The efficacy of pomegranate peel extract on *Eimeria* shedding and growth indices in experimental coccidiosis in broiler chickens

Pedram Khorrami1 | Majid Gholami-Ahangaran2 | Elham Moghtadaei-Khorasgani3

1 Graduated of Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
2 Department of Poultry Diseases, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
3 Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

**Abstract**

**Background:** Coccidiosis is the most common enteric parasitic disease in poultry that is caused by different species of *Eimeria*. Some chemical drugs were utilized for the control of this infection but drug residue and resistance-induced concerns in chicken consumers.

**Objective:** The present study was performed to evaluate the effect of pomegranate peel extract (PPEx) on the control of coccidiosis in chickens.

**Methods:** Totally, 300 broiler chicks were divided into 10 groups and 5 groups challenged with a mixture of common *Eimeria* of 21 days old in Iran. Five days after the challenge, as soon as clinical signs appeared, the oocyte per gram (OPG) of faeces was measured, and chickens were treated with 100, 200 and 400 ppm PPEx (for 7 days) and toltrazuril (for 2 days) in separate groups. Another five groups were only treated with these compounds without challenge. All challenged and non-challenged have a control group. The OPG was measured in the final treatment in chickens. At 35 days of age, all chickens were euthanized and the intestinal and liver samples were collected in 10% formalin for histopathological examination. The growth indices were recorded and analysed all over the growing period, weekly.

**Results:** The results revealed that the addition of PPEx especially in 400 ppm can reduce the intestinal lesions and OPG but in this dose, the growth indices can influence negatively, while the administration of toltrazuril with the best effect on OPG and intestinal lesions, no effect on growth indices in challenged chickens. Also, supplementation of 400 ppm PPEx can induce injuries in the liver while the lower dose has no effect on liver tissue.

**Conclusion:** It is necessary for finding effective ingredients in PPEx and preparation of serial dilution for access to optimal dose with best anti-coccidial effect and lowest negative effect on growth indices in chickens.

**Key words**

chickens, coccidiosis, *Eimeria*, pomegranate peel

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Authors. Veterinary Medicine and Science published by John Wiley & Sons Ltd.
1 | INTRODUCTION

Coccidiosis is a protozoan parasitic disease that is identified by lesions in the intestines. Coccidiosis is the most common parasitic disease of poultry that is caused by different species of *Eimeria* (Conway & McKenzie, 2008). Nine species of *Eimeria* can cause intestinal lesions in chickens that comprise *E. tenella*, *E. acervulina*, *E. maxima*, *E. brunetti*, *E. necatrix*, *E. mitis*, *E. praecox*, *E. mivati* and *E. hagani*. Usually, each of these species multiplies and causes complications in the epithelium tissue of different parts of the intestine via ingestion (Pop et al., 2019). Recent studies represent that contaminated larva and mealworms can transfer some pathogens as vector, for example, *Eimeria* spp. and avian pox virus (Gholami-Ahangaran et al., 2014). The economic losses caused by this disease are significant due to the global spread and relatively high prevalence in poultry-producing countries. The economic losses of this disease include weight loss, reduced food efficiency, anti-coccidial therapy and mortality (Blake et al., 2020). Furthermore, the *Eimeria* infections can influence digestion and absorption in gastrointestinal tract that lead to immunosuppression and low performance (Gholami-Ahangaran et al., 2021). In addition, the *Eimeria* infection can increase the removal of carcasses in the slaughterhouse (Gholami-Ahangaran, Karimi-Dehkordi, Miranzadeh-Mahabadi, et al., 2021; Rahimi et al., 2012). The infection appears clinical or sub-clinical forms, but it seems that in Iran, as in other countries of the world, the sub-clinical form has a high prevalence and causes great losses to the poultry industry by reducing food efficiency and weight gain (WG) (Shirzad et al., 2011).

In the current situation, the most important strategy to control coccidiosis in poultry farms is to use chemical coccidiostats, vaccines or the use of natural plant compounds (Gholami-Ahangaran, Karimi-Dehkordi, Namjoo, et al., 2021). Although the strategy of using preventive chemical compounds has been very effective in controlling parasitic infections, the emergence of drug resistance and drug residues in animal and poultry tissues has caused great concern (Bahmani et al., 2014) and researchers have been looking for healthy alternatives (Pop et al., 2019). However, in European countries, the use of anti-coccidial compounds as a preventive agent of coccidiosis has been very limited since 2006 and is expected to be completely banned in the coming years (Pop et al., 2019).

Numerous studies have been performed to evaluate the beneficial effects of plant-derived (phytogenic) compounds on broiler performance, gastrointestinal function, as well as meat quality. The addition of some plants and their derivatives to poultry diets has been shown to improve feed conversion and meat production (Ahangaran et al., 2019; Ghasemian et al., 2021; Gholami-Ahangaran, Karimi-Dehkordi, Akbari Javar, et al., 2021).

In the control program of coccidiosis, phytogenic compounds can be considered as a suitable alternative to chemical compounds. The phytogenic anti-coccidial compound, further inhibitory effects against *Eimeria* species can have low side-effects on vital organs (Deghghani-Ashkezari et al., 2019). Recently, there are many reports regarding anti-coccidial effects of phytogenic compounds comprising spices, plant extracts and essential oils (Bozkurt et al., 2013; Deghghani-Ashkezari et al., 2019; Pop et al., 2019).

Although the use of plant origin products is a major option for the prevention and treatment of coccidiosis in the production of poultry without antibiotics (Gholami-Ahangaran, Karimi-Dehkordi, Akbari Javar, et al., 2021), if these compounds are prepared from agricultural residues and have a lower cost and stronger effect than chemical compounds, it will be more welcomed in all poultry production farms (Gholami-Ahangaran, Karimi-Dehkordi, Miranzadeh-Mahabadi, et al., 2021). One of the wastes of the pomegranate paste industry is pomegranate peel, which is not used properly.

Pomegranate with the scientific name of *Punica granatum* is a native fruit to Iran, Africa, China and India. Iran is one of the largest producers of this fruit with an annual production of 990,000 tons. Pomegranate peel has high phenolic compounds, therefore, it has more antioxidant properties than other parts, and anti-fungal, anti-parasitic and anti-cancer compounds are attributed to it (Singh et al., 2019). Pomegranate fruit contains minerals, amino acids, acidic compounds (citric acid, tartaric acid, malic acid, ascorbic acid), anti-cancer compounds and antioxidants (tannins, anthocyanins, ponica and laginine) (Singh et al., 2018). Previous studies have shown that pomegranate peel has anti-parasitic properties and can affect different stages of growth and development in different parasites. Previous studies have shown the effect of pomegranate peel on the adult parasites of *Schistosoma mansoni* (Fahmy et al., 2009), *Trichomonas tenax* (El-Sherbini & Shoukry, 2012), *Cryptosporidium parvum* (Al-Mathal & Alsalem, 2013), *Giardia* (Al-Megrin, 2017) and some nematodes as well as *strongyloides* (Boonmasawai et al., 2013).

According to previous researches, it seems that pomegranate peel can have a good anti-parasitic property against *Eimeria*, which has been examined in this study. In this study, the effect of pomegranate peel extract (PPEX) on *Eimeria* was evaluated with indicators such as reduced oocyt excretion, reduced intestinal pathology lesions and the effect of this treatment on growth indices of broiler chickens. In addition, the possible effects of PPEX on liver tissue were monitored by pathological evaluation of the liver.

2 | MATERIALS AND METHODS

2.1 | Preparation of PPEX

After separating the edible part of the pomegranate fruit, the pomegranate peel was dried in the shade and ground. A total of 100 g of pomegranate peel was soaked in a mixture of water and ethanol (50:50) in a shaking water bath for 24 hours according to Shan et al. (2007). After 24 hours, it was passed through Whatman No. 2 paper. The resulting solution was left in the oven at 70°C for 48 hours until the alcohol was completely evaporated to give a dry extract.

2.2 | Preparation of *Eimeria* oocytes

A mixture of four different strains of *Eimeria* that are most prevalent in Iran, including 10⁵ oocytes of *E. acervulina*, 4 × 10⁴ oocytes of
E. maxima, 4 × 10³ oocytes of E. necatrix and 10 × 10³ of E. tenella, was prepared.

2.3 | Study design and grouping

In this study, 300 Ross broiler chicks were prepared and randomly divided into 10 groups with three equal replications. All chickens were reared until 21 days old under the same nutritional and management conditions. All chickens get a continuous lighting program and they access feed and water freely (ad libitum). Chickens received vaccines according to the local vaccination program. Each chicken in groups 1, 2, 3, 4 and 5 received the mixture of sporulated Eimeria oocysts in 0.5 mL normal saline at 21 days old (challenged chickens). The challenged chickens were monitored for coccidiosis symptoms (chocolate diarrhoea and haemorrhage in the epithelium of the intestine). As soon as there is an appearance of coccidiosis symptoms, the faecal samples were collected in all groups and examined for oocyst per gram (OPG) of faeces. After confirmation of infection, chickens in groups 2, 3, 4 and 5 were treated and group 1 as control did not receive any treatments. The chickens in groups 2, 3 and 4 received 100, 200 and 400 ppm PPEX in drinking water (respectively) for 7 days and the chickens in group 5 received toltrazuril (according to manufacturer instructions; 1 mL/L of drinking water, for 2 days). In this study, five non-challenged groups were considered for comparing data in challenged and non-challenged chickens. The non-challenged chickens in group 6 as negative control were considered for comparing data in challenged and non-challenged chickens. The non-challenged chickens in group 8 received toltrazuril (according to manufacturer instructions; 1 mL/L of drinking water, for 2 days). The chickens in groups 2, 3, 4 and 5 were treated and group 1 as control did not receive any treatments. The chickens in groups 2, 3 and 4 received 100, 200 and 400 ppm PPEX in drinking water (respectively) for 7 days and the chickens in group 5 received toltrazuril (according to manufacturer instructions; 1 mL/L of drinking water, for 2 days). In this study, five non-challenged groups were considered for comparing data in challenged and non-challenged chickens. The non-challenged chickens in group 6 as negative control did not receive any treatment, but non-challenged chickens in groups 7, 8 and 9 received PPEX at 100, 200 and 400 ppm, for 7 days. The non-challenged chickens in group 8 received toltrazuril (1 mL/L of drinking water, for 7 days).

The growth indices comprising feed intake (FI), WG and feed conversion ratio (FCR) were measured weekly all over the growing period.

The faecal samples were collected from all groups at the end of the treatment period, for analysis of OPG. The OPG was measured according to the modified McMaster method (Haug et al., 2006).

All of the chickens were euthanized at 35 days old. The intestine and liver samples were collected and fixed in 10% formalin for histopathological examination. The tissue samples were embedded in paraffin and the tissue sections were prepared by microtome in 5 µm thickness. All of the sections were stained with haematoxylin and eosin (H & E) according to Bancroft and Cook (1994). The intestinal lesions were graded from 0 to 4 based on the severity of lesions; 0 (no lesion), 1 (mild), 2 (moderate), 3 (severe) and 4 (very severe) according to Conway and McKenzie (2008).

2.4 | Statistical analysis

All quantitative data were sorted and analysed using SigmaPlot 12.3 statistical software. One-way ANOVA method was used to compare the means of the data. If there was a significant difference between the data, the Tukey method was utilized. The significant value was selected at the level of p < 0.05.

3 | RESULTS

3.1 | Clinical observation

The chickens were challenged at 21 days old and they showed the clinical signs of coccidiosis comprising anorexia, depression, inability to move, chocolate diarrhoea and haemorrhage on the surface of the intestinal epithelium, at 26 days old (5 days after the challenge).

3.2 | The excretion of Eimeria oocyte (OPG)

The results of oocyte count showed that in the positive control group, the oocyte excretion (OPG) increased significantly after the challenge. In other groups challenged and treated with PPEX and toltrazuril, there was a significant difference in OPG between before and after treatment. This difference between before and after treatment was more pronounced in the groups treated with PPEX at a concentration of 400 ppm and toltrazuril. Comparison of post-treatment results showed that there was no significant difference between the rate of oocyte excretion in the groups treated with 400 ppm of PPEX and toltrazuril. Also, there was no significant difference in the OPG, in chickens receiving the PPEX at doses of 100 and 200 ppm (Table 1). After treatment, the OPG in chickens that received 100 and 200 ppm PPEX was significantly higher than chickens treated with toltrazuril or PPEX at 400 ppm.

3.3 | Growth indices

Comparison of growth indices in different groups at 28 days of age showed that in challenged chickens, higher FCR was obtained than non-challenged chickens. After the challenge, the lowest FI and WG were obtained in positive control. At 28 days of age, the negative control chickens had the highest weight (Table 2).

At 35 days of age, chickens receiving 400 ppm PPEX that were not challenged by Eimeria had the lowest FI and WG. There was no significant difference in FI between challenged chickens and chickens treated with PPEX or toltrazuril. At 35 days of age, the lowest WG was shown in group 5.

| Groups                      | Before challenge | After challenge |
|-----------------------------|------------------|-----------------|
| Positive control            | 14000 ± 2207     | 14966 ± 659     |
| 100 ppm PPEX                | 16333 ± 1074     | 853 ± 203       |
| 200 ppm PPEX                | 15133 ± 2802     | 400 ± 81        |
| 400 ppm PPEX                | 14500 ± 1386     | 269 ± 65        |
| Toltrazuril                 | 15033 ± 2276     | 127 ± 34        |
| Negative control            | 0 ± 0            | 0 ± 0           |

Different superscripts in each row and column represents significant differences between treatments (p < 0.05).
| Groups          | FI (gr)         | WG (gr)         | FCR   |
|---------------|----------------|----------------|-------|
| Non-challenged| Negative control 1570 ± 62<sup>a</sup> | 1150 ± 35<sup>a</sup> | 1.35 ± 0.03<sup>a</sup> |
|                | 100 ppm PPEX 1525 ± 53<sup>a</sup> | 1120 ± 49<sup>a</sup> | 1.36 ± 0.01<sup>a</sup> |
|                | 200 ppm PPEX 1520 ± 49<sup>a</sup> | 1120 ± 55<sup>a</sup> | 1.34 ± 0.03<sup>a</sup> |
|                | 400 ppm PPEX 1510 ± 45<sup>a</sup> | 1120 ± 33<sup>a</sup> | 1.36 ± 0.03<sup>a</sup> |
|                | Toltrazuril 1560 ± 53<sup>a</sup> | 1155 ± 38<sup>a</sup> | 1.35 ± 0.02<sup>a</sup> |
| Challenged     | Positive control 1425 ± 50<sup>b</sup> | 1010 ± 29<sup>b</sup> | 1.39 ± 0.03<sup>b</sup> |
|                | 100 ppm PPEX 1465 ± 63<sup>b</sup> | 1025 ± 45<sup>b</sup> | 1.42 ± 0.04<sup>b</sup> |
|                | 200 ppm PPEX 1450 ± 58<sup>b</sup> | 1020 ± 40<sup>b</sup> | 1.42 ± 0.04<sup>b</sup> |
|                | 400 ppm PPEX 1445 ± 65<sup>b</sup> | 1025 ± 69<sup>b</sup> | 1.40 ± 0.05<sup>b</sup> |
|                | Toltrazuril 1480 ± 67<sup>ab</sup> | 1080 ± 53<sup>ab</sup> | 1.37 ± 0.02<sup>ab</sup> |

Different superscripts in each column represent significant differences between treatments (p < 0.05).

| Groups          | FI (gr)         | WG (gr)         | FCR   |
|---------------|----------------|----------------|-------|
| Non-challenged| Negative control 850 ± 44<sup>a</sup> | 465 ± 38<sup>a</sup> | 1.82 ± 0.06<sup>a</sup> |
|                | 100 ppm PPEX 830 ± 35<sup>a</sup> | 450 ± 35<sup>a</sup> | 1.84 ± 0.05<sup>a</sup> |
|                | 200 ppm PPEX 800 ± 26<sup>ab</sup> | 440 ± 22<sup>a</sup> | 1.82 ± 0.06<sup>a</sup> |
|                | 400 ppm PPEX 750 ± 37<sup>b</sup> | 415 ± 27<sup>ab</sup> | 1.81 ± 0.03<sup>a</sup> |
|                | Toltrazuril 860 ± 67<sup>a</sup> | 475 ± 53<sup>a</sup> | 1.80 ± 0.03<sup>a</sup> |
| Challenged     | Positive control 700 ± 66<sup>b</sup> | 365 ± 50<sup>b</sup> | 1.90 ± 0.05<sup>b</sup> |
|                | 100 ppm PPEX 770 ± 76<sup>ab</sup> | 420 ± 65<sup>a</sup> | 1.84 ± 0.06<sup>a</sup> |
|                | 200 ppm PPEX 750 ± 70<sup>b</sup> | 410 ± 52<sup>ab</sup> | 1.83 ± 0.4<sup>a</sup> |
|                | 400 ppm PPEX 725 ± 37<sup>b</sup> | 395 ± 29<sup>b</sup> | 1.84 ± 0.06<sup>a</sup> |
|                | Toltrazuril 825 ± 47<sup>a</sup> | 445 ± 51<sup>a</sup> | 1.85 ± 0.05<sup>a</sup> |

Differential superscripts in each column represent significant differences between treatments (p < 0.05).

the positive control group (challenged and untreated) and the highest WG was shown in the negative control group (un-challenged and untreated), which showed a significant difference (p < 0.05). In the challenged groups, the lowest FCR was observed in the chickens treated with toltrazuril, which is not significantly different from the chickens receiving 200 and 400 ppm PPEX. Also, in the challenged groups, the highest FI and WG were observed in the chickens treated with toltrazuril, which showed a significant difference with the chickens receiving 200 and 400 ppm PPEX (p < 0.05) (Table 3).

### 3.4 Histopathological observations

Histopathological examination of intestinal epithelium in challenged-un-treated chickens showed that necrosis and haemorrhage were predominant lesions in these chickens. In these chickens, different developmental stages of *Eimeria* and inflammatory cells (mononuclear and eosinophils) were observed in the intestinal epithelium (Figure 1). Chickens treated with different concentrations of the PPEX as well as toltrazuril showed fewer lesions. Analysis of quantitative data on the degree of intestinal lesions showed that in challenged-treated chick-

![Degeneration of hepatocyte and leukocyte infiltration in liver of chickens treated with PPEX 400 ppm (H & E staining; ×100)](image-url)

ens, the lowest intestinal lesion score was observed in chickens treated with toltrazuril, which was significantly lower than other groups. Groups treated with different concentrations of the PPEX had significantly fewer lesions than the positive control chickens (challenged-un-treated chickens). Chickens receiving 400 ppm of the PPEX had...
The present study showed that PPEx reduces intestinal lesions following challenges with *Eimeria* oocysts and can reduce the excretion of *Eimeria* oocysts from the gastrointestinal tract. These effects are mainly seen following the administration of PPEx at a concentration of 400 ppm and are the same as toltrazuril. However, a comparison of growth indices shows that in non-challenged groups, the lowest FI and WG were observed in chickens receiving 400 ppm PPE. Also in the challenged-treated chickens, the lowest FI and WG are seen in chickens treated with 400 ppm PPE. In addition, the study of pathological lesions in the liver shows that the highest liver lesions are observed at a dose of 400 ppm PPE. These findings may indicate that PPE at a dose of 400 ppm can have acceptable anti-coccidial activity, but since it has a negative effect on the histological structure of the liver, and growth indices is not proposed suitable alternative to chemical anti-coccidial drugs. Therefore, it is necessary to design other studies on the effective dose of PPE based on serial dilution to select the most effective dose on *Eimeria* with the least side-effect on growth indices and liver tissue.

Evaluation of 100 and 200 ppm doses of PPE shows that although this extract has relative anti-coccidial effects at these doses, these effects are significantly less than toltrazuril in reducing oocyte excretion and reducing intestinal lesions. In addition, the evaluation of the effects of doses of 100 and 200 ppm of PPE shows that in these doses, no pathological effects on the liver are induced and no negative effects on growth indices are observed. Therefore, in doses of 100 and 20 ppm of PPE, the anti-coccidial effects were significantly less than toltrazuril and the effectiveness anti-coccidial activity was not observed in comparison with toltrazuril.

Researchers believe that the use of phenolic compounds in the food chain can be effective in controlling parasitic diseases and is indirectly a cheap and easy way to improve the health of livestock and poultry against parasitic diseases. Pomegranate peel is a valuable source of bioactive compounds such as phenolic acids, hydrolysable tannins and flavonoids (Conway & McKenzie, 2008). Elfalleh, et al. (2012) compared pomegranate peel, flower, seed and leaf for total phenolics, flavonoids, anthocyanins and hydrolysable tannins levels and reported that the highest level was found in Pomegranate peel. Various studies have shown the anti-bacterial, anti-fungal and anti-parasitic effects of pomegranate peel in vivo and in vitro (Elfalleh et al., 2012). For example, the anti-bacterial effect of pomegranate on *Staphylococcus aureus* and the antifungal effect on *Aspergillus niger* (Oraki et al., 2011) and the anti-parasitic effect on the adult worm of *S. mansoni* (Fahmy et al., 2009) and *Trichomonas tonax* (El-Sherbini & Shoukry, 2012) has been proved. There are some reports related to the potential anti-parasitic activity of PPE (Al-Megrin, 2016, 2017). Al-Megrin (2016) reported the anticestodal activity of PPE against *Hymenolipes nana* (a dwarf tapeworm infection) (Al-Megrin, 2016). In another study, the effectiveness of PPE in the prevention and treatment of *Giardia lamblia* infection was proved (Al-Megrin, 2017). Also, in another study by Bunviboolvat et al. (2013) in Thailand, the anti-parasitic effect of alcoholic PPE on nematode eggs in the gastrointestinal tract of lambs was reported and this extract was able to reduce the excretion of nematode eggs (Bunviboolvat et al., 2013). The anti-parasitic effect of PPE in the recent study agreed with other previous studies but it seems the access to an effective dose with the least negative effect on growth and vital organs requires more detailed study.

Various studies have been performed on the effect of PPE on growth indices in chickens (Qnais et al., 2007; Rezvani & Rahimi, 2017). Hamady et al. (2015) studied the addition of 0.1% PPE powder in the chicken diet for 6 weeks and showed continuous supplementation

**TABLE 4** The microscopic lesion score of intestine in examined chickens 7 days after treatment

| Groups          | Lesion scores of intestine |
|-----------------|----------------------------|
| Positive control| 2.10 ± 0.72<sup>a</sup>    |
| 100 ppm PPEx    | 1.20 ± 0.91<sup>a</sup>    |
| 200 ppm PPEx    | 1.00 ± 0.80<sup>b</sup>    |
| 400 ppm PPEx    | 0.80 ± 0.65<sup>b</sup>    |
| Toltrazuril     | 0.30 ± 0.21<sup>c</sup>    |
| Negative control| 0 ± 0<sup>c</sup>          |

Different superscripts in each column represents significant differences between treatments (p < 0.05).

**FIGURE 2** The developmental stages of *Eimeria* oocyte (black arrow) and infiltration of inflammatory cells (red arrow) in the intestinal epithelium of challenged chickens (H & E staining; 400x)

significantly fewer lesions than other challenged-treated chickens with different concentrations of the PPE (p < 0.05) (Table 4).

Liver histopathological examination showed that chickens receiving 100 and 200 ppm of PPE had normal liver tissue but in chickens receiving 400 ppm PPE, the degeneration of hepatocytes and infiltration of leukocytes were observed (Figure 2). In chickens treated with toltrazuril, congestion in the central vein, leukocyte infiltration and hepatocyte swelling was observed.

**4 | DISCUSSION**

In the present study, the effect of PPE on growth indices, excretion of *Eimeria* oocyte and intestinal and liver pathology in an experimental infection with *Eimeria* species in broiler chickens was investigated. The present study showed that PPE reduces intestinal lesions following challenges with *Eimeria* oocysts and can reduce the excretion of *Eimeria* oocysts from the gastrointestinal tract. These effects are mainly seen following the administration of PPE at a concentration of 400 ppm and are the same as toltrazuril. However, a comparison of growth indices shows that in non-challenged groups, the lowest FI and WG were observed in chickens receiving 400 ppm PPE. Also in the...
of PPEx powder in chicken diets can reduce FI and increase WG. In this study, the FCR value was not influenced by PPEx supplementation (Hamady et al., 2015). A study by Rezvani and Rahimi (2017) designed the effect of PPEx on WG, digestibility of food, microbial flora and antibody titre of broiler chickens. In this study, PPEx improved daily WG and FI throughout the growing period but had no effect on FCR (Rezvani & Rahimi, 2017). Also, PPEx improved the digestibility of nutrients, beneficial microbial flora of Lactobacillus and increased antibody titre at 39 days old. However, the effect of PPEx on FI and WG is inconsistent with the mentioned studies and the results of the present study showed that although PPEx 100 and 200 ppm does not significantly change in WG and FI, a dose of 400 ppm can significantly reduce WG and FI. Decreased FI and ultimately decrease in WG in these chickens can be attributed to the presence of tannins in pomegranate peel. High tannins in pomegranate peel change the taste of water and chickens consume less water. Taking into account, feed and water consumption in chickens is interdependent, not consuming enough water leads to reduced FI and ultimately decreases WG. Also, Qnais et al. (2007) demonstrated the beneficial effect of tannins and flavonoids of PPEx on reducing intestinal motility and intestinal fluid accumulation. The decrease of intestinal motility can reduce appetite and lead to decrease FI and WG (Qnais et al., 2007). However, no improvement of FCR following the administration of PPEx in different doses is consistent with previous studies (Hamady et al., 2015; Rezvani & Rahimi, 2017).

In general, the results of the recent study showed that PPEx reduces the excretion of Eimeria oocysts in the faeces, which is dose-dependent, and with increasing the dose of the extract, an increase in anti-coccidial effect is observed. Since, increasing the dose of the extract has negative effects on liver tissue, FI and WG of chickens, it is recommended to adjust the effective dose for anti-coccidial effects without a negative effect on growth indices. Phytochemical studies on the purification of beneficial components are also recommended in future studies.

ACKNOWLEDGEMENT
The authors are grateful for the cooperation of the Vice Chancellor for Research of Shahrekord Branch, Islamic Azad University, and the Director of the Poultry Breeding Research House in Shahrekord Branch, Islamic Azad University.

ETHICAL STATEMENT
The chickens reared and cared according to animal care instruction of Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University.

AUTHORS’ CONTRIBUTION
Pedram Khorrami: Funding acquisition, Resources, Validation, Visualization; Majid Gholami-Ahangaran: Conceptualization, Methodology, Supervision, Writing original draft, Writing review & editing; Elham Moghtadaei-Khorasgani: Methodology, Visualization

DATA AVAILABILITY STATEMENT
All data are available and reserved in near of corresponding author. The requests are answered.

PEER REVIEW
The peer review history for this article is available at https://publons.com/publon/10.1002/vms.3714

ORCID
Pedram Khorrami https://orcid.org/0000-0002-3599-4514
Majid Gholami-Ahangaran https://orcid.org/0000-0002-2725-1091
Elham Moghtadaei-Khorasgani https://orcid.org/0000-0002-8666-5104

REFERENCES
Al-Mathal, E. M., & Alsaleem, A. A. (2013). Pomegranate ( Punica granatum ) peel is effective in a murine model of experimental Cryptosporidium parvum ultrastructural studies of the ileum. Exp Parasitol, 134(4), 482–494.
Al-Megrin, W. (2016). Efficacy of pomegranate ( Punica granatum ) peel extract against Hymenolepis nana in infections mice. Biosciences Biotechnology Research Asia, 13(1), 103–108.
Al-Megrin, W. A. (2017). In vivo study of pomegranate ( Punica granatum ) peel extract efficacy against Giardia lamblia in infected experimental mice. Asian Pacific Journal of Tropical Biomedicine, 7(1), 59–63.
Bancroft, J. D., Cook, H. C. (1994). Manual of histological techniques and their diagnostic application (p. 53). Churchill Livingstone.
Bamhani, M., Abdi, F., Adineh, A., Hassanazadazar, H., Eghbali, B., Gholami-Ahangaran, M., & Rafieian-Kopaei, M. (2014). The anti-leech effect of ethanolic extract of achillea millefolium I. Compared to levamisole and niclosamide on limnatis nilotica. Studia Universitatis Vesile Goldis Arad, Seria Stiintele Vietii, 24(3), 293–297.
Bunviboolvat, P., Taechaarponkul, N., Saratham, J., Sungpradit, S., Jirapattharasate, C., Nakthong, C., Piasai, L., Thongjui, O., & Boonmasawai, S. (2013). Anthelmintic effects of ethanolic extracts from pomegranate peels, mangosteen peels and tamarind seeds on gastrointestinal nematode egg counts in lambs. Journal of Applied Animal Science, 6(2), 39–48.
Blake, D. P., Knox, J., Dehaeck, B., Huntington, B., Rathinam, T., Ravipati, V., Ayoade, S., Gilbert, W., Adebambo, A. O., Jatau, I. D., Raman, M., Parker, D., Rushton, J., & Tomley, F. M. (2020). Re-calculating the cost of coccidiosis in chickens. Veterinary Research, 51(1), 1–14.
Boonmasawai, S., Sungpradit, S., Jirapatharasate, C., Nakthong, C., & Piasai, L. (2013). Effects of alcoholic extract from pomegranate ( Punica granatum L.) peels on gastrointestinal nematode egg counts in doe. Journal of Applied Animal Science, 6, 27–37.
Bozkurt, M., Giannenas, I., Küçükyılmaz, K., Christaki, E., & Florou-Paneri, P. (2013). An update on approaches to controlling coccidia in poultry using botanical extracts. British Poultry Science, 54(6), 713–727.
Conway, D. P., McKenzie, M. E. (2008). Poultry coccidiosis diagnostic testing procedures (p. 72). Blackwell Publishing.
Dehghani-Askarzai, A. A., Gholami-Ahangaran, M., & Fathi-Hafshejani, E. (2019). The effect of garlic extract on reducing of side effects of Eimeria tenella oocyte on the growth indices and mucosal tissue of cecum in broiler chickens. Iran J Anim Biol, 11(2), 45–54.
Elfalleh, W., Hannachi, H., Tlili, N., Yahia, Y., Nasri, N., & Ferchichi, A. (2012). Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. Journal of Medicinal Plant Research, 6(32), 4724–4730.
El-Sherbini, G. T., & Shoukry, N. M. (2012). In vitro effect of pomegranate peel extract on Trichomonas tenax. Life Science Journal, 9(3), 791–797.
Fahmy, Z. H., El-Shennawy, A. M., El-Komy, W., Ali, E., & Hamid, S. S. A. (2009). Potential antiparasitic activity of pomegranate extracts against Schistosomules schis and mature worms of Schistosoma mansoni: in vitro and in vivo study. Australian Journal of Basic and Applied Sciences, 3(4), 4634–4643.
Ghasemian, S. O., Gholami-Ahangaran, M., Pourmahdi, O., & Ahmadi-Dastgerdi, A. (2021). Dietary supplementation of proteixin and artichoke extract for modulating growth performance and oxidative stress.
in broilers, Ankara Üniversitesi Veteriner Fakültesi Dergisi, https://doi.org/10.33988/avsf.833094
Gholami-Ahangaran, M., Fathi-Hafshejani, E., & Seyed-Hosseini, R. (2013). Seromolecular study of chicken infectious anemia in chickens, ostriches, and turkeys in Iran. Journal of Applied Poutry Research, 22(3), 404–409.
Gholami-Ahangaran, M., Zia-Jahromi, N., & Namjoo, A. (2014). Molecular detection of avian pox virus from nodular skin and mucosal fibro-necrotic lesions of Iranian backyard poultry. Tropical Anim Health and Product, 46(2), 349–353.
Gholami-Ahangaran, M., Peiman, N., & Ahmadi-Dastgerdi, A. (2019). The effect of thyme (Thymus daenensis) supplement on growth and hygienic parameters of broilers meat. Iraqi Journal of Veterinary Sciences, 33(1), 87–92.
Gholami-Ahangaran, M., Karimi-Dehkordi, M., Akbari Javar, A., Haj Salehi, M., & Ostadpoor, M. (2021). A systematic review on the effect of Ginger (Zingiber officinale) on improvement of biological and fertility indices of sperm in laboratory animals, poultry and humans. Veterinary Medicine and Science, 7, 1959–1969.
Gholami-Ahangaran, M., Karimi-Dehkordi, M., Miranzadeh-Mahabadi, E., & Ahmadi-Dastgerdi, A. (2021). The frequency of tetracycline resistance genes in Escherichia coli strains isolated from healthy and diarrheic pet birds. Iranian Journal of Veterinary Research, 22(4), 277–282. https://doi.org/10.22099/ijvr.2021.38454.5592
Gholami-Ahangaran, M., Karimi-Dehkordi, M., Namjoo, A., Shojaei, H., & Ahmadi-Dastgerdi, A. (2021). The co-administration effects of flordenicol and lusalocid on performance, biochemical and pathological parameters of muscle, heart, liver, kidney and sciatic nerve in broiler chickens. Veterinary Medicine and Science, https://doi.org/10.1002/vms3.626
Hamady, G. A., Abdel-Moneim, M. A., El-Chaghaby, G. A., Abd-El-Ghany, Z. M., & Hassanin, M. (2015). Effect of Pomegranate peel extract as natural growth promoter on the productive performance and intestinal microbiota of broiler chickens. African Journal of Agricultural Science and Technology, 3(12), 514–519.
Haug, A., Williams, R. B., & Larsen, S. (2006). Counting coccidial oocysts in chicken faeces: a comparative study of a standard McMaster technique and a new rapid method. Vet Parasitol, 136(3-4), 233–242.
Qnais, E. Y., Elokia, A. S., Abu Ghalyun, Y. Y., & Abdulla, F. A. (2007). Antidiarrheal activity of the aqueous extract of Punica granatum (Pomegranate) peels. Pharmaceutical Biology, 45(9), 715–720.
Oraki, H. H., Demirci, A. S., & Gümüş, T. (2011). Antibacterial and antifungal activity of pomegranate (Punica granatum L. cv) peel. Electronic Journal of Environmental, Agricultural and Food Chemistry, 10(3), 100–110.
Pop, L. M., Varga, E., Coroiu, M., Nedijan, M. E., Mircean, V., Dumitruche, M. O., Farcădă, L., Fülop, L., Croitoru, M. D., Fazakas, M., & Győrke, A. (2019). Efficacy of commercial herbal formula in chicken experimental coccidiosis. Parasit Vectors, 12(1), 343–351.
Rahimi, E., Hormozipoor, H., Gholami Ahangaran, M., & Yazdi, F. (2012). Prevalence of Arcobacter species on chicken carcasses during processing in Iran. Journal of Applied Poutry Research, 21(2), 407–412.
Rezvani, M. R., & Rahimi, S. (2017). Effects of adding pomegranate peel extract and commercial antioxidant to diets on performance, nutrient digestibility, gastrointestinal micro flora and antibody titer of broilers. Journal of Veterinary Research, 72(2), 110–123.
Shan, B., Cai, Yi-Z, Brooks, J. D., & Corke, H. (2007). The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology, 117, 112–119.
Shirzad, M. R., Seifi, S., Gheisari, H. R., Hachesoo, B. A., Habibi, H., & Bujmehrani, H. (2011). Prevalence and risk factors for subclinical coccidiosis in broiler chicken farms in Mazandaran province, Iran. Trop Anim Health Product, 43(8), 1601–1604.
Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2018). Phenolic compounds as beneficial phytochemicals in pomegranate (Punica granatum L) peel: A review. Food Chem, 261, 75–86.
Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2019). Antimicrobial potential of pomegranate peel: a review. International Journal of Food Science & Technology, 54(4), 959–965.

How to cite this article: Khorrami, P., Gholami-Ahangaran, M., & Mohtadaei-Khorasgani, E. (2022). The efficacy of pomegranate peel extract on Eimeria shedding and growth indices in experimental coccidiosis in broiler chickens. Veterinary Medicine and Science, 8, 635–641. https://doi.org/10.1002/vms3.714