

DIAGNOSIS AND CONTROL OF POULTRY COCCIDIOSIS: AN UPDATE

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SUMMARY

Coccidiosis, caused by various *Eimeria* species, is one of the most important infectious diseases of poultry, which occurs mainly in caecal and intestinal forms. Diagnosis of coccidiosis is usually based on post-mortem lesions and faecal examination for oocysts, when damage has already been occurred. For combating coccidiosis, anticoccidials are continuously added in feed and water which may result in development of resistant strains. Number of vaccines are in use; however, almost all of these are either live virulent or live attenuated vaccines. There is an urgent need to develop a subunit/recombinant vaccine, which may provide multi-species protection. Molecular biology techniques are being exploited for diagnosis and control of poultry coccidiosis. Additionally other means like good managemental practices, dietary modulations by using probiotics, herbal polysaccharides, vitamins and poly-herbal coccidiostats are being exploited to control coccidiosis.

Key words: Coccidiosis, anti-coccidials, vaccines, probiotics, dietary modulations

Small scale poultry production is very important in developing countries and it seems that it will be important for many years to come because of the high population densities and the enormous numbers of small farms (Ellis, 1992). Chickens in India are usually reared under intensive conditions particularly in the rural areas which are conducive to infections by opportunistic pathogens. Among the infectious diseases of poultry, coccidiosis caused by the intracellular protozoan parasite of *Eimeria* spp, is the major parasitic disease. World-wide losses to poultry industry due to coccidiosis have been estimated > US \$ 3 billion per year (Shirley *et al.*, 2004). Though nine species of *Eimeria* have been identified as causative agents of poultry coccidiosis, but only seven of them have been reported to be pathogenic (Kahn, 2008). The species important in broiler production include *Eimeria tenella*, *E. maxima*, *E. acervulina* and *E. mivati*; whereas, the species important in breeder and egg- layers are *E. brunetti* and *E. necatrix*. However, under stress conditions, the mild species may also turn pathogenic.

Till date in majority of the cases the major tools used for the diagnosis of coccidiosis in poultry include faecal examination for oocyst findings and typical post-mortem lesions. The oocysts are passed in the faeces, when the damage due to other developmental stages of *Eimeria* spp. has already been caused. Molecular

biological techniques are being exploited for an early and accurate diagnosis of coccidiosis.

Eimeria species affecting poultry are strictly host specific and various species parasitize the specific parts of intestinal tract. The short, direct life cycle and high reproductive potential of coccidia intensifies the potential for severe outbreaks of disease. Though, extensive use of anticoccidials in-feed has a check on clinical outbreaks, the sub-clinical coccidiosis due to emerging drug resistance is still the most important cause of performance loss in the world poultry industry today. Due to development of resistant strains of the parasite, most of the laboratories are engaged in research on alternate approaches of controlling coccidiosis like vaccination, herbal medicines, dietary modulations etc.

Coccidiosis is largely a disease of young birds because immunity is low and it quickly develops after exposure and gives protection against later disease outbreaks. However, birds can be infected at any age if not exposed earlier. Chickens get the infection by eating oocysts once these are sporulated after being shed in the droppings of infected birds. They pick them up by pecking on the ground or in litter used for bedding in the house. Oocysts can also be spread by insects, dust, wild birds and humans through shoes and equipment. A coccidial infection differs from bacterial

and viral infections because coccidia are 'self-limiting'. Once sporulated, the oocyst remains infective for months if protected from very hot, dry or freezing conditions. In large poultry houses, oocysts do not last long in the litter because of the action of ammonia released by decomposition of litter and manure and by the action of molds and bacteria. However, there are usually so many oocysts that birds continue to pick up and get sick. Outbreaks of coccidiosis usually occur when birds are between 3 and 8 weeks of age.

Eimeria spp. multiply in the intestinal tract and cause tissue damage. This damage can interfere with the food digestion and nutrient absorption as well as cause dehydration and blood loss. The tissue damage can also expose the bird to bacterial infections, like *Clostridium* and *Salmonella*. Diseases that suppress the bird's immune system may act with coccidiosis to produce a more severe problem. Marek's Disease may interfere with the development of anticoccidial immunity and Infectious Bursal Disease may exacerbate a coccidial infection. An outbreak of coccidiosis if left untreated, eventually runs its course and survived birds will gain immunity but production may never recover. If the infection is severe, the gut remains scarred and impaired and stunted broilers do not catch up in weight gain.

DIAGNOSIS

It includes both conventional as well as molecular/modern methods of diagnosis.

Traditional diagnosis: Diagnosis in majority of the cases is based upon examination of faecal samples for oocyst findings and typical post-mortem lesions. Each *Eimeria* species develops in a particular location within the chick digestive tract. The oocysts of six species including *E. acervulina*, *E. maxima*, *E. tenella*, *E. brunetti*, *E. mitis*, and *E. praecox* are commonly seen in litter samples during first 6 weeks (Williams, 1995). Further most of the species can easily be identified on post-mortem examination as they produce characteristic gross lesions at specific sites in the intestine (Conway and McKenzie, 2007, Kahn, 2008) Their pathogenicity ranges from moderate to severe. Various criteria used to differentiate *Eimeria* spp include clinical signs, characteristic lesions at particular sites of infection in intestine, pre-patent period, size of oocysts, sporulation

time, morphology of intracellular stages etc. Outward signs of coccidiosis include droopiness and listlessness, loss of appetite, loss of yellow colour in shanks, pale combs and wattles, ruffled, unthrifty feathers, huddling or acting chilled, blood or mucus in the faeces, diarrhoea, dehydration and even death. Other signs include poor feed digestion, poor weight gain and poor feed efficiency. Some symptoms can be confused with other diseases, like necrotic enteritis, that also causes bloody diarrhoea. It is advised that on suspicion, do a necropsy and look for the developmental stages of the parasite and/or the characteristic lesions or sores in the gut. Coccidiosis causes a thickening of the intestines, which make them feel like a sausage. *Eimeria acervulina* affects the upper part of the small intestine. One may see small red spots and white bands on it. *Eimeria maxima* affects the entire small intestine. The intestines look watery and in later stages have blood and mucus. The intestine may look thickened and ballooned with red pinpoint lesions. *Eimeria tenella* affects the blind sacs (caeca) of the gut. They may be filled with blood and pus and turn into a solid core. *E. necatrix* produces major lesions in the anterior and middle portions of small intestine. Small white spots usually intermingled with rounded, bright- or dull-red spots of various sizes can be seen on the serosal surface. In severe cases, the intestinal wall is thickened, and the infected area dilated to 2-2.5 times the normal diameter. The lumen may be filled with blood, mucus and fluid. *Eimeria brunetti* is found in the lower small intestine, rectum, caeca and cloaca. In moderate infections, the mucosa is pale and disrupted but lacking in discrete foci and may be thickened. In severe infections, extensive coagulative necrosis and sloughing of the mucosa occurs throughout most of the small intestine. *Eimeria mitis* is recognized as pathogenic in the lower small intestine. Lesions resemble moderate infections of *E brunetti* but can be distinguished by finding small, round oocysts associated with the lesion. *Eimeria praecox*, which infects the upper small intestine, does not cause distinct lesions but may decrease rate of growth. It is considered to be of less economic importance than the other species. *Eimeria hagani* and *E. mivati* are of dubious status but are thought to develop in the anterior

part of the small intestine.

Molecular diagnosis: Traditional methods have major limitations in the specific diagnosis of coccidiosis; there have been significant advances in the development of molecular diagnostic tools. The use of molecular biological approach to differentiate various *Eimeria* species on the basis of iso-enzyme patterns of oocysts by starch block electrophoresis was first done by Shirley (1975). Later on Ellis and Bumstead (1990) demonstrated that rRNA and rDNA probes could be used to identify individual species through characteristic restriction fragment patterns. To differentiate *E. acervulina* and *E. tenella*, Proconier *et al.* (1993) used a randomly amplified polymorphic DNA assay. Recombinant DNA techniques were also used to discriminate different strains of *E. tenella* and to develop markers for precocious and drug-resistant strains (Shirley and Harvey, 1996). Schnitzler *et al.* (1997) used PCR amplification of internal transcribed spacer region 1 from genomic DNA to detect and differentiate six *Eimeria* species. Later, same technique was used by Su *et al.* (2003) for differential diagnosis of five *Eimeria* species in Taiwan. Similarly, eight species including *E. hagani* were differentiated by Tsuji *et al.* (1997) using a two-step PCR procedure. PCR-linked restriction fragment length polymorphism approach has also been used to characterize six *Eimeria* spp in Australia (Woods *et al.*, 2000). Fernandez *et al.* (2003) reported the development of a novel multiplex PCR assay based on Sequence-Characterized Amplified Region (SCAR) markers for the simultaneous diagnosis of seven *Eimeria* species. They found it as a rapid and cost-effective diagnostic method for the detection and discrimination of *Eimeria* species that infect domestic fowl. A PCR-based capillary electrophoresis approach has been evaluated for the identification of seven currently recognized *Eimeria* species affecting poultry and has been reported another time and cost effective tool (Gasser *et al.*, 2005, Morris *et al.*, 2007). Based on 422 base pairs derived from the 3 prime region of the 18S rRNA gene, containing polymorphic DNA sequences of *Eimeria*, the technique of PCR-RFLP was developed by Choosaksangthong *et al.* (2005). This technique was found to be useful in identifying species-specific DNA profiles of four

important *Eimeria* species. An improved PCR-based method for determining the species composition of *Eimeria* in poultry litter was developed by incorporating species-specific internal standards in the assay (Jenkins *et al.*, 2006). Haug *et al.* (2007) compared three steps such as oocyst wall rupturing methods, DNA extraction methods and identification of species-specific DNA sequences with PCR of five protocols for molecular identification of chicken *Eimeria* species in field samples. The studies indicated that rupturing the oocysts by mini-pestle grinding, preparing the DNA with Gene Releaser followed by optimised single species PCR assays, makes a robust and sensitive procedure for identifying chicken *Eimeria* species in field samples. Importantly, it also provides minimal hands-on-time in the pre-PCR process, lower contamination risk and no handling of toxic chemicals. Recently, Haug *et al.* (2008) used morphometric and polymerase chain reaction technique (targeting the ITS-1 region of the genome) for the identification of *Eimeria* species and reported that PCR technique is better than morphometric technique. It seems that these PCR methods will prove very useful for detection and differentiation of various *Eimeria* species in the near future.

CONTROL MEASURES

Managemental control: 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. Management has always been important to coccidiosis control and it focuses on reducing the number of coccidia to keep infection at a minimum until immunity is established (Fanatico, 2006).

Natural immunity: A small-scale, low-density production system can allow a low level of exposure to coccidia, which permits the chicks to develop immunity without triggering the disease. However, birds may not pick up enough parasites to cause immunity or they may be overwhelmed by too many. In addition, immunity is only species-specific (Chapman, 2000). Exposure to one type of coccidia will not protect a chicken from the other types that can infect it. Early detection is a management method to avoid the use of

preventative medication. High-density, large-scale production almost always requires the use of anticoccidial medication. Many small-scale producers do not use anticoccidial medication; however, as the size of the flocks grows, more problems are encountered and more management is required to attain natural immunity. Immunity is especially important in turkeys, layers, breeders, and slow-growing broilers that are kept longer than fast-growing broilers marketed at a younger age.

Brooder and grower management: If chicks are brooded separately before moving them to grower facility, they stay clean of infective oocysts as fast growing broilers do not remain past three weeks of age. However, chicks are at risk for coccidiosis if they stay in the brooder longer than three weeks. Pullet chicks for egg laying grow slowly and stay in the brooder longer. If chicks are brooded and grown out in the same facility, they seed the area with coccidia. However, the good brooding practices can reduce the need for medication. Give birds adequate floor space to prevent overcrowding. There should be at least one square foot of floor space per chick (Plamondon, 2003). Keep the feeders full, if feeders go empty, birds forage in the litter and ingest oocysts. The longer they peck at contaminated litter the more oocysts they will ingest.

Sanitation: It focuses on good hygiene and removing infected droppings. Put waterers and feeders at a height level with the backs of the birds, so that they cannot defecate or scratch litter into them. Clean waterers and feeders frequently (Kahn, 2008).

Litter: Add fresh litter or rake litter frequently to cover parasites. Keep the litter dry to reduce sporulation of oocysts. Remove any wet or crusted litter. In the large-scale industry, 'new-house coccidiosis syndrome' sometimes occurs when birds are placed on brand-new litter. There is no low-level population of coccidia to establish immunity, so the flock is more susceptible. Some small flock producers are interested in the built-up or composting litter as an ecosystem of microbes. Poultry-house litter becomes significantly anti-coccidial after about six months' use as organisms that eat coccidia start to thrive and knock down the coccidia population. By never removing more than half the brooder house litter at a time, it can keep its anti-

microbial properties indefinitely. However, little scientific information is available on composting litter (Fanatico, 2006).

Ventilation: Housing should prevent drafts but not be airtight. Humidity, along with ammonia and other gases, needs to escape.

Pasture: Some producers provide outdoor access to allow poultry to express natural behaviour, increase space, and to provide fresh air and sunlight. Outside, birds may pick up fewer oocysts, since they are more likely to peck forage instead of droppings; however, access to the outdoors has both advantages and disadvantages for coccidial control. Extreme heat and cold outdoors can reduce sporulation or kill oocysts. Yet warmth and moisture are favorable conditions for coccidia. The locations of the waterers and feeders, the pasture, and the house itself, if possible, should be rotated (Sainsbury, 1984).

Cage system: A change from floor to cages greatly reduces the exposure to coccidia. Outbreaks of coccidiosis rarely occur in laying hens maintained in cages. Even prophylactic use of anticoccidials is not required if the cages are kept clean and the faeces do not contaminate watering and feeding system (Kahn, 2008).

Anticoccidial drugs: The effective use of anticoccidials over the past 50 years has played a major role in the growth of poultry industry and has allowed the increased availability of high-quality, affordable poultry products to consumers. During this period a number of drugs have been introduced, many of which are available and used today. However, there is increasing concern about rising levels of drug resistance (Chapman 1997). In India also varying degree of efficacy has been reported against various anticoccidials including ionophores (Yadav and Gupta, 2001, Gautam and Gupta, 2004). Anticoccidials can be classified as (i) Synthetic drugs (chemicals) - the first to be introduced and have specific modes of action against parasite metabolism and these include amprolium, clodol, decoquinate, halofuginone, nicarbazin, robenidine etc. The recent among them are diclazuril and toltrazuril. Roxarsone was found to have anti *E. tenella* activity and worked well in combination with ionophores (Chapman *et al.*, 2004). (ii) Polyether ionophores : They kill coccidia by

interfering with the balance of important ions such as sodium and potassium. The host cells are able to manage these ions in the presence of ionophores, but the parasites cannot. These include monensin, lasalocid, salinomycin, narasin, maduramycin and semduramicin. These are now an important component of coccidiosis control. Though no new drug has been introduced in the market in recent years, two potential drug targets i.e. enzymes of sporozoite mannitol cycle (Allocco *et al.*, 1999) and trophozoite histone deacetylase have been identified (Schmatz, 1997). Emergence of resistant strains of coccidia is a great problem with most of the drugs, which in due course, limit their use. To minimize the resistance alteration of drugs i.e. rotation of different compounds (having different mode of action) between flocks and shuttle programme i.e. using one compound for the starters and another for the growers are quite effective. Further, some ionophores can be used in combination with live virulent vaccines, therefore, the use of ionophores-tolerant resistant strains would probably have a wider application of the development of anticoccidial vaccines for suitable control of

coccidiosis (Danforth, 2000).

Immunoprophylaxis: In the current scenario, vaccines do cost more than in-feed anticoccidials. However, considering the bleak prospect of these drugs comprising resistance, anxiety about residuals, costs and time involved in new discoveries, vaccines are set to become the primary and, in some cases, exclusive means of control. *Eimeria* spp are highly immunogenic and primary infections can stimulate solid immunity to homologous challenges. So, immunological control has been recognized as the major practical alternative to chemotherapy for coccidiosis control. Efforts to develop various types of vaccines have been continuous over the past several decades, but progress has been slow (Lillehoj and Lillehoj, 2000).

The use of live vaccines for the control of coccidiosis in broiler breeders or layers is well established. Their use in broilers has been slow to gain acceptance considering the economics, perceived adverse effect on chick growth with virulent vaccines and concerns about timely onset of protective immunity in short lived birds.

Table
Details of available vaccines against poultry coccidiosis

Vaccine	Description	Mainly meant for
Coccivac® D	Wild type, 7 species, oral	Breeders/Layers
Coccivac® B	Wild type, 4 species, oral	Broilers
Immucoc® C ₁	Wild type, 4 species, oral	Broilers/Breeders
Immucoc® C ₂	Wild type, 6 species, oral	Broilers/Breeders
ADVENT®	Wild type, 3 species, oral	Broilers
Nobilis® COX-ATM	Wild type, 3 species, oral (ionophore resistant)	Broilers
VAC M®	Wild type, single species, oral	Broilers
Inovocox	Wild type, 3 species, <i>in ovo</i> injection	Broilers
Livacox® D	Attenuated, 2 species, oral	Caged chicken
Livacox® T	Attenuated, 2 species, oral	Breeders/Broilers
Livacox® Q	Attenuated, 4 species, oral	Broilers
Paracox®	Attenuated, 7 species, oral	Breeders/Layers
Paracox® 5	Attenuated, 4 species, oral	Broilers
Eimervax® 4m	Attenuated, 4 species, oral	Breeders/Layers/Broilers
Eimerivac® Plus	Attenuated, 3 species, oral	Breeders/Layers/Broilers
Immuner® Gel-Coc	Wild type and attenuated, 3 species, oral	Breeders/Layers/Broilers
Supercoc®	Wild type and attenuated, 3 species, oral	Broilers
CoxAbic®	Subunit vaccine from <i>E. maxima</i> gametocytes	Breeders/ Broilers

Live-virulent vaccines: Comprise a variable number of wild type strains depending on their formulation and field of application (Lee, 1987). They induce long-lasting protective immunity leading to equal or superior performance compared with prophylactic medication. Commercial vaccines of this type are CocciVac® and Immucox®. However, there are certain drawbacks like possible introduction of new pathogenic species in the environment and secondly, there may be antigenic variability between *Eimeria* species present in the vaccines and those in the field.

Live-attenuated vaccines: These vaccines are prepared by attenuation of live parasites. Attenuation may be done by the following methods :

- i. Selection for precocious strains: This is achieved by repeated *in vivo* passage of virulent parasite and selection for early maturing, less pathogenic oocysts with each passage (Shirley, 1993). Precocious strains, when compared with their parental strains, exhibited reduced pre-patent time, modified intracellular development, decreased reproductive potential and diminished infectivity (McDonald *et al.*, 1986). Two vaccines using biologically attenuated *Eimeria* strains are Paracox® and Livacox®.
- ii. Attenuation by serial passage in embryonated eggs: Though loss of virulence usually occurs upon parasite's passage in eggs and this method of vaccine development has consequently been discontinued. An alternative method is adapting the parasite to grow in cells grown in tissue culture. Brake *et al.* (1997) reported that the tissue culture derived parasite antigens obtained from *E. tenella* infected SB-CEV-1/F7 cell line are immunogenic and can provide partial protection against *E. tenella* clinical coccidiosis.

Live tolerant to ionophores vaccines: These vaccines comprise of strains of different species of *Eimeria*, which are relatively tolerant to ionophores. The advantage of these vaccines is that they allow the use of ionophores during the first 3-4 weeks when immunity is not complete. Such use limits the increase of infection pressure due to expanding field strains during the period of development of immunity, which further reduces the overall risk of contracting coccidiosis.

(Vermeulen *et al.*, 2001).

The formulations of all other commercially available live anticoccidial vaccines are currently based upon the scientific principles established for Coccivac®, Paracox® or Livacox®. Number of species included in these vaccines also varies. VAC M contains single species whereas others have two or more *Eimeria* species. It is always desirable to choose a vaccine containing species prevalent in the particular geographical area/farm. Because of their low productive potential, they induce optimal immunity with minimal tissue damage (Williams, 1994). However, the higher production cost associated with lower yield of oocysts in chickens used for generating vaccine adds to their disadvantage.

Subunit/Recombinant vaccines: Live vaccines have the problems of safety, short shelf-life and need for large scale production. Recent efforts to develop a genetically engineered vaccine against *Eimeria* have given some encouraging results. Identification of stage specific, protective antigens as well as devising suitable delivery methods have been pivotal in subunit vaccine development. A lot of progress has been made regarding antigenic characterization of different developmental stages of *Eimeria* species and also regarding expression vectors. CoxAbic®, a subunit vaccine with affinity purified proteins of *E. maxima* gametocytes when used in breeding hens, protected their broilers against three species (*E. maxima*, *E. tenella* and *E. acervulina*) upon challenge (Ziomko *et al.*, 2005). The chicken IFN- γ and cytokines have also been exploited as vaccine immunomodulators. Song *et al.* (1997) cloned IFN- γ gene which when transfected into chicken fibroblast cells, inhibited intracellular development of *E. tenella* after *in vitro* infection. These findings related to IFN- γ raise the exciting possibility of using IFN- γ immuno-prophylactically to control coccidiosis in commercial poultry flocks. The Et1A (*Eimeria tenella* refractile body gene) ligated with mammalian expression vector pcDNA3-SO7 resulted in significant reduction in caecal lesions and weight loss upon subsequent *E. tenella* challenge (Kopko *et al.*, 2000). Recently, Wu *et al.* (2004) constructed two DNA vaccines with genes encoding *E. tenella* sporozoite antigens, which on following administration resulted into reduced oocyst shedding and decreased weight loss.

DNA vaccines: *In-ovo* administration of oocysts, sporocysts or spozoites has been shown to induce protective immunity in newly-hatched chicks (Weber and Evans, 2003). With this finding in sight, *in ovo* vaccination with DNA vaccines has been the focus of recent research. *In ovo* immunization using recombinant gene 3-1E from *E. acervulina* alongwith cytokine genes reduced faecal oocyst shedding and enhanced body weight gain following infection with *E. acervulina*. (Lillehoj *et al.*, 2005). Further, it has been demonstrated that EtMIC2 gene has got the prospect of a viable candidate for recombinant vaccine at least by *in ovo* delivery method (Ding *et al.*, 2005). Exploiting the flexibility of DNA vaccines with respect to mode of administration, Du and Wang (2005) tested the efficacy of an oral DNA vaccine carrying *E. tenella* 5401 antigen gene delivered by attenuated *Salmonella typhimurium* and reported induction of strong humoral and cell mediated immunity. The vaccine also offered partial protection to chickens against *E. tenella* challenge thus demonstrating that an attenuated strain of *S. typhimurium* could be utilized as the oral delivery vector for recombinant eukaryotic expression plasmids as DNA vaccines. Recently, Hafeez *et al.* (2007) reported that maternal immunization with egg-propagated gametocyte vaccine can control *E. tenella* infections in offspring chicks.

Designing of synthetic peptide-based vaccines based on T and B cell epitope mapping strategy and use of cytokine adjuvants may give further fillip to the current research undergoing in subunit vaccines. There remains the challenge of designing peptide vaccines with multispecies protection, possibly through better epitope mapping and combination of multiple reactive epitopes.

Genetically resistant birds: It is well documented that avian genetic factors greatly influence immunity to coccidiosis. This is known for both outbred and inbred chicken lines. Pinard-Van Der Laan *et al.* (1998) compared five outbred lines for resistance to experimental infection with *E. tenella* as a tool to search for genetic markers of disease resistance. Large differences in disease resistance were observed between the lines although no effect of the major histocompatibility complex (MHC) was detected. On

the other hand a number of studies have documented the effect of MHC on acquired immunity to *Eimeria* (Nakai *et al.*, 1993).

Dietary modulations: The natural products were in use even before the availability of effective anticoccidial drugs. Supplements of skim milk, butter milk or whey were effective in reducing the severity of coccidial infection (Beach and Corl, 1925, Becker, 1937). Certain feed components directly or indirectly enhance severity of coccidiosis e.g. vitamins (biotin, riboflavin and niacin), non-starch polysaccharides, salt content etc. On the other hand, certain components restrict the severity of coccidiosis like vitamins (A, E, K), n-3 fatty acids, betaine, fibres etc (Allen *et al.*, 1998).

Phytochemicals and herb polysaccharides: Polysaccharide extracts from two mushrooms (*Lentinus edodes* and *Tremella fuciformis*), and a herb (*Astragalus membranaceus*) when given as feed supplement to chickens, significantly enhanced both cellular and humoral immunity to *E. tenella* (Guo *et al.*, 2004). Artemisinin from *Artemisia annua* has been found effective in reducing oocyst output from both *E. acervulina* and *E. tenella* (Allen *et al.*, 1997). Extracts from *Sophora flavescens* have shown promising results against *E. tenella* (Youn and Noh, 2001). Neem fruit @150gm/50 kg in feed was found to have good anticoccidial efficacy against *E. tenella* (Tipu *et al.*, 2002). Dakpogan (2005) used papaya leaf powder at 15 % level in feed against *E. tenella* infections and found favourable results in terms of weight gain, FCR and reduced oocyst output. Recently, Hadimani and Gupta (2010a) demonstrated the anticoccidial efficacy against *E. tenella* of both papaya and neem leaf powders and possible humoral immunomodulation of papaya when included as feed additive. Similarly, green-tea based diets were evaluated in chickens following oral infection with *Eimeria maxima*. Though the faecal oocyst counts were significantly reduced as compared to infected control, however, such diets did not improve the body weights (Jang, 2007). In India, the effect of polyherbal anticoccidials has been seen against various life cycle stages viz., schizonts and gametocytes (Kurkure *et al.*, 2006). The polyherbal coccidiostat AV/CCP/22 containing *Holerrhena antidysentrica*, *Barberia* spp. and *Alium* spp., has

been found effective against endogenous stages of *E. tenella*. Being eco-friendly in nature and due to less chances of development of resistance these can be better option for chemoprophylaxis (Pangasa *et al.*, 2007). Further, herbal immuno-modulators have given the best performance in terms of weight gain and lower mortality (Singla *et al.*, 2007).

Seed oils: Seed oils from wheat, corn, soybean containing γ -tocopherol, and the spice turmeric appeared effective in reducing coccidiosis by *E. acervulina* and *E. maxima* (Allen *et al.*, 1998).

Unsaturated fatty acids: Sources containing high concentration of n-3 fatty acids like fish oil, flaxseed oil and whole flaxseed, when added to starter rations, effectively reduced lesions from *E. tenella* but not from *E. maxima* (Allen *et al.*, 1997).

Betaine: A naturally occurring amino acid derivative, when used in conjunction with salinomycin was found to have a significant effect on invasion by *E. acervulina* and *E. tenella* (Augustine *et al.*, 1997).

Vitamins: Vit K is now universally added to feed formulas as it reduces mortality from *E. tenella* and *E. necatrix* by decreasing clotting time. Deficiency of vit A impaired the local immune defenses within the gut lymphoid tissues of broilers by reducing the intraepithelial lymphocyte (IEL) subpopulations, which lowered the ability of broilers to resist *E. acervulina* infection. Further, vit A deficiency affected the systemic immunity and lowered IFN- γ secretion (Dalloul *et al.*, 2002).

CpG oligodeoxynucleotides (ODNs): Dalloul *et al.* (2004) have identified short ODNs containing unmethylated CpG motifs for chickens and reported that these do activate innate immunity and enhance protective immune response against coccidia.

Probiotics: Probiotic supplementation of the intestinal microflora has been shown to enhance gut defensive mechanisms in poultry. Studies involving commercial probiotics fed in *E. acervulina* infected broilers have proven beyond doubt that direct fed-microbials significantly enhance the gut and systemic CMI response (Dalloul *et al.*, 2005). Further, the addition of *Pediococcus acidilactici* based probiotic in broilers diet effectively enhanced the resistance of birds and partially protected against the negative growth effects

associated with coccidiosis (Lee *et al.*, 2007). Recently, Hadimani and Gupta (2010b) have reported the possible antagonistic action of probiotic cultures of *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* with *E. tenella*, atleast during a heavy experimental challenge of the latter.

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