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In vitro antiparasitic effects of carob, grape and sainfoin extracts on rabbit *Eimeria* oocysts sporulation and excystation

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Abstract

Coccidiosis caused by *Eimeria* species is a common, occasionally severe infection resulting in economic losses in the rabbit industry. The potential anticoccidial effect of tannin-containing plant resources, fed in rabbits has been previously reported *in vivo*. However, the mode of action remains unknown. During this study, we investigated *invitro* the anticoccidial effect of selected plant extracts by targeting two developmental phases of *Eimeria* life cycle: based on 1) an oocysts sporulation assay (OSA), 2) a sporozoites excystation assay (SEA). For this purpose, *Eimeria* oocysts of different species were harvested from naturally infected rabbits. Overall, 6 extracts of 3 plants (carob (*Ceratonia siliqua*) pods, grapes (*Vitis vinifera*) (skins and stems) and sainfoin pellets (*Onobrychis viciifolia*, var. *Perly*) harvested on 3 occasions) were prepared in PBS and tested at 0 to 1200 µg/ml for both OSA and SEA. For the OSA, freshly harvested oocysts were incubated with the extracts for 4 days at 26°C in order to enable sporulation. The sporulation rate reached 95% in all cases showing no significant effect of extracts. For SEA, oocysts were left to sporulate up to 98% before adding extracts. Solution was

suspended first for 20h at 39°C in 20% CO₂ with cysteine and NaHCO₃, then 4h more with an excystation solution (0.4% trypsin/HBSS and 40% rabbit bile/PBS). Thereafter, excystation progress was checked every 4 hours until excystation rate reached at least 85% in controls. After 4 hours, grape extracts inhibited oocyst excystation from - 53% (150 µg/ml) to - 85% (1200 µg/ml) (p<0.001). Carob extract at 1200 µg/ml reached an excystation of 37%. Sainfoin effect varied depending on the harvest cut and year, with excystation rate ranging between 60 to 93% (lower excystation rate for a third-cut). These results suggest some disturbing effects of plants extracts tested on the coccidial excystation process.

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