Sci* 35:351-359.
- Lee JT, Broussard C, Fitz-Coy S, Burke P, Eckert NH, Stevens SM, Anderson PN, Anderson SM, Caldwell DJ 2009 Evaluation of live oocyst vaccination or salinomycin for control of field-strain *Eimeria* challenge in broilers on two different feeding programs. *J Appl Poult Res* 18:458-464.
- Lee BH, Kim WH, Jeong J, Yoo J, Kwon YK, Jung BY, Kwon JH, Lillehoj HS, Min W 2010a Prevalence and cross-immunity of *Eimeria* species on Korean chicken farms. *J Vet Med Sci* 72:985-989.
- Lee KW, Lee SH, Lillehoj HS, Li GX, Jang SI, Babu US, Park MS, Kim DK, Lillehoj EP, Neumann AP, Rehberger TG, Siragusa GR 2010b Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. *Poultry Sci* 89:203-216.
- Lee KW, Lillehoj HS 2011 Antimicrobials, gut microbiota and immunity in chickens. *Korean J Poult Sci* 38:155-164.
- Lee SH, Lillehoj HS, Jang SI, Lee KW, Bravo D, Lillehoj EP 2011a Effects of dietary supplementation with phytonutrients on vaccine-stimulated immunity against infection with *Eimeria tenella*. *Vet Parasitol* 181:97-105.
- Lee KW, Lillehoj HS, Jang SI, Pagès M, Bautista DA, Pope CR, Ritter GD, Lillehoj EP, Neumann AP, Siragusa GR 2011b Effects of *in ovo* vaccination and anticoccidials on

- the distribution of *Eimeria* spp. in poultry litter and serum antibody titers against coccidia in broiler chickens raised on the used litters. Res Vet Sci. in press.
- Lee YK, Mazmanian SK 2010 Has the microbiota played a critical role in the evolution of the adaptive immune system? Science 330:1768-1773.
- Lillehoj HS, Lillehoj EP, Weinstock D, Schat KA 1988 Functional and biochemical characterizations of avian T lymphocyte antigens identified by monoclonal antibodies. Eur J Immunol 18:2059-2065.
- Lillehoj HS, Trout JM 1996 Avian gut-associated lymphoid tissues and intestinal immune responses to *Eimeria* parasites. Clin Microbiol Rev 9:349-360.
- Munir K, Muneer MA, Tiwari A, Chaudhry RM, Muruganandan S 2007 Effects of polyether ionophores on the protective immune responses of broiler chickens against Angara disease and Newcastle disease viruses. Vet Res Commun 31:909-929.
- Munir K, Muneer MA, Tiwari A, Masaoud E, Chaudhry RM 2009 Effects of salinomycin on cell-mediated immunity of broiler chickens against hydropericardium syndrome and Newcastle disease viruses. Poultry Sci 88:86-91.
- Murata H, Shimada N, Yoshioka M 2004 Current research on acute phase proteins in veterinary diagnosis: An overview. Vet J 168:28-40.
- Murwani R, Murtini S 2009. Effect of chlortetracycline additive in broilers fed local diets on antibody titers to NDV vaccine. Int J Poult Sci 8:755-759.
- Nematollahi A, Moghaddam G, Pourabad RF 2009 Prevalence of *Eimeria* species among broiler chickens in Tabriz (northwest of Iran). Mun Ent Zoo 4:53-58.
- Sun XM, Pang W, Jia T, Yan WC, He G, Hao LL, Bentue M, Sue X 2009 Prevalence of *Eimeria* species in broilers with subclinical signs from fifty farms. Avian Dis 53:301-305.
- Talebi A, Amirzadeh B, Mokhtari B, Gahri H 2008 Effects of a multi-strain probiotic (PrimaLac) on performance and antibody responses to Newcastle disease virus and infectious bursal disease virus vaccination in broiler chickens. Avian Pathol 37:509-512.
- Williams RB 1999 A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. Int J Parasitol 29:1209-1229.
- Williams RB 2005 Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. Avian Pathol 34:159-180.
- Williams RB, Bushell AC, Reperant JM, Doy TG, Morgan JH, Shirley MW, Yvore P, Carr MM, Fremont Y 1996 A survey of *Eimeria* species in commercially-reared chickens in France during 1994. Avian Pathol 25:113-130.
- Wlodarska M, Finlay BB 2010 Host immune response to antibiotic perturbation of the microbiota. Mucosal Immunol 3:100-103.
- Yin Y, Lei F, Zhu L, Li S, Wu Z, Zhang R, Gao GF, Zhu B, Wang X 2010 Exposure of different bacterial inocula to newborn chicken affects gut microbiota development and ileum gene expression. ISME J 4:367-376.

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