

## **M52, a novel natural feed additive, preserved mucosal immune and intestinal microbial homeostasis of broilers challenged with *Eimeria* spp.**

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M52 is a natural anticoccidial feed additive featuring a select blend of bioactive phytochemicals. Recent research suggests the formulation has potential as an alternative to ionophores and chemical coccidiostats to help prevent coccidiosis while improving gut health and maintaining bird performance. This study further explored the potential effects of the product on host coccidial immunity, the composition and structure of the gut microbiome, and intestinal integrity of broilers challenged with experimental coccidiosis.

In this 28-day study, day-old Cobb broiler chicks were randomly assigned to 1 of 3 groups: 1) unchallenged control; 2) *Eimeria*-infected control; and 3) *Eimeria*-infected + M52 (70g/MT feed). On d14 chickens from Treatment 2 & 3 were challenged with 100X Bio-Coccivet R Vaccine consisting of sporulated oocysts of seven *Eimeria* spp. Peripheral blood mononuclear cell phenotype, ceca-cecal tonsil cytokine mRNA expression, gut microbiome of cecal content and duodenal/jejunum histopathology were examined.

M52 markedly alleviated *Eimeria*-induced histopathological changes, i.e., prominent villus hyperplasia, heterophil mucosal infiltration, and hemorrhagic or necrotic foci on d19. On d28, M52 treatment better preserved the competence of protective mucosal immunity by significantly increasing the abundance of mucosal cytotoxic (CD4-TCRVβ1+) and helper (CD4+TCRVβ1+) T cell and immune-responsive CD8-CD28+ helper T cell subsets (vs. *Eimeria*-infected control,  $P < 0.05$ ). Further, on d19, M52 dampened *Eimeria* challenge-associated upregulation of cecal IL-10 (vs. *Eimeria*-infected control,  $P < 0.01$ ), which may help overcome parasite immune evasion. *Eimeria* challenge significantly decreased the  $\alpha$ -diversity of the cecal microbiome (vs. unchallenged control,  $P < 0.05$ ), which was completely normalized with M52 treatment (vs. *Eimeria*-infected control,  $P < 0.05$ ). Furthermore, M52 conferred challenge-independent protection to the intestinal homeostasis via promoting the relative abundance of *Blautia* and *L-Ruminococcus* genera, two short-chain fatty acid producers.

Collectively, M52 treatment promoted a well-balanced immune homeostasis, dampened intestinal damage, and preserved the microbiota diversity, which all contribute to an enhanced resilience to *Eimeria* spp. challenge.

**Key Words:** coccidiosis, anti-*Eimeria* immunity, IL-10, microbiome, intestinal integrity