

***In vitro* Anticoccidial Effects of Oregano and Garlic Essential Oils Against *Eimeria tenella* and *in vivo* Results on Growth Performance, Faecal Oocyst Output and Intestinal Microbiota in Broilers - Abstract**

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Summary

This study investigated the *in vitro* effect of oregano and garlic essential oils on sporozoites of *Eimeria tenella* (Wisconsin strain) invasion into Madin-Darby bovine kidney (MDBK) cells, as well as the *in vivo* effects of the combined dietary use of these essential oils on growth performance of broiler chickens, intestinal microflora composition and *Eimeria* oocyst output. An inhibition assay was performed *in vitro* using sporozoites of *E. tenella* pre-treated for 1 hour at 41°C and 5% CO₂ with essential oils of garlic or oregano at different concentrations (100, 50, 20, 5 µg/ml), then added to MDBK cells for invasion. Dymethyl sulfoxide (10µl/ml) and robenidine (5 µg/ml) were used as controls; no morphological changes were observed in MDBK cells when incubated for 24 hours with any of the oils at their higher concentration (100 µg/ml). Intracellular invasion of pre-treated sporozoites was assessed by detection of *E. tenella* DNA by qPCR from MDBK monolayers harvested at 2 hours and 24 hours after invasion. The relative level of inhibition of *E. tenella* showed that parasite invasion was inhibited by the oregano essential oil at the concentration of 100 µg/ml by 83% or 93% after 2 hours or 24 hours, respectively. Garlic essential oil reached a maximum inhibition of 70% after 24 hours with the 50 µg/ml concentration. For the *in vivo* trial, 180 male broiler chicks (Ross-308) were allocated into two treatments (6 pens of 15 chicks per treatment). The CONTROL treatment was fed commercial diets (starter, grower, prefinisher, finisher) based on corn and soybean meal without antibiotics or anticoccidials. The second treatment (OREGAR) received the same diets, further supplemented with a premix (1 g/kg) containing oregano essential oil (50 g/kg premix)

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and garlic essential oil (5 g/kg premix). Feed and water were offered to birds *ad libitum*. At the end of the trial (day 37), all birds were slaughtered under commercial conditions, and intestinal samples were collected. Cecal microbiota were determined by conventional microbiological techniques. Oocyst output in faeces was also determined. OREGAR treatment compared to the CONTROL treatment showed: improved final body weight (1834 vs 1686 g; $P<0.01$) and feed conversion ratio (1.489 vs 1.569; $P<0.01$); lower oocysts in faeces (3.48 vs 4.01 log₁₀ CFU/g; $P<0.001$); lower counts of anaerobes (8.22 vs 8.82 log₁₀ CFU/g; $P<0.001$) in the ceca; lower counts of *Clostridium perfringens* (2.56 vs 2.88 log₁₀ CFU/g; $P<0.01$) and higher counts of *E. coli* (5.03 vs 3.53 log₁₀ CFU/g; $P<0.01$) and *Enterobacteriaceae* (5.34 vs 3.83 log₁₀ CFU/g; $P<0.01$) in the jejunum. Based on these trial results, the oregano and garlic essential oils exhibits a potent anticoccidial effect *in vitro* and their combined supplementation in broiler chicken diets can results in an effective growth promoting effect, even in the absence of anticoccidial drugs.

Keywords: Oregano essential oil; garlic essential oil; broiler chicken; performance; *Eimeria tenella*.

JEL Codes: N50; Q10; Q13.

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