Recent Advances in Biology and Immunobiology of Eimeria Species and in Diagnosis and Control of Infection with These Coccidian Parasites of Poultry

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INTRODUCTION

Coccidiosis is recognized as the parasitic disease that has the greatest economic impact on poultry production. The annual worldwide cost is estimated at about $800 million (107), and that for the American broiler industry about $450 million. These estimates include the costs of prophylactic in-feed medication for broilers and broiler-breeders, alternative treatments (e.g., with amprolium) if the medications fail, and losses due to mortality, morbidity, and poor feed conversions of birds that survive outbreaks.

LIFE CYCLE, GENETICS, AND BIOCHEMISTRY

Avian Eimeria spp. have homoxenous life cycles, which have been well described (38). A web site (http://www.iah.bbsrc.ac.uk/eimeria/) that clearly details the biology of coccidia has also become available. In general, oocysts shed in feces undergo sporogony (a meiotic process) in the external environment, a process requiring oxygen, taking about 24 h. The sporulated oocysts contain four sporocysts, each containing two sporozoites. Upon ingestion by a suitable host, oocysts excyst within the intestinal lumen. This process is aided by trypsin, bile, and CO2. The released sporozoites penetrate the villous epithelial cells. Sporozoites of some species (E. brunetti and E. praecox) develop within cells at the site of penetration. Sporozoites of other species (E. acervulina, E. maxima, E. necatrix, and E. tenella) are transported (1, 49, 95, 97) to other sites, for example the crypt epithelium, where they undergo development. Within the host cells, sporozoites undergo asexual reproduction (schizogony or merogony) in which nuclear division is followed by cytoplasmic differentiation, resulting in merozoites that break free and penetrate other host cells. These may carry out several more merogonic generations. Sexual reproduction or gametogony follows the last merogonic cycle. Merozoites enter host cells and develop into either male (microgamonts) or female (macrogamonts) forms. The microgamonts give rise to many microgametes, that exit, seek, and penetrate (fertilize) the macrogamonts that then develop into oocysts. Oocysts are shed with the feces. Prepatent periods may generally range from 4 to 5 days postinfection. Maximum oocyst output ranges from 6 to 9 days postinfection.

The biochemical and genetic mechanisms that control the development of Eimeria spp. within host cells are not known. However, through study of precocious and drug-resistant lines of E. tenella (86, 87), two linkage groups associated with these traits have been identified and mapped to parasite chromosomes 1 and 2. This information may help identify other genetic loci involved in regulating the life cycle of E. tenella. Genetic segregation data from this study are available through the internet (http://www.genome.org). Other researchers (70) have recently identified a gene (ets3a) whose expression is developmentally regulated and which may be important in controlling the life cycle of E. tenella. More genetic information on Eimeria and many other parasites can be found on the internet. Some of the websites include Eimeria (http://www.iah.bbsrc.ac.uk/eimeria/genome.htm), GenBank (http://www.ncbi.nlm.nih.gov), Parasitology Online (http://www.parasitology-online.com/), and George Washington School of Medicine (http://genome.wustl.edu/gsc/).

The metabolism of Eimeria spp. has come under new scrutiny with the increase in our efforts to define protective anti-
 gens for use in vaccines and to target metabolic pathways for chemotherapy. For example, a mannitol cycle has been characterized in *E. tenella* (12, 80) that apparently provides mannitol as an energy source for sporulation. This pathway may be widespread within *Eimeria* spp. (12). Newer characterized enzymes in *E. tenella* and *E. maxima* have been reported also, as well as a list of enzyme activities previously reported in avian *Eimeria* (108).

**SPECIES DETERMINATION AND DIAGNOSIS**

There are seven valid species of chicken coccidia, *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella* (83), each species developing in a particular location within the chick digestive tract. It is common to find at least six species (e.g., *E. acervulina*, *E. maxima*, *E. tenella*, *E. brunetti*, *E. mitis*, and *E. praecox*) in litter samples from a single flock during its first 6 weeks (106). Five of the species, *E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix*, and *E. tenella*, are well known and identifiable with relative ease, because they produce characteristic gross lesions. Their pathogenicities range from moderate to severe. On the other hand, *E. praecox* and *E. mitis* do not kill chickens or produce pathognomonic lesions and often have been considered to be benign. However, experimental infections result in enteritis, diarrhea, and reduced feed efficiencies (107), indicating that these two species certainly can cause commercial losses and hence need to be controlled.

Generally, the five most pathogenic species listed above can be differentiated in the host on the basis of clinical signs, characteristic lesions at particular sites of infection in the chicken intestine, and consideration of the prepatent period, size of oocysts, and morphology of intracellular stages. However, the less pathogenic species such as *E. mitis* and *E. praecox* might be overlooked. A very useful handbook is available (29) which describes standard diagnostic and testing procedures for *Eimeria* species and includes colored photographs of gross lesions caused by the most commonly found species.

Shirley (82) was the first to use a molecular biological approach to differentiate species on the basis of isoenzyme patterns of oocysts by starch block electrophoresis. Ellis and Bumstead (37) were among the first to demonstrate that rRNA and rDNA probes could be used to identify individual species through characteristic restriction fragment patterns. Procunier et al. (72) used a randomly amplified polymorphic DNA assay to differentiate *E. acervulina* and *E. tenella* and detect within-strain differences. Recombinant DNA techniques have been used to discriminate different strains of *E. tenella* (84) and develop markers for precocious and drug-resistant strains (86), and PCR amplification of internal transcribed spacer region 1 from genomic DNA has been used to detect and differentiate six *Eimeria* species (81). Eight species (including *E. hagani*) are claimed to be differentiated using a two-step PCR procedure (98), and six Australian species have been characterized using a PCR-linked restriction fragment length polymorphism approach (110). These PCR methods should prove very useful for epidemiological surveys of avian coccidiosis.

**IMMUNOBIOLOGY**

Great progress has been made in our understanding of the immune response to coccidial infection. Much of this has come from work on model infections with *E. vermiformis* in genetically altered mice (88). In mice, TCRγδ+ CD4− major histocompatibility complex type II (MHC-II)-restricted T cells that produce gamma interferon (IFN-γ) are essential for antigen-specific resistance to both primary and secondary infections. Additionally, B cells and interleukin-6 IL-6 are minor but necessary components of resistance to primary infections (88). Other, non-class II-restricted cells appear to be involved in effecting good “memory” responses to challenge infections.

Lack of chickens with genetic modifications of the immune effector systems (targeted gene disruptions), as well as the paucity of monoclonal antibody reagents, has made it more difficult to dissect the immune responses to avian coccidiosis. Nevertheless, the following facts are clear. There are genetic components to resistance to primary infections (50, 114). Chickens develop solid immunity to homologous secondary infections (74). Immunity does not prevent sporozoite invasion of cells but does prevent sporozoite development (14).

The effectors of immune responses to primary and challenge coccidial infections are primarily T cells residing in the gut-associated lymphoid tissues (58, 59, 60). Humoral immune responses also occur, but antibodies play a minor role in resistance and immunity to coccidia (51, 74). Chicken intestinal lymphocytes have been phenotyped using monoclonal antibodies, and found to exhibit markers homologous to those of murine and human CD3, CD4, and CD8 T cells (24, 27, 53, 60, 94). The CD3 polypeptide complex is found in association with the antigen-specific T-cell receptor (TCR) (21) characterized by αβ or γδ heterodimers (27). The CD4+ T (helper) cells recognize MHC-II antigens, and CD8+ T (cytotoxic/suppressor) cells recognize MHC-I antigens (60). In chickens, TCRγδ+ T cells can be either CD4+ or CD8− (60).

The roles of these T cells in response to avian *Eimeria* infections appear somewhat different from those in murine *Eimeria* infections. Studies in which these T-cell populations in chickens infected with *E. acervulina* or *E. tenella* have been selectively depleted by treatment with monoclonal antibody (96) show a different contribution of CD4+ and CD8+ T cells to resistance and immunity, which appears, as judged by oocyst output, to be dependent on the infecting species of coccidia. In chickens infected with *E. acervulina*, CD4+ T-cell depletion did not significantly increase oocyst production during either primary or challenge infection (96). Additionally, fewer oocysts were produced during challenge than during primary infection. These results suggest that CD4+ T cells are not significant effectors of resistance or immunity to *E. acervulina*. On the other hand, depletion of CD4+ cells in *E. tenella* infections resulted in increased oocyst output during primary but not challenge infection, indicating that CD4+ cells are important effectors of resistance to primary *E. tenella* infections.

Depletion of CD8− T cells significantly decreased oocyst output during primary infections of both *E. acervulina* and *E. tenella*. It is theorized that this effect is due to a reduction in the number of CD8− cells that serve as transporters for sporozoites (see below). However, CD8− cell depletion results in a larger oocyst output after a challenge infection. These results indicate CD8− cells may not be effectors of resistance in primary infection but are necessary effectors in immunity to challenge infections (59, 96). The importance of CD8− T cells in protective immunity to *E. tenella* infections is also suggested by the observation of a sharp transitory increase in the number of...
these cells in the peripheral blood (19) during primary infection.

In fact, CD8+ T cells seem to play dual role in the immune response to coccidial infections in chickens, as determined by two-color immunofluorescence staining of infected gut from chicken strains differing genetically in susceptibility to coccidiosis (59, 60). First, during primary infections with *E. acervulina* and *E. tenella*, the number of T cells coexpressing TCRβδ and CD8 markers increases in the epithelium. There they appear to be invaded by sporozoites. It is thought that these are the cells that transport sporozoites (1, 49, 95, 97) through the lamina propria to the crypt epithelium, where they exit and invade the crypt cells. Populations of CD8+ cells also increase in the mucosa following challenge infection, where they frequently can be seen in close contact with infected cells. It is theorized that they are functioning as cytotoxic cells, helping to eliminate the parasite challenge by killing the infected cells (59).

Coccidia invade other leukocyte types in the gut mucosa. For example, sporozoites of *E. tenella* have been found in heterophils (75) and merozoites of *E. tenella* have been found in goblet cells and mast cells (34). The relevance of these observations to immune control of avian coccidiosis is not yet clear.

Populations of cells bearing markers (low-level CD8+ and asialo-GM1) for natural killer (NK) cells increase early during primary infections (28, 52). These cells exhibit NK activity in vitro (23) and thus may be involved in immune surveillance.

Mucosal macrophages phagocytize sporozoites during primary and challenge infections, but this activity does not seem enhanced in the immune chicken (97). Macrophages may also function in sporozoite transport (99). Activated macrophages are the source of various inflammatory cytokines that can modulate cellular immune responses.

Increases in the numbers of mucosal mast cells have been noted early and late following primary and challenge infections with *E. acervulina* (76), but the contribution of these cells to effective immunity and resistance is not known.

Cytokines synthesized and secreted by leukocytes play important regulatory roles during the immune response to infection. In avian coccidiosis, it is clear that IFN-γ is produced by the host at sites of infection (78, 112), and IFN-γ release has been used as a measure of the T-cell response to coccidial antigens (68, 73). Treatment of coccidia-infected chickens with recombinant chicken IFN-γ (89) improved weight gains with respect to uninfected controls (55, 64). Tumor necrosis factor (TNF), an inflammatory cytokine, is secreted by activated macrophages. TNF-α-like activity can be detected in stimulated macrophages from infected chickens (22, 114) and in sera from infected chickens (113). Treatment of infected chickens with TNF-α exacerbated the suppression of weight gain due to infection, whereas treatment with polyclonal antibodies to TNF-α partially reversed the weight gain depression (114). These results suggest that TNF-α may play an important role in the pathophysiology of coccidiosis infections in chickens.

Free radicals, including reactive oxygen species and nitric oxide (NO), are products of activated macrophages and other phagocytic leukocytes and are known to be toxic to bacteria and some parasites (77). They are thus implicated as playing significant roles in resistance and immunity to infectious diseases (65, 71).

Peaks in the mucosal activity of NADPH oxidase, which generates superoxide (O2-), and in the levels of NO2- + NO3-, stable metabolites of NO, occur in a qualitatively different time pattern in chickens infected with *E. maxima* (days 1, 3, and 6 postinfection [p.i.] for NADPH oxidase, day 6 PI for NO2- + NO3- (2)). The differences in the time courses of change in these parameters during primary infection suggest the participation of separate cell types in their production. Plasma NO2- + NO3- levels are significantly elevated at about 6 days PI during primary infections with *E. acervulina* and *E. tenella*, as well as *E. maxima* (3, 9), but not during homologous challenge infections of well-immunized chickens. This observation suggests that NO production may be associated more closely with immune responses associated with innate resistance than with acquired immunity to coccidiosis.

There appears to be a genetic link between NO production during primary infection and resistance to *E. tenella* (9). A recent report (36) of in vitro studies suggests that macrophages stimulated by IFN-γ produce NO, which inhibits the replication of *E. tenella* within these cells.

**CONTROL OF COCCIDIOSIS**

**Role of Poultry House Management**

Because coccidial oocysts are ubiquitous and easily disseminated in the poultry house environment and have such a large reproduction potential, it is very difficult to keep chickens coccidia free, especially under current intensive rearing conditions. Oocysts sporulate readily in poultry house litter. However, they can be damaged by bacteria, other organisms, and ammonia that are also present, and their viability can begin to diminish after 3 weeks (106). Many producers in the United States basically remove caked litter, let the houses air out for 2 to 3 weeks, and top dress with fresh litter before placing a new flock (H. D. Danforth, private communication). On the other hand, it is common practice in most European countries and Canada to do a thorough cleanout between flocks. This practice may become more widespread in the United States as the effectiveness of anticoccidials continues to decrease and the use of live vaccines increases. Biocontrol measures such as requiring caretakers to change clothes between houses can minimize the spread of infective oocysts. The practice of strict biosecurity is essential in the care of broiler breeders.

**Prophylactic Control with Anticoccidials**

The effective use of anticoccidial feed additives over the past 50 years has played a major role in the growth of the poultry industry and has allowed the increased availability of high-quality, affordable poultry products to the consumer. These anticoccidials can be classified as (i) chemicals which have specific modes of action against parasite metabolism, such as amprolium, clopidol decoquinate, halofuginone, or (ii) polyether ionophores such as monensin lasalocid, salinomycin, narasin, and maduramicyn, which act through general mechanisms of altering ion transport and disrupting osmotic balance. These latter compounds are now the mainstay of coccidiosis control (41). A compendium of the most frequently used anticoccidials is available through Janssen Pharmaceutica (40).

It is quite clear, however, (25, 26, 79), that some degree of resistance to all anticoccidial drugs, including ionophores, has
developed. To minimize the effects of resistance, poultry producers rotate the use of various anticoccidials with successive flocks, combine chemical and ionophore treatments, or employ shuttle programs during a flock growout. Application of these treatment programs depends on seasonal conditions and prevalence of various species of coccidia (107). In recent years, pharmaceutical companies have not brought new anticoccidials to market. However, two potential drug targets, enzymes of the sporozoite mannitol cycle (12, 80) and trophozoite histone deacetylase, have been recently identified (80).

Vaccination

Avian coccidia are highly immunogenic, and primary infections can stimulate solid immunity to homologous challenges. Therefore, it would seem obvious that vaccines could offer excellent alternatives to drugs as a means of controlling coccidiosis. Efforts to develop various types of vaccines (57, 58, 61) have been continuous over the past several decades, but progress has been slow. Highlights of research on vaccine development are included here.

Recombinant vaccines. Over the past 10 years, much research effort has been spent on the development of recombinant vaccines. Although none are in commercial use, this research has served to highlight the complex nature of the avian host-coccidia interaction. A major hurdle to overcome in the development of a recombinant vaccine is the lack of cross-species immune protection. Other factors impeding the development of a successful vaccine have been recently reviewed (42, 100), the most important of which is the identification of protective antigens. Many potential coccidial antigens have been characterized and cloned. A total of 29 DNA sequences encoding immunity-stimulating *Eimeria* proteins from various species and developmental stages have been listed in a recent review (42). Many of these antigens are surface proteins or internal antigens associated with organelles such as micronemes (92, 93), rhoptries (91), and refractile bodies (101, 102). Recently (90) a low-molecular-weight immunogenic antigen with a single immunodominant epitope was reported to be present in all endogenous stages of *E. tenella*. Metabolic antigens from developing sporozoites (44, 45, 46), merozoite antigens (18, 100), and gamete antigens (103, 104) all elicit various degrees of protective immunity.

A delivery mechanism for coccidial vaccines that produces optimum resistance to challenge infection has yet to be determined. Immunogenic *Eimeria* antigens have been administered as isolated proteins with adjuvants (18, 100), as recombinant antigens in live vectors such as nonpathogenic strains of *Escherichia coli*, *Salmonella enterica serovar Typhimurium*, poxviruses, fowlpox virus, and turkey herpesvirus (93), and by direct plasmid DNA injection (43, 48, 54), with various degrees of success. More research is needed to determine how to target vaccines to the cellular immune effector systems that operate during natural primary and challenge infections and to determine the unique host immune responses to each parasite.

Because T-cell-stimulatory antigens appear to be the most relevant to protective immunity, T-cell proliferation assays (using T cells from recovering chickens) and IFN-γ production have been used to screen for protective antigens against *E. tenella* infections (20). The fraction of antigens that produced the highest T-cell proliferation and macrophage-activating activity, when administered as a vaccine, also yielded chickens with the lowest cecal lesion scores after challenge (20).

Live vaccines. Live vaccines for coccidiosis control have been used to a limited degree by the poultry industry for about 50 years. Their effectiveness hinges on the recycling of initially very low doses of oocysts, and the gradual buildup of solid immunity. They have been used primarily to protect breeder and layer flocks (85). However, their use, particularly in broiler flocks, is increasing.

Live vaccines contain either attenuated or virulent coccidial strains. Paracox (consisting of precocious strains of all seven species of chicken coccidia) and Livacox (consisting of precocious and egg-passaged lines) are attenuated vaccines. All coccidial strains in these vaccines are drug sensitive. Coccivac and Immucox are each composed of several virulent species. All four vaccines are commercially available. An in-depth comparison of these vaccines is provided by Bedrnik et al. (17). All of these vaccines are administered during the first week after hatch and will produce solid immunity when they are used carefully under good rearing conditions (30). Because live vaccines are multivalent (containing more than one species), tests of their efficacies require a different approach from tests of the efficacies of anticoccidial compounds. A standard protocol that is based on growth rates after challenge and that avoids lesion scoring and oocyst output enumeration has been published recently (109).

An advantage of attenuated vaccines is that they have low reproductive potentials, thus avoiding crowding in the specific mucosal areas of infection and resulting in the development of optimal immunity with minimal tissue damage (105). It is believed that the drug-sensitive, attenuated strains and wild, native strains interbred, reducing both virulence and drug resistance in local populations. Thus, the useful period of anticoccidial drugs could be extended by rotating their applications with live vaccines (107).

Coccivac and Immucox have also been used to treat broiler breeders, particularly in the United States and Canada. There has been a general reluctance to try them in broilers and heavy roaster birds, because of reduced weight gain and feed conversion compared to those in prophylactically medicated birds (30). Additionally, there is a risk of introducing unwanted *Eimeria* species into the environment. However, recent battery, floor pen, and broiler house trials (30) have shown that this type of vaccine (e.g., Immucox) can provide equal or superior performance, compared with prophylactic medication, when given in gel form at 1 day of age, because this method ensures the synchronous exposure of all birds to small uniform numbers of oocysts. (32, 33).

Antigenicity of coccidial strains can vary geographically (39, 66). Therefore, it is important to screen live vaccines against local populations before administering them to large flocks. Immunovariant strains can be isolated and substituted to provide region-specific, autogenous vaccines (30). Live vaccines can also be modified by incorporating drug-resistant strains. In floor pen trials, anticoccidially medicated broiler birds that had been immunized at 1 day of age with a drug-resistant strain of coccidia were protected almost completely against homologous challenge (30). When this vaccination regimen was applied to large flocks in two sets of paired broiler houses, vaccinated-
medicated birds had consistently lower (more efficient) feed conversions but slightly more variable final weights than did the medicated controls (30). Thus, this type of vaccine treatment has the potential to extend the life of anticoccidials cleared for use in geographical areas where drug resistance and lack of coccidial control is a problem (30).

Use of cytokines as adjuvants. As mentioned above, cytokines are major modulators of immune responses to infection, and therefore represent natural sources of immunostimulation that could be used as adjuvants in vaccines. IFN-γ has received the most attention as a possible adjuvant for an anticoccidial vaccine. Chicken IFN-γ has been cloned (35, 62, 89). Treatment with recombinant IFN-γ alone has been shown to moderate reductions in weight gain (55, 64) during *E. acervulina* and *E. tenella* infections and to moderate oocyst output (55) during *E. acervulina* infection. One problem with the in vivo use of cytokines is their rapid degradation and clearance (63). Administering them in DNA form or in a viral or bacterial vector could overcome this problem and provide a more practical method for treating large flocks. For example, a recombinant protein (3-1E) from *E. acervulina* merozoites, which is also found in *E. tenella* sporozoites and merozoites, stimulated IFN-γ production in chicken spleen lymphocytes. Direct injection of 3-1E cDNA induced protective immunity against *E. acervulina* challenge. Simultaneous injection of cDNA encoding chicken IFN-γ further enhanced immunity (56). Chickens treated with a fowl adenovirus chicken IFN-γ construct showed increased weight gains compared to untreated controls and exhibited smaller weight gain reductions on challenge with *E. acervulina* (47).

Alternative Controls Including Natural-Product Feed Additives

Many different types of substances have been investigated in the search for alternative controls of coccidiosis. Peptidyl membrane-interactive molecules are known to have antibacterial activities. Three of seven tested produced ultrastructural damage to sporozoite pellicles after a 5- to 10-min exposure to 5 μM concentrations in vitro (67). Oral doses (10, 50, or 75 μM) of two of them, which were methylated to prevent proteolysis, reduced lesion scores from challenge infections with *E. acervulina*, *E. tenella*, and *E. maxima*, suggesting that they have potential for coccidial control.

A number of natural products or feedstuffs have been tested as anticoccidial dietary additives. Some of this work has been recently reviewed (5). Sources of fats containing high concentrations of n-3 fatty acids (n-3 FA) (docosahexaenoic acid, eicosapentaenoic acid, and linolenic acid), such as fish oils, flaxseed oil, and whole flaxseed, when added to starter rations and fed to chicks from 1 day of age, effectively reduced lesions resulting from challenge infections with *E. tenella* (6, 7) but not *E. maxima* (7). The fish oil and flaxseed oil diets significantly reduced the degree of parasitization by and development of *E. tenella* (6) and caused ultrastructural degradation of both asexual and sexual stages, characterized by cytoplasmic vacuolization, chromatin condensation within the nucleus, and lack of parasitophorous vacuole delineation (31). These results are consistent with reports of the effects of high n-3 FA diets on other parasites (5) and suggest that these diets induce a state of oxidative stress (due to the high concentration of easily oxidized double bonds) that is detrimental to parasite development. Further support for the oxidative-stress hypothesis include the observations that (i) n-3 FA diets effective in controlling *E. tenella* reduce plasma carotenoid levels even in uninfected chickens (8), (ii) the effect on *E. tenella* appears related to the concentrations of double bonds in diets supplemented with n-3 FA ethyl esters (4), (iii) antioxidant-stabilized diets supplemented with up to 10% flaxseed do not protect against *E. tenella* (11) and (iv) diets supplemented with 15 and 20% chia seed (another seed with high linolenic acid content) and containing no additional antioxidants reduce *E. tenella* lesion scores (P. C. Allen and J. DeGroot, unpublished observations). Additionally, Michalski and Prowse (69) have shown sporulated oocysts and sporozoites of *E. tenella* to be deficient in superoxide dismutase, an enzyme that would protect them from damage by reactive oxygen species.

Artemisinin is a Chinese herb isolated from *Artemisia annua*; it is a naturally occurring endoperoxide with antimalarial properties. It has been found effective in reducing oocyst output from both *E. acervulina* and *E. tenella* infections when fed at levels of 8.5 and 17 ppm in starter diets (10). The mode of action is also thought to involve oxidative stress.

Most recently, extracts from 15 Asian herbs were tested for anticoccidial activity against *E. tenella*. Of the species tested, extracts from *Sophora flavescens Aiton* was the most effective in reducing lesion scores, maintaining body weight gain, and reducing oocyst production. (111)

Feeding supplementation with antioxidants such as γ-tocopherol (8 ppm), found plentifully in seed oils such as wheat, corn, and soybean, and the spice turmeric (1%), as well as its main medicinal component, curcumin (0.05%), appear effective in reducing upper- and mid-small-intestinal infections caused by *E. acervulina* and *E. maxima* (5). They are not beneficial for *E. tenella* infections, however. The osmoprotectant betaine (from sugar beets) has long been known to have beneficial effects on livestock growth and performance. When fed at 0.15% along with 66 ppm salinomycin in floor pen studies, betaine had beneficial effects on weight gains and feed conversion and resulted in reduced mortality. In tissue culture, betaine and salinomycin significantly reduced cell invasion by *E. acervulina*. It is concluded that this combination may improve performance in coccidially infected chickens directly by its action on *E. acervulina* development and indirectly by its action as an osmoprotectant, supporting intestinal structure and function. (13, 15, 16).

These studies emphasize the unique biology of the individual *Eimeria* species that parasitize chickens, and a pattern of effectiveness of various dietary treatments seems apparent. Products that can generate a state of oxidative stress, such as n-3 FA and artemisinin, are particularly effective against the cecal parasite *E. tenella*. Products that have antioxidant properties, such as γ-tocopherol and curcumin, seem to be more effective against the mid- and upper-small intestinal species *E. maxima* and *E. acervulina*. The osmoprotectant betaine appears to be most active against *E. acervulina*. Practical applications of these findings, such as the use of the products in starter rations or combinations of them with current anticoccidials or vaccines, appear possible and need to be investigated.
DIRECTION OF FUTURE RESEARCH

Over the past several decades, knowledge about the genetic makeup of avian Eimeria spp. and the immunobiological interaction of the avian host with the coccidian parasites has increased dramatically. The impetus for this expansion has come from efforts to find new, effective ways to control coccidiosis in the face of increased resistance of the parasites to traditional anticoccidial pharmaceuticals. Much of the work on both the parasites and the host has been aided by the development of techniques in molecular biology and driven by the search for effective vaccines.

The ultimate key to coccidiosis control may well come in part from research directed toward a better understanding of parasite biology and biochemistry. Some of the questions that need to be answered include the following. Why are avian coccidia host and site specific? What are the important criteria that allow parasites to invade a host cell? What are the biochemical defense mechanisms that promote survival within the host? What genes control the development of coccidia within the host? New developments or improvements in in vitro cultivation methods, the current world-wide push to unravel the parasite genome, and the availability of genetic sequence information in publicly accessible databases, along with new techniques in proteomic research, should help to answer these questions.

Further insight into the control of coccidiosis will also come from research on the avian host that is directed to the following questions. What are the host genetic components associated with innate and acquired resistance to coccidial infections (i.e., identification of qualitative trait loci) (115)? What are the immunological restrictions that limit cross-protection among species? What are the differences in host cellular responses between live infection and vaccination? Current and future research using microarray and proteomic technologies will begin to answer some of these questions. It is anticipated that large advances will be made in the understanding and control of avian coccidiosis within the next decade.

REFERENCES