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ORIGINAL ARTICLE

Assessment of the protective effect of *Artemisia herba alba* Asso against *Eimeria tenella* induced coccidiosis in broilers

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Abstract

Background: The present study was designed to investigate the protective effect of a natural product to control coccidiosis in broilers. We studied the effect of the dried leaves of *Artemisia herba alba* Asso supplement against artificially induced coccidiosis in broilers.

Methods: One hundred twenty chickens were allocated into four equal groups of thirty, negative control (UUG), positive control (IUG), Monensin-treated Group (MTG), and Artemisia-treated group (ATG). The infected chickens with *E. tenella* were treated with 100ppm of monensin and, 5% of Artemisia in the feed.

Results: No mortality was recorded in ATG animals. Oocysts excretion was significantly ($p<0.05$) reduced in ATG animals at the 6th and the 8th days post-infection. The highest reduction of oocysts shedding in fecal samples (79.04%) was in Artemisia-treated chickens. The effect of Artemisia was comparable to that of monensin.

Conclusion: *Artemisia herba alba* Asso is of particular interest in fighting coccidiosis since it has a preventive effect on mortality and reduction of oocysts fecal shedding during caecal coccidiosis.

Keywords: Caecal; Monensin; Natural alternatives; Oocyst shedding

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Introduction

Coccidiosis is still considered the most economically important parasitic disease affecting poultry production worldwide (Shirley et al., 2005). The economic impacts are estimated upward of $3 billion worldwide (Dalloul and Lillehoj, 2006). It is an important enteric disease, which causes poor performance, malabsorption, blood loss, dehydration, and increased susceptibility to other disease agents (Huang et al., 2018). *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* are the most frequent species found in intensively reared chickens, but the latter is highly pathogenic (MacDonald et al., 2017). *Eimeria tenella* parasitizes mainly the caecal mucosa of hosts and is known to cause bloody diarrhea (Matsubayashi et al., 2020).

Currently, the main control methods against avian coccidiosis primarily rely on prophylactic chemotherapy (Constable et al., 2017) and live attenuated vaccines (Thabet et al., 2019; Ma et al., 2021). However, the continual emergence of coccidian drug-resistant strains, coupled with the increasing regulations and bans on the use of anticoccidial drugs in poultry production, urges the need for novel approaches and alternative control strategies (Dalloul and Lillehoj, 2005; Wunderlich et al., 2014). Besides, the effect of vaccines is limited largely due to high production cost and ineffectiveness in the case of poor management conditions. Therefore, there is a strong desire to use some natural alternative agents to replace existing methods (Abudabos et al., 2017).

Scientists over the world are nowadays engaged in research into the use of natural remedies, such as plants and plant-derived products, to reduce the impact of coccidiosis in poultry farms (Abbas et al., 2012). The effect of many medicinal plants, alone or in combination has been studied during coccidiosis. Artemisia species are rich in natural compounds, and their anticoccidial activity has been demonstrated (Arab et al., 2006). Within this context, we describe in this study the coccidiostatic effect of *Artemisia herba alba* Asso in vivo, against *Eimeria tenella* experimental infection in broilers.

Materials and methods

The experimental protocol followed in this study is consistent with the international guidelines of animal care and use in research and teaching (NRC, 2011). Our study was carried out within PADESCA Laboratory research in the Institute of Veterinary Sciences of Constantine, Algeria.

Animals and feeding

A total of 300 one day old Hubbard-ISA15 chickens were reared in floor pens. Strict hygienic practices were followed during the experiment. On day 17 of age, animals were divided into four homogeneous groups of 30 subjects, based on weight. Groups include the negative control uninfected untreated (UUG), the positive control infected untreated (IUG), the group infected treated with monensin (MTG), and the group infected treated with Artemisia (ATG). Birds of the negative and the positive control groups were fed a standard non-medicated poultry ration. Until the end of our experiment, animals of the monensin-treated group received 100ppm of monensin sodium, while birds of the artemisia-treated group received dried leaves (0.05g/g of feed) of *A. herba alba* Asso incorporated in their feed. On days 7 and 14, all chicks were vaccinated against the Gumboro and the Newcastle disease respectively via drinking water administration.

Parasite

The parasitic strain of *Eimeria tenella* used in our study was originally isolated from broiler farms in Constantine region (Algeria). It was maintained in the laboratory and multiplied by passing on broiler chicks. The procedures of preparation of oocysts for infection had been described elsewhere (Conway and McKenzie, 2008). The infective transmission stage is the oocyst which contains, when sporulated, four sporocysts each containing two sporozoites (Chapman et al., 2013). Sporulated oocysts were identified through morphological features and morphometry. On the 18th day of the study, the experimental animals were orally gavaged with 1 mL distilled water containing 105 sporulated

oocysts. The mortality rate and the numbers of *E. tenella* oocysts per gram of feces (OPG) were counted after the infection period, to test the efficacy of the studied plant. The oocyst value and oocyst reduction rate were calculated as cited by Lan et al. (2016).

Data analysis

Data obtained were expressed as mean±SEM. Oocysts fecal shedding data were analyzed using the Kruskal-Wallis test followed by Mann-Whitney Test, by XLSTAT 2010 statistical analysis software (Addinsoft SARL). P-value < 0.05 was considered as significant.

Results

Mortality rate

The record of the mortality cases concerned the period extending until the 9th day post-infection (DPI) in all studied groups. The highest mortality rate was recorded in the IUG (10%), followed by the MTG (3.33%). In the ATG and UUG animals, no mortality was observed during the studied period. This observation showed the beneficial effect of *A. herba alba* Asso in the prevention of mortality.

Fecal shedding of oocysts

The fecal samples were collected from the infected chickens' groups on days 5, 6, 7, and 8 DPI. The numbers of oocysts per gram of feces were counted using the McMaster technique as described previously (Bussieras and Chermette, 1992). Expressed as OPG, the fecal oocysts shedding in all infected groups showed a gradual increase from day 5 until day 7 DPI (Table 1). The oocyst value and oocyst reduction rate are shown in Fig. 1.

Table 1: Fecal oocysts shedding in infected animals (10^6OPG).

Post-Infection Period (Days)	Untreated	Treated Groups	
	IUG	MTG	ATG
5	2.45±0.94[a]	0.88±1.02[a]	3.03±1.89[a]
6	15.54±6.34[a]	3.80±4.12[b]	5.34±6.59[b]
7	77.77±72.44[a]	14.89±22.39[a]	36.20±37.67[a]
8	27.57±17.82[a]	8.24±8.37[b]	5.78±6.13[b]

Values are expressed as mean ± standard deviation (n=6). UUG: uninfected untreated, IUG: infected untreated, MTG: infected treated with monensin, ATG: infected treated with Artemisia

[a,b] Values within each row with different superscripts are significantly different (P < 0.05).

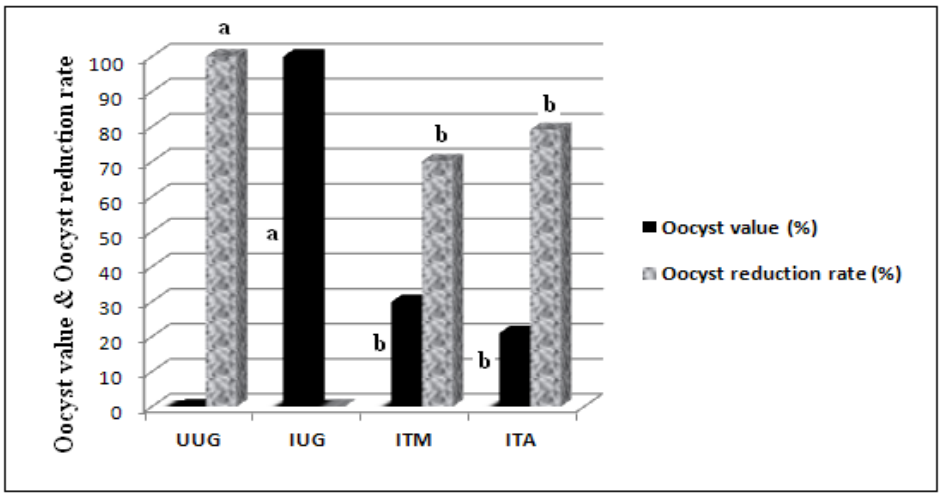

Fig. 1: Effect of treatments on oocyst value and oocyst reduction rate at 8 DPI. **a** and **b** lowercase letters indicate significant differences. UUG: uninfected untreated, IUG: infected untreated, MTG: infected treated with monensin, ATG: infected treated with Artemisia

Discussion

We studied the effect of the dried leaves of *Artemisia herba alba* Asso supplement against artificially induced coccidiosis in broilers. Due to the preventive effect on mortality and reduction of oocysts fecal shedding dried leaves of Artemisia herba alba Asso can be used as an alternative of chemical coccidiostats. *Eimeria tenella* is one of the most pathogenic Eimeria species (Chapman et al., 2010). It invades and damages the caecal epithelium causing severe injuries and economic loss. The economic loss has been mainly attributed to poor performance, increased mortality, and the costs of medication (Rasheed and Matsler, 2020). It was concluded from many studies that natural products are very effective in comparison with anticoccidial drugs to control poultry coccidiosis (Abudabos et al., 2018). To prove their effectiveness, several test parameters are commonly utilized in research. Oocyst shedding is a useful way to determine the level of Eimeria infection (Jordan et al., 2011). Quantification of OPG provides information at the infection level and reproduction of Eimeria, which may be used to monitor treatment impact on the overall Eimeria life cycle (Chasser et al., 2020). In our study, the peak of fecal oocyst shedding was reached by the seventh DPI, where the OPG recorded in the positive control group was $77.77 \pm 72.44 \times 10^6$.

The present study was planned to examine the effect of *A. herba alba* Asso dried leaves against *Eimeria tenella*-induced changes in fecal shedding of oocysts. At 6 and 8 DPI, oocyst shedding was significantly reduced ($p<0.05$) in ATG, treated with Artemisia compared to the positive control untreated group. Also, the oocyst reduction rate in the Artemisia-treated group (79.04%) was significantly different ($p<0.05$) from the infected untreated group (IUG: 0%), but not from the monensin treated group (MTG: 70.11%). This observation was expected and agrees with findings published in several studies on the anticoccidial effect of different species of the genus Artemisia. Among the most studied species, Artemisia annua (Del Cacho et al., 2010; Drăgan et al., 2010), Artemisia absinthium (Kostadinovic et al., 2012), and Artemisia sieberi (Arab et al., 2006). All these studies attributed the anticoccidial effect to their artemisinin content (Lans et al., 2007). The therapeutic applications of artemisinin are numerous and varied (Wynn and Fougère, 2007; Efferth, 2009). Artemisinin is a sesquiterpene lactone with an endoperoxide group that causes oxidative stress in coccidia and leads to their destruction (Ivanescu et al., 2015). This sesquiterpene has been identified in many Artemisia species (Tang et al., 2000; Houari and Ferchichi, 2009; Akrout et al., 2010). From the species Artemisia herba-alba Asso, various secondary metabolites were isolated, the most important being lactone sesquiterpenes (Talbi et al., 2015). Messaï et al. (2008) reported that Artemisia herba-alba Asso, widespread in Algeria (Tebessa) is rich in sesquiterpenes lactones. In our study, the effect of the plant on the reduction of oocysts shedding could be attributed to its possible artemisinin content. However, phytochemical studies are needed to determine the amount of artemisinin in the plant under study.

Otherwise, the effect of other medicinal plants was also studied against the species *Eimeria tenella* (Al-Quraishy et al., 2020). Reduction of the fecal oocysts shedding was observed and was attributed to several chemical compounds, which were identified in the species Artemisia herba-alba Asso. These compounds include mainly; phenols (Ultee et al., 1999; Qasem et al., 2020), tannins (Zaman et al., 2011), flavonoids (Nweze and Obiwulu, 2009), and saponins (Hassan et al., 2008). The chemical compounds which have

been shown to have a reducing effect on oocyst shedding against *Eimeria tenella* are of a very varied nature. In our study, it is difficult to talk about the exact nature of the active ingredient responsible for this effect. Nevertheless, the molecules mentioned above have been isolated in studies of the chemical composition of the different chemotypes of A. herba-alba Asso in Algeria, and throughout the world. Among the common compounds in A. herba-alba Asso: phenols (Gharzouli et al., 1999; Mohamed et al., 2010), flavonoids (Salah and Jäger, 2005; Bora and Sharma, 2011), tannins (Khlifi et al., 2013) and saponins (Aziz et al., 2018). It was therefore assumed that Artemisia herba-alba Asso could contain one or all of these compounds, which would be responsible for the reduction of oocysts shedding.

Note that the infection of birds with E. tenella began with the invasion of the intestinal caecum, destroying the caecal epithelium due to the multiplication of the parasite stages, and finally the developed oocysts were released in faeces (Mehlhorn, 2016). Feces containing coccidia oocysts are a source of reinoculation for chickens (Hassan et al., 2008). In addition to its direct destructive effect on oocysts, the reduction of oocysts excretion by A. herba-alba Asso also helps to limit the effects of parasitism by reducing litter contamination.

In our study, monensin was used as a reference drug. Since the introduction of monensin, this ionophore antibiotic has had a remarkable effect in the fight against coccidiosis (Augustine et al., 1987). Monensin leads to a programmed death of E. tenella parasites by inducing autophagy as a mechanism of anticoccidial action (Qi et al., 2020). In our study, monensin remains numerically, the most effective molecule in reducing oocysts shedding. During all the period of infection, the number of oocysts in the monensin-treated group was significantly lower ($p<0.05$) compared to the positive control group, but not significantly different from the Artemisia-treated group. However, at 8 DPI, the highest reduction of oocysts shedding in fecal samples was recorded in Artemisia-treated chickens.

Conclusion

To limit the ubiquity of coccidiosis in poultry production farms, effective alternative treatment and prevention strategies should continue to be developed. Our findings suggested that A. herba-alba Asso was able to control the mortality rate and to reduce fecal oocysts shedding in chickens infected with *Eimeria tenella*. Collectively, A. herba-alba is a promising medicinal plant with anticoccidial properties and could be used for the treatment of coccidiosis as a food additive.

Competing Interest

The authors declare that they have no competing interests.

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