



Full Length Article

Evaluation of pomegranate (*Punica granatum*) pericarp aqueous extract on *Eimeria* spp. from Japanese quails (*Coturnix japonica*)



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Abstract Antibacterial, anti-inflammatory and antiparasitic properties have been associated with the extract of pomegranate (*Punica granatum*) in several animals and conditions. The Japanese quail (*Coturnix japonica*), originated from North Africa, Europe and Asia, is used worldwide as an experimental animal and model for aviculture. The current study investigated the effects of the pomegranate pericarp aqueous extract on the shedding, viability and morphometry of three *Eimeria* spp. from Japanese quails, besides the weight gain and genotoxic activity. Although the pomegranate is recognized by multiple properties, including anti-coccidial, in the current study the results are contrary. The treated group shed greater amount of oocysts; the sporulation times and viability were similar in both groups; despite some morphometric differences, these were not expressive; weight gains were similar; and the pomegranate had insignificant effect genotoxic. Finally, these results suggest that the pomegranate pericarp extract did not influence on *Eimeria* spp. from Japanese quails; therefore, the pomegranate pericarp extract is not suggested in the prevention/treatment of coccidiosis in Japanese quails, or at least not using methods of preparation and administration applied in this study.

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1. Introduction

Parasitoses represent a limiting factor in the breeding of poultry species, both in high-production commercial rearing farms or even in rustic breeding systems, where economic losses can be significant. Among the most pathogenic parasites, coccidiosis caused by *Eimeria* spp. is distinguished by severe enteropathy, which promotes anorexia, decreased reproduction and posture in adults, and is responsible for high levels of mortality in young animals [1–5].

Poultry coccidiosis is mainly controlled by the use of chemotherapeutic agents. However, several studies have targeted the use of herbal medicines, which reduces costs and must be effective in the prevention and/or treatment of coccidiosis. Studies have reported anti-inflammatory, antioxidant, antibacterial and antiparasitic (including anti-coccidial) properties associated with the extract of pomegranate (*Punica granatum*) in several animals, parasites and conditions [6–12].

Japanese quails (*Coturnix japonica*) has excelled in aviculture, due to increased consumption of exotic meats and eggs, and represents an alternative to chicken production. Furthermore, in recent decades, it has become an important experimental animal for scientific research, due to short life cycle and greater resistance to many poultry diseases [13–19].

Berto et al. [20] characterizes *Eimeria bateri*, *Eimeria tsunodai* and *Eimeria uzura*, which are commonly encountered on farms breeding Japanese quails provisioning an algorithm designed to enable reliable identification during routine diagnosis and experimental studies.

In this context, the present study investigated the effects of the aqueous extract of pomegranate pericarp on the shedding, viability and morphometry of three *Eimeria* spp. from Japanese quails, besides the weight gain and genotoxic activity.

2. Material and methods

2.1. Experimental Japanese quails and treatments

Eight one-day-old Japanese quails were obtained from a commercial rearing farm located in the Municipality of Seropédica in the State of Rio de Janeiro, Brazil. The chicks were transported to the Universidade Federal Rural do Rio de Janeiro (UFRRJ), and were reared and fed a single cage without anticoccidial additives. Feed and water were administered *ad libitum*. Chicks were randomly assigned to two experimental groups consisting of 8 individuals per group. Group 1 was fed with aqueous extract of pomegranate pericarp and were challenged (treated); Group 2 received an untreated standard diet and were challenged (control). Sample processing and data analysis were conducted at the Laboratório de Coccidios e Coccidioses and in the Departamento de Biologia Animal (Biology area), both located at UFRRJ. The experiments were approved by Ethics Committee in Research of the UFRRJ.

2.2. Preparation of the pomegranate pericarp extract

Pomegranate pericarps were obtained from fruit collected from trees in the municipality of Seropédica, state of Rio de Janeiro, Brazil. The samples were authenticated by Herbaria Technician Thiago Azevedo Amorim (Departamento de

Botânica, Instituto de Biologia, UFRRJ, Brazil) and the exsiccate was placed in the herbaria under protocol RBR No. 35804. Pomegranate pericarp aqueous extract was prepared according to the method described by Amorim and Borba [21] with some modification. Pericarps were cold-dried under ambient conditions, pulverized and dissolved (5 g) under boiled (90 °C/194°F) distilled water (100 ml). This pomegranate aqueous extract were supplied for drinking water from 5 to 11 days of age.

2.3. *Eimeria* spp. challenge

The adult Japanese quails from the commercial rearing farm used to provide the chicks were naturally parasitized by *E. bateri*, *E. tsunodai* and *E. uzura*. An inoculum for experimental infection was produced by recovering and isolating oocysts from fecal samples of positive Japanese quails by flotation in Sheather's sugar solution (S.G. 1.20) according to the protocol of Duszynski and Wilber [22]. The oocysts were preserved in a 2.5% (w/v) solution of K₂Cr₂O₇ to induce sporulation and maintained in a refrigerator (2–5 °C) until use. *C. japonica* chicks were inoculated at the age of 7 days, using inocula, quantified using a Neubauer chamber, containing approximately 6.5×10^4 sporulated oocysts per chick, at the approximate proportion of: 50% *E. bateri*; 25% *E. tsunodai*; and 25% *E. uzura*.

2.4. Assessment of the shedding, viability and morphometry of the *Eimeria* spp. oocysts

The number of oocysts shed in the feces was determined on days 2, 3, 4, 5, 7, 9, 11, 14, 16, 18, 21, 23, 25 and 28 after infection (DAI). Enumeration of oocysts per gram of faeces (OoPG) was performed according to Menezes and Lopes [23], as modified by Cardozo et al. [18]. The viability of the oocysts was evaluated from the shedding of the oocysts until 120 h after, maintaining the fecal sample in a thin layer (~5 mm) of K₂Cr₂O₇ 2.5% solution in Petri plates, and incubated at 23–28 °C. During this period was observed daily the proportion of oocysts non-sporulated, with sporoblasts and sporulated within of the same sample. *Eimeria* spp. oocysts from Japanese quails were identified using the characteristics and the algorithm designed by Berto et al. [20]. Morphological measurements, given in micrometres, were made using a Carl Zeiss binocular microscope with an apochromatic oil immersion objective lens and an ocular micrometer (K-15X PZO) Poland. Size ranges are shown in parentheses followed by average and shape index (L/W ratio).

2.5. Assessment of the genotoxic activity

For estimating genotoxic property, a Japanese quail from each group, chosen randomly, were used to collect blood samples on days 2, 4 and 28 post infection. Smears slides were prepared and all were code, fixed with methanol and stained with Giemsa solution. For micronucleus (MN) presence, two thousand erythrocytes from each animal (one thousand/slide) were scored and the total of micronucleated polychromatic erythrocytes (MNPCEs) and micronucleated normochromatic erythrocytes (MNNCEs) was determined.

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