



Efficacy of an Aqueous Extract of *Stellaria media* (L.) Cyr. against *Eimeria legionensis* Infection (Apicomplexa: Eimeridae) in Red-legged Partridges (*Alectoris rufa*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SP, G. Fichi and G. Flamini designed the study, wrote the protocol and wrote the first draft of the manuscript. Author G. Fichi performed the statistical analysis. All authors managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Coccidiosis is one of the most frequent causes of morbidity and mortality in farmed red-legged partridges (*Alectoris rufa*). The aim of this study was to evaluate the effects of the plant *Stellaria media* (Caryophyllaceae) on coccidia-infected red-legged partridges.

Methodology: Of 9 replicates of red-legged partridges, each composed by 6 coccidia-infected birds (*Eimeria legionensis*), 3 replicates (S group) received an aqueous extract (12 ml/l) of *S. media* with drinking water for 3 consecutive days, while 3 further replicates (D group) received 20% sodium sulfaquinoxaline (2 g/l) with drinking water for 3 consecutive days. The remaining 3 replicates did not receive any treatment (C group). The day before the beginning of the treatment (day 0), the last day of the treatment (day 3) and 7 days after the end of the treatment (day 10), individual fresh faecal samples were collected from all examined birds and analysed for presence and number of coccidian oocysts/gram of faeces (OPG). Data were statistically elaborated with the Analysis of

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Variance (ANOVA) and the Test of Student-Newman-Keuls for multiple comparisons. The percentage of reduction of the mean OPG number was also assessed. In addition, all birds were clinically observed in order to evaluate the appearance of diarrhoea and of other signs of clinical coccidiosis.

Results: Seven days after the end of the treatment, birds of the C group showed diarrhoea and significant ($P<0.05$) higher OPG numbers respect to those of S and D groups, while no significant differences resulted between S and D groups. Moreover, percentages of OPG reduction of 98.61% and 99.60% and of 99.23% and 78.46% were found at day 3 and day 10 for group S and group D, respectively, while an increased mean OPG number was observed in the untreated group (C).

Conclusion: After oral administration to the examined red-legged partridges *S. media* was able not only to prevent the clinical form of coccidiosis, but also to give a significant reduction of *E. legionensis* OPG number in faecal samples of birds treated with this plant. This reduction resulted comparable to that observed in animals treated with the commercial drug.

Keywords: *Eimeria legionensis*; *Stellaria media*; coccidiosis; *Alectoris rufa*; anticoccidian activity.

1. INTRODUCTION

The red-legged partridge (*Alectoris rufa*) is a very popular game bird species native to the Iberian Peninsula, but currently present in southern and central France, and in north-western Italy [1]. This game species grows and reproduces easily in captivity, if effective hygienic and measures to prevent diseases are taken. However, the concentration of farmed birds enhances disease risk and intestinal coccidiosis is one of the most frequent causes of morbidity and mortality in farmed red-legged partridges [2]. In poultry and game birds, coccidiosis is a protozoan disease caused by specific protozoan species of the genus *Eimeria* (Apicomplexa: Eimeriidae) that localise in the intestine of the infected host and can be responsible for reductions of feed utilization, weight gain and egg production and also for diarrhoea and eventually mortality [2,3]. *Alectoris rufa* is the host of at least three different *Eimeria* species, namely *Eimeria kofoidi*, *Eimeria caucasica* and *Eimeria legionensis* [2]. Coccidian infection is initiated by oral ingestion of sporulated (mature) oocysts by the susceptible host. Currently, very few preventive and curative drugs are available in Europe for the control of coccidiosis in game birds with a consequent high risk of treatment resistance and treatment failure [2,4].

Since ancient times, plants and plant extracts have been a source of drugs for indigenous poultry production systems all over the world [5]. In poultry, the anticoccidian properties of a number of natural feed additives have been recently shown [6-9].

Stellaria media (L.) Cyr. commonly known as chickweed, is an edible and nutritious plant of the family Caryophyllaceae native to Europe [10]. It

is known that wild and caged birds eat the seeds as well as the young tops and the leaves of *S. media*, while little caged birds, especially linnets, are refreshed with "chickweed". This weed plant is also reported for its tonic proprieties in caged birds and poultry and in folk medicine it is used as food integrator and for stimulating the production of eggs [11,12]. Furthermore, the use of *S. media* as a nutraceutical compound for human diet has been proposed, for the excellent antioxidant properties of this plant [13].

Considering the positive effects on bird health and productions reported for *S. media* [11,12], the aim of this study was to evaluate the effects of this plant on naturally *Eimeria*-infected red-legged partridges (*Alectoris rufa*). Even if fresh greenstuffs are appreciated by growing game birds, an aqueous extract of *S. media* that preserved the fresh plant characteristics was used in this study.

Considering that the susceptibility to a same anticoccidian compound may vary among different coccidian species [14], the identification of the *Eimeria* species involved in the infection of examined partridges was a further aim of this study.

2. MATERIALS AND METHODS

2.1 Plant Materials

Flowering aerial parts of *S. media* were collected in Pisa (Italy). A voucher specimen of the plant was deposited at the Dipartimento di Farmacia of Pisa University. After the collection, the aerial parts of *S. media* was coarsely cut, then 133 g of the plant material were inserted in a conical glass

flask and 1,000 ml of room temperature distilled water were added. With the help of a shaft-drive agitator, an aqueous extract was prepared by maceration at 40°C for two hours. The extract was filtered with Whatman n.5 paper filter and preserved at – 25°C until used.

2.2 Animals

Examined birds lived in a farm where red-legged partridges are reared for their release in the natural habitat. As a routine treatment to control clinical coccidiosis, in this farm birds are treated with 20% sodium sulfaquinoxaline [15] administered with drinking water (2 g/l) at 16-18 and 46–48 days of age. From a 45 days old group of 500 animals, born in the same hatching and reared in brooder house and outside pen with a litter of wood chip and found infected by a subclinical form of coccidiosis at faecal examination, 54 red-legged partridges of both sexes, were randomly selected. All partridges were placed into single cages of 82.5 cm x 38.5 cm x 30 cm in dimensions and with a wire netting bottom of 10-12 mm little meshes. Cages were placed about 70 cm above the ground, to permit the falling of faeces. Water was administered *ad libitum*. Prior (day 0) and at the end of the observation period (day 10), all selected birds were found negative for pathogenic bacteria, including *Salmonella* spp., and other parasite species causing intestinal disorders.

2.3 Research Design

In order to have three replications for each treatment regime, 9 replicates of 6 partridges each were randomly formed. When the animals were 47 days old, 3 replicates (18 birds) received *ad libitum* drinking water containing 12 ml of *S. media* aqueous extract/l for three consecutive days (S. group), further 3 replicates (18 birds) received *ad libitum* drinking water containing 2 g/l of 20% sodium sulfaquinoxaline for three consecutive days (D group), while the 18 animals of the remaining 3 replicates (C group) did not receive any treatment with *ad libitum* drinking water and served as untreated control. All treatments lasted for 3 consecutive days because this is the usual period of chemotherapy for coccidiosis using the standard drug sulfaquinoxaline.

Through the treatment period and in the following 7 days, all examined birds were clinically observed in order to evaluate the appearance of diarrhoea or a reduction of faecal consistency

and of other signs indicating a clinical form of coccidiosis.

2.4 Parasitological Analysis

For the evaluation of number of *Eimeria* oocysts/gram of faeces (OPG), on day 0, just before the treatment, on day 3, the last day of the treatment, and on day 10, 7 days after the end of the treatment, from all examined birds individual fresh faecal samples were collected by means of cardboards placed under the cages the day before, after cleaning the wire netting. Faecal samples were labeled, preserved at 4°C and microscopically analysed within 24 h for coccidian oocysts with a LEICA ICC50W microscope. More specifically, collected samples were quali-quantitatively analysed by flotation test and by a modified McMaster-technique [16], by using as flotation fluid a saturated sodium chloride solution with 50% glucose monohydrate [17]. With the McMaster-technique used in this study, each oocyst counted in the McMaster slide corresponded to 20 oocysts per gram of faeces (OPG).

For the identification of the *Eimeria* species, an aliquot of each faecal sample collected at day 0 from each examined animal was dissolved in 2.5% $K_2Cr_2O_7$ solution and maintained at room temperature until the sporulation of oocysts. Oocysts, sporocysts and all other structures of sporulated (mature) oocysts were microscopically evaluated at 400X and 1000X, measured by an eye-piece micrometer and identified by their morpho-metrical features according to previously reported data [2,18].

2.5 Statistical Analysis

Data from the counts of the number of *Eimeria* OPG were statistically elaborated with the Analysis of Variance (ANOVA) and the Test of Student-Newman-Keuls for multiple comparisons with $P < 0.05$ significance [19].

2.6 Evaluation of Therapeutic Efficacy

For each group, arithmetic means of the pre- and post-treatment faecal oocyst counts were used to calculate the percentages of faecal oocyst reduction at the end of the treatment (day 3) and 7 days after the end of the treatment (day 10) according to the formula:

Efficacy (percentage of faecal oocyst reduction) = [(OPG pre-treatment - OPG post-treatment) x100] /OPG pre-treatment.

3. RESULTS AND DISCUSSION

Among birds of the genus *Alectoris*, coccidiosis is a disease caused by intracellular protozoans of the genus *Eimeria* that is prevalent in game farms and often responsible for weight loss and poor feed conversion [2,14]. Clinically manifest disease or even epidemic-like outbreaks with considerable mortality are frequently observed [2,14]. The present study was designed to evaluate possible effects of an aqueous extract of the plant *S. media* on naturally *Eimeria*-infected red-legged partridges. Parasitological analysis showed that at the beginning of the experiment (day 0) all examined animals were positive for the presence of faecal *Eimeria* oocysts. More specifically, at day 0 the untreated group (C) showed the lowest mean oocyst number (11,733 OPG), the group treated with the extract of *S. media* showed the highest mean oocyst number (13,150 OPG), while the sulfaquinoxaline- treated group showed an intermediate value of mean oocyst number (12,588) (Table 1). However, from ANOVA analysis the observed differences in OPG number among the three groups were not statistically significant ($P= 0.967$), thus showing the homogeneity of all the groups. At day 3, the last day of the treatment, and at day 10, seven days after the end of the treatment, significant ($P<0.05$) lower OPG numbers were observed in faecal samples of S and D groups with respect to the C group, while no significant differences resulted between S and D groups (Tab. 1). At the end of the treatment (day 3), 98.61% and 99.60% percentages of reduction of OPG number were found for group S and group D, respectively. However, seven days after the end of the treatment (day 10), a higher percentage of reduction of OPG was observed in group S

(99.23%) compared to group D (78.46%). On the contrary, in the untreated control group (group C) higher numbers of mean OPG were found at day 3 and at day 10 compared to that observed at the beginning of the study (day 0) (Table 1).

For dimensions and morphology, mature oocysts of the species found in faecal samples of the red-legged partridges examined in this study was identified as *Eimeria legionensis* [2,14]. In fact, they were sub-spherical to elliptic in shape, showed a micropyle and one or two granulations, measured $21.5 \mu\text{m} \times 15.5 \mu\text{m}$ and contained four sporocysts of $9.9 \mu\text{m} \times 6.0 \mu\text{m}$ in size with a small Stieda body at the pointed end. *E. legionensis* is a highly pathogenic coccidian species invading the caeca of *A. rufa* [2,4] that can be responsible for caecal inflammation and diarrhoea with watery faeces, light brown in color and often containing a great amount of urates, caseous cores stuffed with oocysts or even blood [2,4,14]. Coccidiosis, caused by *E. legionensis* alone or in association with other *Eimeria* species infecting *A. rufa*, is a serious limitation to productivity. If left untreated, infected animals may die quickly or may show prostration, alteration of water consumption and reduction of food intake with considerable muscular losses, high rates of mortality may be observed in infected farms [2,4,18]. From clinical examination, before the beginning of the experiment and trough the treatment period no bird showed clinical signs of coccidiosis. However, on day 10 partridges of the C groups emitted not formed, white and liquid faeces with a preponderance of urates, while these symptoms were not observed in the red-legged partridges treated with sulfaquinoxaline (D groups) and in those treated with the aqueous extract of *S. media* (S groups).

Table 1. Mean number \pm standard deviation (SD) and standard error (SE) of oocysts per gram of faeces (OPG) observed on day 0 (the day before the beginning of the treatment period), on day 3 (the last day of the treatment period) and on day 10 (7 days after the end of the treatment period) in the 3 groups of red-legged partridges (3 replications of 6 birds/group) left untreated (C) or treated for three consecutive days with 12 ml of a *Stellaria media* aqueous extract/l of drinking water (S) or with 2 g of 20% sodium sulfaquinoxaline (D)

	Day 0			Day 3			Day 10		
	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE
S	13,150	$\pm 17,235$	4,062	183 ^a	± 185	44	100 ^a	± 145	34
D	12,588	$\pm 9,518$	2,243	50 ^a	± 78	18	2,711 ^a	$\pm 3,131$	738
C	11,733	$\pm 20,547$	4,775	42,08 ^b	$\pm 4,919$	9,919	34,116 ^b	$\pm 9,440$	222

a, b: $P < 0.05$

The activity showed by *S. media* in this study was very interesting, because after oral administration to the examined red-legged partridges it was able not only to prevent the clinical form of coccidiosis, but also to give a significant reduction of *E. legionensis* OPG number in faecal samples of birds treated with this plant. These reductions resulted comparable to that observed in animals treated with the commercial drug normally used in the farm to prevent clinical coccidiosis, showing the anticoccidian efficacy of the extract of *S. media* evaluated in this study. However, a higher percentage of reduction of mean OPG number was observed a week after the end of the treatment in the partridges treated with *S. media* compared to that observed in birds treated with the reference drug, possibly indicating a longer lasting anticoccidian activity of this plant extract. These interesting results should be confirmed on a larger number of coccidian infected red-legged partridges and on other species of birds, with the aim of proposing the use of this plant as an alternative and natural anti-coccidian compound. Meanwhile, we think that it is important to evaluate the effects of this plant on farmed bird productions and also the chemical, toxicological and nutritive characteristics of *S. media* should be studied in depth.

S. media has been identified to contain high concentration of phenols and saponins [20-22]. Plant phenolic components are included among the main factors responsible for the inhibitory effects against coccidia of a number of plants extracts [3], including their ability to inactivate the oocysts of the poultry species *Eimeria tenella* and to inhibit the cells invasions by *E. tenella* sporozoites *in vitro* and *in vivo* [3]. Furthermore, the anticoccidian activity of some plant-derived saponins has been reported in previous studies [9]. Therefore, the presence of these compounds in the composition of *S. media* extract might have been responsible for the reduction in oocyst count or the absence of clinical presentation observed in birds examined in this study.

4. CONCLUSION

Results from the present study led to suppose that the beneficial refreshing and tonic properties of *S. media* reported in birds could depend also on its direct and/or indirect anticoccidian properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the experiments were carried out in accordance with the guidelines given by the European law on the use of animals in research and were approved by the animal welfare committee of Pisa University and by the Italian Minister of Health (n. 15/2003).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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