Effects of dietary supplementation with phytoneutrients on vaccine-stimulated immunity against infection with *Eimeria tenella*†

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**A B S T R A C T**

Two phytoneutrient mixtures, VAC (carvacrol, cinnamaldehyde, and *Capsicum* oleoresin), and MC (Capsicum oleoresin and turmeric oleoresin), were evaluated for their effects on chicken immune responses following immunization with an *Eimeria* profilin protein. Chickens were fed with a non-supplemented diet, or with VAC- or MC-supplemented diets, immunized with profilin, and orally challenged with virulent oocysts of *Eimeria tenella*. Immunity against infection was evaluated by body weight, fecal oocyst shedding, profilin antibody levels, lymphocyte recall responses, cytokine expression, and lymphocyte subpopulations. Following immunization and infection, chickens fed the VAC- or MC-supplemented diets showed increased body weights, greater profilin antibody levels, and/or greater lymphocyte proliferation compared with non-supplemented controls. Prior to *Eimeria* infection, immunized chickens on the MC-supplemented diet showed reduced IFN-γ and IL-6 levels, but increased expression of TNFSF15, compared with non-supplemented controls. Post-infection levels of IFN-γ and IL-6 were increased, while IL-17F transcripts were decreased, with MC-supplementation. For VAC-supplemented diets, decreased IL-17F and TNFSF15 levels were observed only in infected chickens. Finally, immunized chickens fed the MC-supplemented diet exhibited increased MHC class II⁺, CD4⁺, CD8⁺, TCR1⁺, or TCR2⁺ T cells compared with nonsupplemented controls. Animals on the VAC-containing diet only displayed an increase in K1⁺ macrophages. In conclusion, dietary supplementation with VAC or MC alters immune parameters following recombinant protein vaccination against avian coccidiosis.

Abbreviations: BSA, bovine serum albumin; WST, water-soluble tetrazolium; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mAb, monoclonal antibody; MHC, major histocompatibility complex; TNFSF15, tumor necrosis factor super family 15; F, forward primer; R, reverse primer.

† Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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1. Introduction

Coccidiosis is an intestinal disease caused by multiple species of *Eimeria* (Protozoa, Apicomplexa). Avian coccidiosis is an economically important infection for the poultry industry worldwide that has traditionally been controlled with coccidiostatic drugs and antibiotic-based growth promoters (Lillehoj and Lillehoj, 2000; Lillehoj and Lee, 2007a,b; Lee et al., 2008a). Recent interest has focused on developing drug-free disease control strategies due, in part, to the emergence of drug-resistant parasites. In particular, an increasing reliance has been placed on live, attenuated *Eimeria* vaccines for control of avian coccidiosis (Williams et al., 1999; Williams and Catchpole, 2000). However, the potential development of additional diseases, such as necrotic enteritis due to the interaction of coccidia parasites with *Clostridium* bacteria, limits the utility of live parasite vaccines (Williams, 2002; Pogonka et al., 2003).

*Eimeria* recombinant protein vaccines, while less efficacious than coccidiostats and live vaccines, offer an alternative control strategy against avian coccidiosis (Lillehoj et al., 2000). For example, *Eimeria* profilin was identified in the merozoites of the parasite as an immunogenic protein which increased antigen-specific proliferation of chicken spleen lymphocytes and augmented cytokine production by splenic and intestinal lymphocytes (Lillehoj et al., 2000; Ding et al., 2004; Lillehoj and Okamura, 2003; Yarovinsky et al., 2005; Plattner et al., 2008; Lee et al., 2010b,d). Subcutaneous immunization of chickens with recombinant profilin expressed in *Escherichia coli* increased protective immunity to subsequent challenge infection by virulent *Eimeria acervulina* (Lillehoj et al., 2000, 2004). However, the effectiveness of profilin immunization was dose-dependent, such that only partial protection against challenge infection was generated at low vaccine doses (≤100 μg).

A variety of approaches has been pursued to improve the effectiveness of recombinant protein vaccines, for example by coadministration of the vaccine with adjuvants or recombinant chicken cytokines, or by feeding plant-based phytonutrients (Min et al., 2001; Park et al., 2004; Lee et al., 2005, 2007a, 2008a,b, 2009c, 2010a; Kim et al., 2010; Jang et al., 2010). Previously, we showed that in vitro exposure of spleen cells to an extract of turmeric (*Curcuma longa*) increased lymphocyte proliferation compared with cells treated with vehicle alone (Lee et al., 2010a). Additionally, dietary supplementation of chickens with carvacrol, cinnamaldehyde, or *Capsicum* oleoresin altered the in vivo expression of a family of innate immune response genes compared with chickens fed a non-supplemented diet (Kim et al., 2010). Therefore, we hypothesized that these phytonutrients would enhance vaccine-induced immunity against experimental *Eimeria* infection. To test this hypothesis, chickens were fed from hatch with normal diets, or with diets supplemented with carvacrol, cinnamaldehyde and *Capsicum* oleoresin (VAC) or with *Capsicum* plus turmeric oleoresins (MC), and the ability of recombinant profilin immunization to induce immunity against subsequent *Eimeria* challenge infection was compared between the experimental and control groups.

2. Materials and methods

2.1. Experimental animals and diets

A schematic diagram of the experimental protocol is shown in Fig. 1. One-day-old broiler chickens (Ross/Ross, Longenecker’s Hatchery, Elizabethtown, PA) were housed in Petersime starter brooder units and randomly assigned to 4 groups (12 chickens/group). Chickens were continuously fed a standard diet or with the standard diet supplemented with VAC (5 mg/kg carvacrol, 3 mg/kg cinnamaldehyde, and 2 mg/kg *Capsicum* oleoresin) or MC (4 mg/kg turmeric oleoresin and 4 mg/kg *Capsicum* oleoresin) for 26 days after hatching. The concentration of supplements in the diet was based on our previous report (Lee et al., 2010a). All diets contained 24.2% protein, 54.0% carbohydrate, 3.7% vitamin and mineral mixture, 4.7% fat, and 2.4% fiber (USDA/FeedMill, Beltsville, MD). The birds were maintained in a temperature-controlled environment at 29°C and fed with diets and water ad libitum. All experiments were approved by the USDA-Agricultural Research Service Institutional Animal Care and Use Committee (IACUC).

2.2. Recombinant profilin immunization and experimental infection

*E. acervulina* recombinant profilin was expressed in *E. coli* and purified as described (Jang et al., 2010). Chickens were mock immunized subcutaneously with PBS or vaccinated with 50 μg of recombinant profilin at 7 days post-hatch. At 17 days post-hatch, chickens were orally...
infected with $2.0 \times 10^4$ sporulated virulent oocysts of *E. tenella*, WLR-1 strain ([Lee et al., 2008c, 2010b,d]). The birds were kept in brooder pens in an *Eimeria*-free facility for 2 weeks prior to infection and transferred to hanging cages (2 birds/cage) at a separate location where they were infected and kept until the end of the experimental period.

### 2.3. Body weight and fecal oocyst shedding

Body weights were measured at 17 and 26 days post-hatch (0 and 9 days post-infection). Fecal oocysts were collected daily between 22 and 26 days post-hatch (5 and 9 days post-infection). Oocyst numbers were determined as described ([Lee et al., 2010b,c,d]) using a McMaster chamber according to the formula: total oocysts/bird = (oocyst count × dilution factor × [fecal sample volume/counting chamber volume])/2.

### 2.4. Anti-profilin antibody levels

Blood samples (4 chickens/group) were collected by cardiac puncture immediately following euthanasia at 17 and 26 days post-hatch, sera were obtained by low speed centrifugation, and used in an ELISA to measure profilin-specific antibody responses as described ([Lee et al., 2007c,d, 2010b]). Briefly, 96-well microtiter plates were coated overnight with 1.0 µg/well of purified recombinant profilin. The plates were washed with phosphate-buffered saline containing 0.05% Tween (PBS-T) and blocked with PBS containing 1% BSA. Diluted sera (1:50) were added (100 µl/well), incubated with agitation for 2 h at room temperature, and washed with PBS-T. Bound antibodies were detected with peroxidase-conjugated rabbit anti-chicken IgG and 3′,3′,5′-tetramethylbenzidine substrate (Sigma, St. Louis, MO). Optical density at 450 nm (OD₄₅₀) was measured with an automated microplate reader (Bio-Rad, Richmond, CA). All samples were analyzed in quadruplicate.

### 2.5. Spleen lymphocyte proliferation

At 26 days post-hatch, chickens (4/group) were euthanized by cervical dislocation, spleens were removed and placed in Petri dishes with 10 ml of Hanks’ balanced salt solution (HBSS) supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma). Cell suspensions were prepared by gently flushing through a cell strainer and lymphocytes were purified by density gradient centrifugation through Histopaque-1077 (Sigma). The cells were adjusted to 5.0 × 10⁶ cells/ml in RPMI medium containing 10% fetal bovine serum (Thermo Scientific HyClone, Logan, UT), 100 U/ml penicillin, and 100 µg/ml streptomycin and incubated with medium alone or with 20 µg/ml of profilin in 96-well plates in a humidified incubator (Forma, Marietta, OH) at 41 °C and 5% CO₂ for 24 h. Cell proliferation was measured using WST-8 (Dojindo Molecular Technologies, Gaithersburg, MD) at OD₄₅₀ using a microplate spectrophotometer (Bio-Rad) as described ([Lee et al., 2010a,b]). WST-8 is reduced by dehydrogenases in cells to give a yellow-colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. Lymphoproliferation was expressed as stimulation index equal to the mean OD₄₅₀ value of the profilin-stimulated group divided by the mean OD₄₅₀ value of the medium-only stimulated group.

### 2.6. Intestinal cytokine mRNA levels

Cecal tonsils were obtained from non-infected and infected chickens at 17 and 23 days post-hatch (6 days post-*Eimeria* infection) (4/group), respectively, the tissues were cut longitudinally, and washed three times with ice-cold HBSS containing 100 U/ml of penicillin and 100 µg/ml of streptomycin as described ([Lee et al., 2010a]). The mucosal layer was carefully scraped away using a surgical scalpel and total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA). Five micrograms of total RNA were treated with 1.0 U of DNase I in 1.0 µl of 10 × reaction buffer (Sigma) for 15 min at room temperature, 1.0 µl of stop solution was added to inactive DNase I, and the mixture was heated at 70 °C for 10 min. RNA was reverse-transcribed using the StrataScript first-strand synthesis system (Stratagene, La Jolla, CA) according to the manufacturer’s recommendations. Quantitative RT-PCR oligonucleotide primers for chicken interferon-γ (IFN-γ), interleukin-6 (IL-6), IL-17F, and tumor necrosis factor superfamily 15 (TNFSF15) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) internal control are listed in Table 1. Amplification and detection were carried out using equivalent amounts of total RNA using the Mx3000P system and Brilliant SYBR Green qPCR master mix (Stratagene). Standard curves were generated using log₁₀ diluted standard RNA. Levels

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Oligonucleotide primers used for quantitative RT-PCR of chicken cytokines.</th>
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<tbody>
<tr>
<td>RNA target</td>
<td>Primer sequences</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: 5′-GGTGTGTGCTAAAGGTTAT-3′ &lt;br&gt; R: 5′-ACCTCTTGATCTCCACA-3′</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>F: 5′-AGCTGGACGCGAAGCACTATTAT-3′ &lt;br&gt; R: 5′-GGGTGGCCTGGATCC-3′</td>
</tr>
<tr>
<td>IL-6</td>
<td>F: 5′-CACGGTACGAGGAGGCGAC-3′ &lt;br&gt; R: 5′-TGGCGAGAGGATTTCT-3′</td>
</tr>
<tr>
<td>IL-17F</td>
<td>F: 5′-TCCGATCTTATTTCTCCCT-3′ &lt;br&gt; R: 5′-AAGCGGTGGTGGTGGCCAT-3′</td>
</tr>
<tr>
<td>TNFSF15</td>
<td>F: 5′-CTGAGATTTCCACCAAGCCA-3′ &lt;br&gt; R: 5′-ATCCACACGCTTGACTAC-3′</td>
</tr>
</tbody>
</table>
of individual transcripts were then normalized to those of GAPDH and analyzed by the Q-gene program (Muller et al., 2002). Each analysis was performed in triplicate. To normalize individual replicates, the logarithmic-scaled threshold cycle (Ct) values were transformed to linear units of normalized expression prior to calculating means and the SEM for the references and individual targets, followed by determination of the mean normalized expression using the Q-gene program (Lee et al., 2008b).

2.7. Flow cytometric analysis

At 26 days post-hatch, chickens (4/group) were euthanized by cervical dislocation and bled by cardiac puncture using a heparinized syringe. Single cell suspensions of peripheral blood lymphocytes (PBL) were prepared as described (Lee et al., 2007a,b), resuspended in HBSS without phenol red supplemented with 3% fetal bovine serum and 0.01% sodium azide (FCA buffer), and adjusted to 1.0 × 10⁷ cells/ml in FCA buffer. The cells were incubated for 30 min on ice with mouse monoclonal antibodies (mAbs) specific for chicken major histocompatibility complex (MHC) class II, CD4, CD8, K1, T cell receptor 1 (TCR1), or TCR2 surface proteins (Lillehoj and Chai, 1988; Lee et al., 2008c). An antibody against chicken MHC class I antigens, C6B12, was used as a positive control and HB2, an antibody specific for human T cells (American Type Culture Collection, Manassas, VA), was used as a negative control. Following incubation, the cells were washed three times with FCA buffer and incubated with 100 μl of fluorescein isothiocyanate–labeled goat anti–mouse IgG secondary antibody (Sigma) for 45 min on ice. The cells were washed three times with FCA buffer, and fluorescence was then analyzed with 1.0 × 10⁴ viable cells using a FACScalibur (BD, Boston, MA).

2.8. Statistical analyses

Statistical analyses were performed using SPSS software (SPSS 15.0 for Windows, Chicago, IL) and all data were expressed as the mean ± SEM values. Comparisons of the mean values were performed by one-way analysis of variance, followed by the Duncan’s multiple range test, and differences were considered statistically significant at P < 0.05.

3. Results

3.1. Effect of dietary supplementation with VAC or MC on body weight and fecal oocyst shedding

Our previous studies demonstrated that immunization of chickens with a suboptimal dose (<100 μg/chicken) of recombinant profilin failed to provide complete protection against subsequent challenge infection with live parasites (Lillehoj et al., 2000, 2004). Therefore, we selected a 50 μg dose to evaluate the ability of the VAC and MC phytonutrients to augment the efficacy of profilin vaccination. Initially, we determined that neither phytonutrient mixtures exhibited gross toxic effects when used at the denoted concentrations. Further, continuous feeding with VAC- or MC-supplemented diets during the first 17 days post-hatch and prior to E. tenella infection did not affect body weights compared with the non-supplemented diet group (data not shown). Following immunization with recombinant profilin and E. tenella infection, chickens fed the VAC- or MC-supplemented diets exhibited increased body weights by 8% and 21%, respectively, compared with immunized and infected animals given the non-supplemented diet (Fig. 2A). Indeed, there was no significant difference in body weights between non-infected controls and infected chickens fed the VAC- or MC-supplemented diets. As shown in Fig. 2B, fecal oocyst shedding was not affected in profilin-immunized and E. tenella-infected chickens by dietary supplementation with VAC or MC, compared with immunized and infected animals given the non-supplemented diet.

3.2. Effect of dietary supplementation with VAC or MC on profilin serum antibody levels

Anti-profilin serum antibody levels increased in immunized chickens and were significantly higher in the chickens fed the VAC- or MC-supplemented diets, com-
pared with immunized and infected animals given the non-supplemented diet (Fig. 3). MC was superior to VAC in augmenting profilin antibody levels.

3.3. Effect of dietary supplementation with VAC or MC on spleen lymphocyte proliferation

Profilin-driven spleen lymphocyte proliferation was increased in the profilin-immunized and parasite-infected group given the MC-supplemented diet compared with immunized and infected animals fed the non-supplemented diet (Fig. 4). VAC supplementation had no effect on the splenocyte proliferative response compared with the non-supplemented controls.

3.4. Effect of dietary supplementation with VAC or MC on intestinal cytokine mRNA levels

Fig. 5 illustrates the effect of dietary supplementation with VAC or MC on the levels of mRNAs for IFN-γ, IL-6, IL-17F, and TNFSF15 in intestinal tissues of profilin-immunized chickens either prior to *Eimeria* infection (17 days post-hatch) or at 6 days following infection (23 days post-hatch). Profilin-immunized chickens fed the MC-supplemented diet displayed reduced levels of transcripts for IFN-γ and IL-6, but increased TNFSF15 transcripts, prior to *Eimeria* infection, compared with non-supplemented and immunized controls. By contrast, the levels of IFN-γ and IL-6 transcripts were increased, while IL-17F transcripts were decreased in parasite-infected animals given the MC-supplemented diet compared with non-supplemented and immunized controls. For VAC-supplemented diets, decreased levels of transcripts for IL-17F and TNFSF15 compared with non-supplemented and immunized controls were observed only in infected chickens.

3.5. Effect of dietary supplementation with VAC or MC on PBL subpopulations

Chicken lymphocytes play a critical role in the host immune response to *Eimeria* infection. Therefore, we next assessed the changes in PBL subpopulations in profilin-immunized and parasite-infected chickens given the VAC, MC or control diets. Animals fed the MC-supplemented diet exhibited increased percentages of MHC class II-, CD4-, CD8-, TCR1-, and TCR2-positive cells compared with non-supplemented and immunized controls, while animals
given the VAC diet only displayed an increased fraction of K1-staining macrophages (Table 2).

4. Discussion

This study demonstrated that dietary supplementation of broiler chickens with phytonutrient mixtures, carvacrol, cinnamaldehyde, *Capsicum* and/or turmeric enhanced immune responses that were induced by vaccination with an *Eimeria* recombinant profilin protein. Birds on the VAC- or MC-supplemented diets exhibited higher body weights, compared with the non-supplemented control group. Analysis of a series of parameters of innate and adaptive immunity revealed a more robust post-immunization response stimulated by the MC-supplemented diet compared with the VAC diet. Thus, immunized chickens given the MC diet displayed increased serum anti-profilin antibody levels, greater profilin-induced lymphocyte proliferation, altered levels of mRNAs encoding IFN-γ, IL-6, IL-17F, and TNFSF15 in the intestine, and increased percentages of MHC class II-, CD4-, CD8-, TCR1-, and TCR2-staining PBLs, compared not only with non-supplemented controls, but also with the VAC diet group. Given that both phytonutrient mixtures contained similar amounts
of Capsicum, the differences between MC and VAC diets likely represents the presence of turmeric in the former. Earlier studies have investigated the beneficial effects of dietary phytonutrients in mammals and chickens. In mammals, extracts from plants of the genus Curcuma, including C. longa (turmeric), exhibited anti-oxidant and anti-inflammatory properties (Policegoudra et al., 2007; Sodsai et al., 2007; Mannangatti and Narayanasamy, 2008). In chickens, dietary phytonutrients have improved the digestibility of feed, reduced intestinal pathogenic bacterial load, and counteracted Eimeria parasite-induced body weight loss (Jamroz et al., 2003, 2005; Lee et al., 2010a,c). Our recent report showed increased levels of mRNAs encoding IL-6 and IFN-γ in chicken macrophages treated in vitro with turmeric extract, as well as increased proliferation of chicken spleen cells treated with the extract, compared with non-treated controls (Lee et al., 2010a). We also have demonstrated that dietary Capsicum oleoresin reduced the expression of intestinal proinflammatory cytokines, improved body weight, and reduced parasite fecundity in chickens infected with E. acervulina, compared with animals provided with a non-supplemented diet (Lee et al., 2010c). Carvacrol is one of the most common components of essential oils and possesses a wide range of anti-microbial activities (Casewell et al., 2003; Ben Arfa et al., 2006; Chami et al., 2005; Veldhuizen et al., 2006). Carvacrol has been approved as a safe food additive in the US and Europe (Center for Food Safety and Applied Nutrition, 2006). Cinnamaldehyde, the other phytonutrient used in this study, has been shown to exhibit anti-fungal, anti-pyretic, anti-oxidant, anti-microbial, and larvicidal activities, in addition to modulating T cell differentiation (Lin et al., 2003; Cheng et al., 2004; Kim et al., 2004; Koh et al., 1998).

We observed that markers of both humoral immunity (profilin-specific antibody) and cell-mediated immunity (T cell proliferation, lymphocyte subpopulations) were enhanced by the MC diet. Cell-mediated immunity has been shown to play a major role in protection against avian coccidiosis (Lillehoj and Ruff, 1987; Lee et al., 2007a,b, 2008c, 2010a,c). Antibody-mediated immunity also appears to play a role in passive immune protection against this disease (Lillehoj et al., 2004; Lee et al., 2009a,b). The increased level of antibodies against parasite antigens of survival importance, such as profilin, likely enhances local protection against coccidiosis. Current studies in our laboratory are directed at measuring anti-profilin antibody levels in intestinal secretions of chickens given phytonutrient-supplemented diets.

The host cell-immune response to Eimeria is initiated and amplified through the production of multiple proinflammatory cytokines in the intestine, including IFN-γ, IL-6, IL-17F, and TNFSF15 (Lillehoj et al., 2001, 2007; Hong et al., 2006a,b; Lee et al., 2008b). Cytokines are critical for local immunoregulation and protection against intracellular parasites in general and coccidia in particular. IFN-γ is a common marker of cellular immunity and has been shown to be associated with protective immune responses to avian coccidiosis (Min et al., 2003; Lillehoj et al., 2004; Lee et al., 2008c). For example, administration of purified recombinant IFN-γ to chickens increased resistance to experimental coccidiosis, significantly reduced intracellular development of Eimeria parasites, and exerted an immune adjuvant effect when given simultaneously in a DNA vaccine (Lillehoj and Choi, 1998; Min et al., 2001). IL-6, a cytokine produced by T cells and macrophages, acts as both a pro-inflammatory and an anti-inflammatory cytokine, depending upon the experimental context (Kanegane and Tosos, 1996; Choi and Lillehoj, 2000). IL-6 and IFN-γ transcript levels in the gut were significantly up-regulated following E. tenella infection, with maximum responses at 6 days post-infection (Hong et al., 2006b), and our current results indicate that MC supplementation further increases the expression of these two cytokines at this time. IL-17 was originally described as a cytokine secreted exclusively by activated memory T cells that induced fibroblasts to secrete other cytokines involved in proinflammatory or hematopoietic processes, such as IL-6, IL-8 and granulocyte-colony stimulating factor (Yao et al., 1995). Subsequently, a number of homologous proteins comprising an IL-17 family were identified, including IL-17A (original IL-17), -17B, -17C, -17D, -17E, and -17F (Pappu et al., 2008). IL-17F is a cytokine secreted exclusively by activated memory T cells that induces fibroblasts to secrete additional soluble mediators of inflammation. Following primary infection with E. acervulina or E. maxima, but not E. tenella, intestinal IL-17F expression at the mRNA level was highly up-regulated (Lee et al., 2010b; Hong et al., 2006a,b). While the present study corroborates the inability of E. tenella to increase IL-17F expression, the responsible molecular and cellular mechanisms are unknown. TNFSF and TNFSF receptor proteins are essential for the differentiation, proliferation, and function of immune cells (Collette et al., 2003). In chickens, recombinant TNFSF15 exhibited a dose-dependent cytotoxic effect on HTC and LSCC-R9 tumor cells (Park et al., 2007). In addition, a previous study provided evidence that chicken TNFSF15 decreased feed intake, increased rectal temperature, and exerted a direct cytotoxic effect against murine fibroblasts or primary chicken cell cultures (Takimoto et al., 2005).

Table 2

<table>
<thead>
<tr>
<th>mAb/%</th>
<th>CON</th>
<th>CON-V</th>
<th>VAC-V</th>
<th>MC-V</th>
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<tr>
<td>MHC class II</td>
<td>10.6 ± 1.3*</td>
<td>21.3 ± 1.8*</td>
<td>18.6 ± 1.1*</td>
<td>54.7 ± 4.1*</td>
</tr>
<tr>
<td>CD4</td>
<td>7.9 ± 1.0*</td>
<td>17.3 ± 1.6*</td>
<td>16.2 ± 1.1*</td>
<td>32.8 ± 1.8*</td>
</tr>
<tr>
<td>CD8</td>
<td>11.4 ± 1.4*</td>
<td>23.3 ± 1.8*</td>
<td>23.3 ± 1.9*</td>
<td>44.2 ± 3.5*</td>
</tr>
<tr>
<td>K1</td>
<td>1.9 ± 0.2*</td>
<td>2.1 ± 0.1*</td>
<td>3.9 ± 0.2*</td>
<td>1.8 ± 0.1*</td>
</tr>
<tr>
<td>TCR1</td>
<td>2.1 ± 0.3*</td>
<td>3.6 ± 0.3*</td>
<td>2.8 ± 0.1*</td>
<td>9.0 ± 0.6*</td>
</tr>
<tr>
<td>TCR2</td>
<td>6.9 ± 0.6*</td>
<td>9.1 ± 0.3*</td>
<td>13.4 ± 0.8*</td>
<td>30.0 ± 1.6*</td>
</tr>
</tbody>
</table>

One-day-old broiler chickens were fed a standard diet alone (CON, CON-V) or a standard diet supplemented with VAC (VAC-V) or MC (MC-V). At 7 days post-hatch, chickens were immunized with 50 μg of recombinant profilin. At 17 days post-hatch, chickens were infected with 2.0 × 10⁴ sporulated oocysts of E. tenella. At 26 days post-hatch, PBLS were analyzed by flow cytometric staining with the indicated monoclonal antibody (mAb). Each value represents the mean ± SEM (n = 4). Values not sharing the same letter within a row are significantly different (P < 0.05) according to the Duncan’s multiple range test.
on profilin-stimulated avian immunity against coccidiosis. In particular, we observed that major T lymphocyte subsets (CD4⁺, CD8⁺, TCR⁺, and TCR⁻) were increased in profilin-immunized chickens fed the MC-supplemented diet and infected with *E. tenella*. CD4⁺ T cells are the main IFN-γ-producing cells in response to antigen challenge (McSorley et al., 2000). In chickens, CD8⁺ cells play an important role as direct effector cells in mediating protective immunity during avian coccidiosis (Lillehoj and Trout, 1996). In murine experimental models of apicomplexan parasite infections, both CD4⁺ and CD8⁺ T cells serve as effector cells and local producers of cytokines that regulate host responses (Sher and Coffman, 1992). TCR⁺ lymphocytes that express the γδ polypeptide receptor for antigen recognition are capable of mediating specific cellular immune functions without antigen processing, and can directly recognize invading pathogens or damaged cells (Schild et al., 1994; Wallace et al., 1995). These cells are also capable of modulating the secretion of immunoregulatory cytokines as well as other soluble factors that influence epithelial cell growth and repair at mucosal surfaces (Boismenu et al., 1996). Unlike mammals, TCR⁺ cells are a major circulating T cell subset in chickens and, in avian coccidiosis, are involved in cytotoxic effector mechanisms that are considered vital for disease control (Lee et al., 2008c; Trout and Lillehoj, 1996; Lillehoj and Ruff, 1987). TCR²⁺ (α/β) cells secrete important immunoregulatory cytokines and have been suggested to play a critical role in mediating a cytotoxic effect against intracellular parasites such as *Eimeria* (Trout and Lillehoj, 1996).

In conclusion, the results of this investigation demonstrate that profilin-immunized chickens fed the VAC- or MC-supplemented diets and infected with *E. tenella* had increased body weights, greater anti-profilin antibody levels, heightened profilin-induced lymphocyte proliferation, altered intestinal cytokine transcript levels, and augmented percentages of PBL subpopulations, compared with immunized and infected animals provided with a non-supplemented diet. Although the underlying mechanisms that are responsible for the dietary immune enhancing effects remain to be determined, these results provide the foundation for further studies to identify a safe and effective alternative to prophylactic medication for control of avian coccidiosis in commercial poultry production facilities.

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References


