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Nutritional Comparison of Different Levels of Pomegranate Peel Extracts on Production Values and Performance in Female Rabbits

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Abstract:

Pomegranates (*Punicagranatum*) are common plants that are related to the *Lythraceae* family. These plants and their extracts have used in medicinal properties including antioxidant, anti-inflammatory, antimicrobial activities. The present study carried out for 75 days and used 24of local-breed female rabbits that were aged 6 - 8 months. These animals were divided into three equal groups, each includes 8 females.

The pomegranate peel extracts (PPE) were prepared and reached two aqueous concentrations, which are 200 and 100 mg/ml (w/v) for each group. This study indicated using PPE at 100 mg/ml orally led to significant effects more than 200 mg/ml, which were applied to the female rabbits. The parameters showed improvement of the body weight and gain, FCR values and feed intake at a low dose.However, the high dose of PPE had no significant difference in the trials of growth performance compared to control. Furthermore, the low dose of PPE illustrated an interesting impact of the pH values in the stomach and caecum.

Nonetheless, this study reported that the administration of PPE orally led to a significant decrease in total counting of two pathogenic bacteria such as *E. coli* and Salmonella spp.In conclusion, the 100 mg/ml of PPE may promote the production and performance of the female rabbits.

Keywords: Pomegranate peel extracts, pH values, performance values, female rabbits, pathogenic bacteria

Introduction:

Traditional medicine has used plants and herbs as treatment of diseases throughout the world for centuries (Dakheel and Al-saigh, 2012). Food and dietary additives, for humans and animals, have been applied through herbal infusion, decoction and/or aqueous and alcoholic

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extracts (Mills and Bone, 2000). These plants contain many beneficial actions of therapeutical properties that could treat some disorders; thus, several studies have widely investigated for their biological activities such as reduction of diarrhoea, ulceration and poisoning effect (Gurib-Fakim, 2006), antioxidants (Mills and Bone, 2000),anti-inflammatory (Atshanet al., 2020), antimicrobial (Dakheel et al., 2020), anthelmintic effects (Williams et al., 2014), anticancer (Al-Bedhawi and Dakheel, 2021), and cardiovascular and diabetes affected on innate immune responses (Tolossa et al., 2013).These medicinal plants have well-known bioactivities that may provide a useful action for research, especially when they combined with other compounds (Mueller-Harvey, 2010; Charlier et al., 2014). These bioactive compoundscould, therefore, contribute to the stability of active ingredientsand played a vital role to prevent the impact of free radicals that might cause cell damage (Mansouri et al., 2014; Mushtaq et al., 2015).

Pomegranates(*Punicagranatum*) are shrub plants of the family *Lythraceae* that has a fruit-bearing deciduous. They are grown in wild areas that extend from Iran, Iraq and Syria throughout Mediterranean countries (Badawi and Gomaa, 2016).The ancient people have used this plant, besidesother fruits, in medicinal activities because it contains a lot of substantial compounds such as polyphenols e.g. flavonoids,terpenoids, phenols, anthocyanin, coumarins, alkaloids and tannins and these compoundsshowedantioxidant activity thatassociated with its fruit (Negi et al., 2003; Afaq et al., 2005; Zahin et al., 2010).

Moreover, pomegranate extract reported preventing many of major health issues, including anti-microbial effect, inflammatory activity (Julie Jurenka, 2008) and anti-oxidant activity (Ibrahim et al., 2017) anti-cancer (Jihadi and Hadi, 2018). Several studies have also mentioned that pomegranate extract could be a significant food supplement that canimprove the growth which leads to protected from the diseases (Shams Ardekani et al., 2011; Kharchoufi et al., 2018). However,this study had a concern that pomegranate extract may have a negative influence on animal performance. Therefore, this study aimed to investigate the *in vivo* effect of different levels of pomegranate peel extracts (PPE) on production values and performance in healthy female rabbits.

Materials and methods:

Collecting and drying the pomegranate peel samples

Thelocal pomegranate fruits were purchased from Baghdad markets and washed by clean water. The fruits were then peeled and their edible portions were manually separated from the

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fruits carefully. The peel samples were left in a shade to dry byair.Afterwards, these samples weregrounded well in an impeller cutting mill to pass a <1 mm as a powder. Thepowdered peels were stored at room temperature in airtight containers until the extraction was processed for the next experiment.

Pomegranate peel extraction and preparation

The extracts preparation method was followed and applied according to Ma et al. (2011). To reach these two different concentrations, which is 200 and 100 mg/ml (w/v) for each group;a100 and 50 g of dried pomegranate peel powder was dissolved in500ml of sterilise boiling water, separately. The aqueous extract was performed on the milled peels in a conical flask witha magnetic stirrer for 3h at 40°Cwithspinning the liquid at 70ppm. The liquid samples were filtered using a filter paper (Whatman No.) in a Buchner funnel to removethe extra residues fromtheextracts; theseliquid extracts werekept in the fridge at 4°C untilthey have beenused at the same day freshly.

Animal housing and the experimental design

The current study carried out in the Animal-house at the College of Veterinary Medicine for around 75 days. Twenty-four of local-breed female rabbits were purchased from the local market, aged 6 - 8 months; the animals, then, were divided into three equal groups, each contains 8 females. The rabbits were left for 14 days as preliminary and adaptation time.

Afterwards, the first group of rabbits weighted average 1515 ±100g that fed a basal diet (0% of PPE), which include a commercial pellets % (crude of proteins=18.5; crude fibre=14.5; fat=2.5). These animals were kept as control and were named (C);the second group weighted average 1520 ±100g that fed a basal diet plus administrated 5ml of liquid extract per rabbit, which contained 100mg/ml of PPE (low dose) and this treatment was named(T1). The third group of these animals that weighted an average 1510 ±100g and fed the same portion of 2nd group, but the animals have orally administrated with 200 mg/ml of liquid extract and this treatment was named (T2).These rabbits were kept in individual cages of commercial type then they provided with feeders and automatic nipple drinkers.

The digestible energy of feeding was calculated approximately 2700 kcal per kilograms, as well as the chemical analysis of the diets, were determined according to AOAC (2007). Thesegroups were also housedat the sameanimal management and hygienic conditions during the experimental time(Marai et al., 2002).

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Ethics approval

The protocols, which applied to the experimental rabbits, was followed the Institutional Animal Care and Use Committee (IACUC). The scientific committee in the College of Veterinary medicine approved this experiment.

Performance and production values

In vivo trials were conducted to detect the production values and healthy performance of rabbits such as body weight, Feed Conversion Ratio (FCR), feed intake (FI) and weight gain as described by Ibrahim et al. (2017).

The bodyweight of the animals was measured every 25 days during the whole experimental time,plus the weight gain was recorded monthly. Feed intake was also determined precisely as grams (a rabbit per day). The leftover diet of the individual cage was collected and weighed daily for the recording of feed consumption; similarly, the FCR was calculated as 1g feed/ 1 g gain (Hassan et al., 2020).The body weight and weight gain of these animals were calculated individually, whereas FI and FCR were deepened on the cage unit.

CaecalpH values

At the end of the experiment, three healthy rabbits from each group were sacrificed andtheir digestaof the caecum wasindividually separated from the body and placed immediately in a sterile container; then, each sample was diluted with 2ml of (7.0 pH) distilled water. Afterwards, the pH values of the samples weremeasured directly for the caecal contents using a pH-meter (China).

Microbial counting of caecalcontent:

At the end of the experiment, the caecal contents of three scarifying rabbits were collected from each group. The caecalingesta was immediately obtained and diluted with 1g sample of digesta to 9 ml nutrient broth (ViadeLevure/VL); the caecal contents were stored immediately in a sterile and anaerobic jar that was kept in an ice-box. Afterwards, these samples were kept at (-20˚C) until they were examined. At the same time, the caeca were washed with sterile and distil water then were kept in a sterile container that contains4% formaldehyde. The ingesta samples were serially diluted 1/10 millilitre (i.e. from 10^1 to 10^7); each ingesta sample was inundated on the suitable agar media, including the duplicated sample for identification

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of the microbial populations. Counting bacteria were achieved utilizing under aerobic conditions of the suitable dilution and plate culture strategies (Younis et al., 2016)

Total numbers of aerobic bacteria were cultured on nutrient agar usinga sterile Petri-dishthat was incubated at 37°C for 48h. Moreover, the coliform bacteria were separated and cultured on a differentiation medium under aerobic conditions for overnight incubation at the same temperature. Afterwards, the visible colonies were counted and expressed in log10 colony-forming units (CFU) related to 1g of the sample according to standard methodology as described previously of Celia et al. (2016).

Characterisation of histological study

For the histological study, pieces (2 mm X 2 mm) of stomach and caecum tissues haveseparately been kept in 10% concentration of formalin buffer phosphate immediately. After two days of fixation, the specimens of these tissues wereprocessed using the alcohol concentrations. The sections of those were embedded in paraffin blocks and sectioned by microtome for(3–5 µm thickness). These tissues were, then, cut andstained to theHematoxylin and Eosin stain (Feldman and Wolfe, 2014) for histopathological detection thatwas examined using a light microscope provided by a digital camera.

Statistical analysis:

Statistically, the data were analysed using Minitab software (2017) version 18.0 (PA, USA). The Significant differences among means of the control and treated groups were compared and obtained using multiple comparisons of Tukey's test adjusted comparisons and one-way ANOVA analysis model at (*P-values*) was set at $P \leq 0.05$.Thedata were based on triplicates (n=3) including control values plus standard error of the means (±SEM).

Results

Performance traits

Table 1 illustrates the results of performance traits that showed the rabbits of treated with PPE recorded a significant increase in the final body weight and the average of weight gain for each rabbit per a day during the experimental time. Thecontrol group recorded 1990±100g and 475.00g for final body weight and weight gain respectively; nonetheless, the body weight of the second and third group was affected significantly ($P \leq 0.05$) compared to control, which measured 2100 ±150g / 590.00g and 2010 ±120 g/ 490.20g for final body weight and weight

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gain, respectively. In general, orally administrated of 100 mg/ml of aqueous PPE improved significantly (P<0.05) the growth performance; however, the high concentrations (200 mg/ml) of this extract was observed a negative impact on growth performance.

Table 1 also showed that treated group with100 mg/ml ofPPE consumed significantly (P<0.05) more diets compared toothergroups; however, the treated group that received 200 mg/ml of PPE showedless significant differences (P<0.05) in feed intake values compared to the control. As a result, a significant enhancement in body weight and gain was observed at P<0.05, as well as, significant improvement in the feed intake and FCR values at the low dose of PPE (100 mg/ml). In contrast, no significant differences (P<0.05) was recorded in the feed intake and FCR of the rabbits that administrated orally with (200 mg/ml) high dose of PPE compared to the control.

Table 1: The results of the performance traits at the end of the experiment

Groups	Bodyweight (g)	Body gain (g)	Feed intake	FCR
Control	1990 ±45 c	475.00 ±20 c	199 ±75 b	2.39 ±0.23 b
T1	2100 ±50 b	590.00 ±22 a	200 ±52 a	2.95 ±0.30 a
T2	2010 ±82 a	490.20 ±25 b	206 ±65 b	2.38 ±0.10 b

The values in the same column that have different letters showed significant differences at (p<0.05); data presented as Mean ±SEM.

The pH values of the stomach and caecum

At the end of the experiment, pH of the stomach and caecalingesta wastaken,the findings in Figure 1 demonstrated that pH values of the stomach and caecalingesta were lower in treated groups, especially T2, compared to the control at 63 days of age.Moreover, theanimals in the T1group had no significant values of the stomach and caecal pH than the rabbits in T2. Asa result, the pH of the stomach and caecal of control was recorded higher values but not significant (*P≤0.05*) for rabbits in the treated groupsthat tended to be similar either in stomach ingesta to (1.80, 1.70, 1.60) or in caecal contents to(6.70,6.50 and 6.30)among the control, T1 and T2treatment, respectively.Consequently, an extract administratedof PPEshowed no impactinpH values of the stomach and caecum. The only interesting finding

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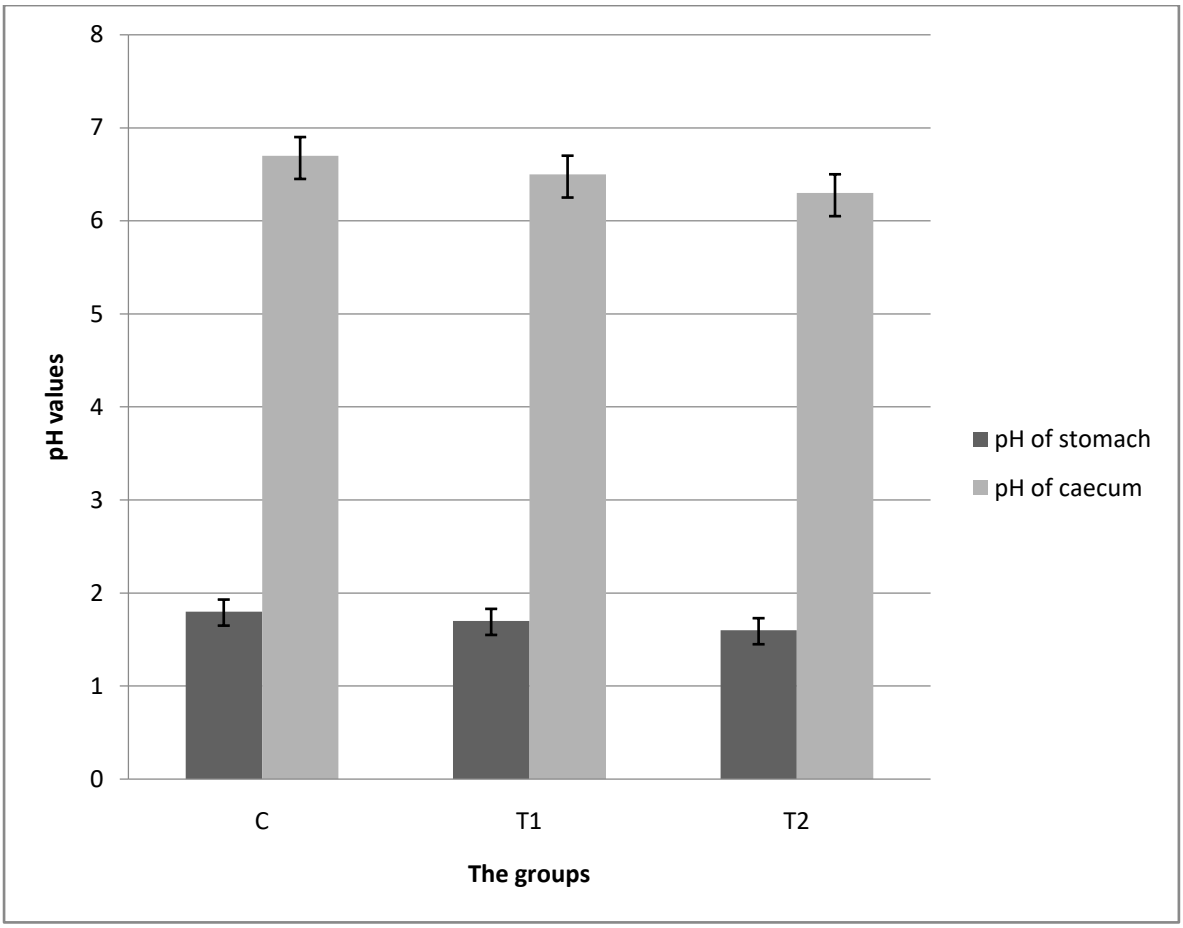
was that the pH was recorded lower values of the stomach content of T2 treatment, but no significant differences among groups (P≥0.05).

Figure 1: The pH values of the stomach and caecalingesta



Data presented as Mean ±SEM.

Microbial population in caecal content

The caecal microbial population was influenced by PPE administration and these results were demonstrated in Figure 2. Giving the rabbits different doses diet containing 0.5ml and 1.0ml of PPE led to significantly (*P<0.05*) changes in the caecal coliforms compared to control. Interestingly, both doses of PPE recordeda reduction of caecalaerobic bacteria such as *E. coli* and Salmonella spp. The total counting of aerobic bacteria ($log10^7$ CFU/ g) was observed as well.Both treated groups were showedclosed findings thatwere found in total microbial counting.

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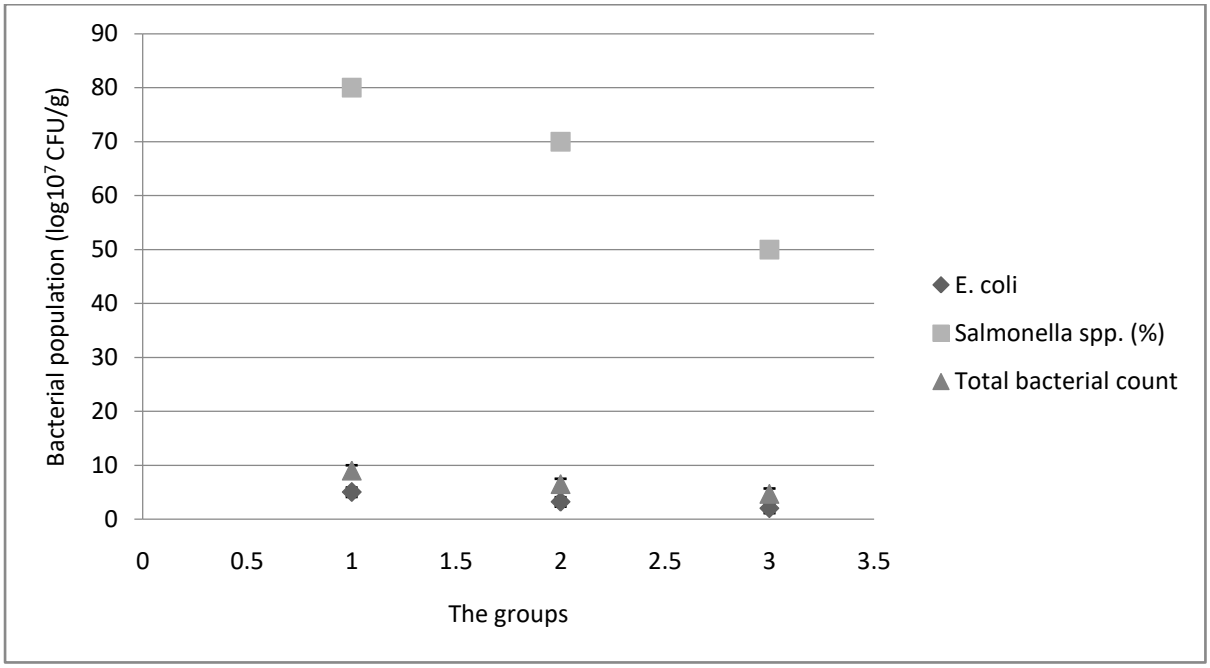
Figure 2: Effect of PPE administration on bacterial population ($log10^7$CFU/g) in caecal content.



* Salmonella spp was detected as a percentage (%); different letters in rows indicate significant differences at *P<0.05*.

Histological sections of the stomach and caecum

Figure 3 demonstrates the changes in the normal stomach tissue and structure in rabbits at the control group; this section revealed a normal gastric mucosa with normal parietal and chief cells as well as muscular mucosa. This section was compared histologically in treated T1 and T2groups (Figure 4 and 5), which demonstrated close results plus more secretory activities in the chief cells.

On the other hands, the evaluation of the normal caecal tissues for rabbits histopathologically illustrated the normal structure of epithelialmucosa, epithelial crypts and diffused lymphoid tissue without any inflammatory alterations (Figure 6) compared with other treated groups that were applied with different doses of PPE. The sections in Figure 7 and 8 of the caecal mucosa were showed enhancement of epithelial mucosa, epithelial crypts.

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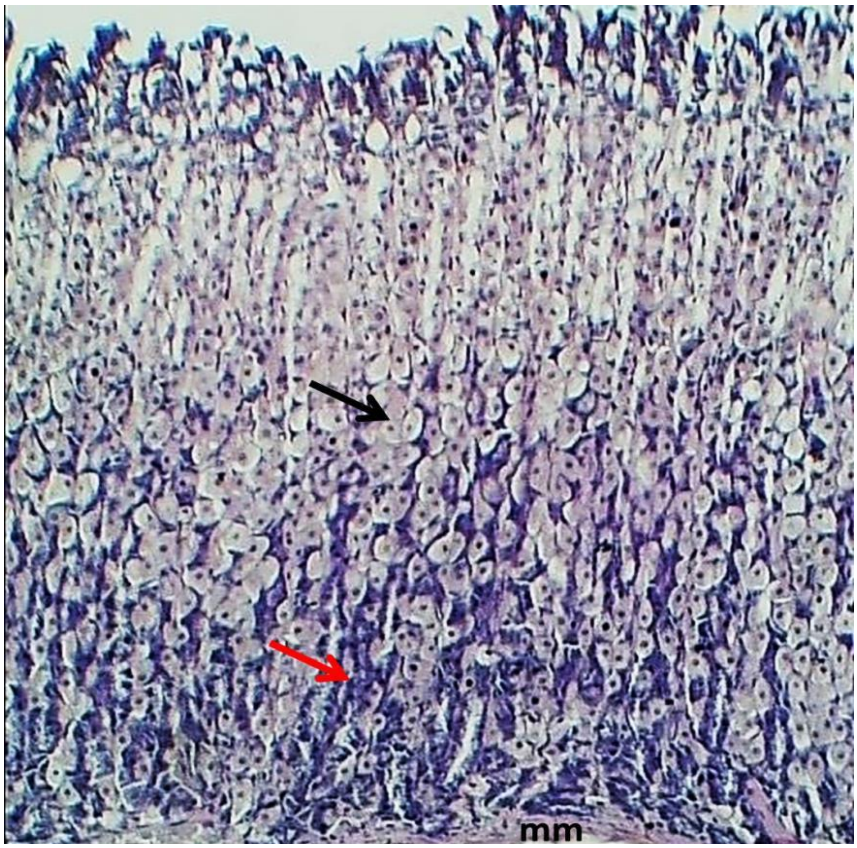

Figure 3: The normal stomach shows tissue and structure in control, parietal cells (Black arrow), chief cells (Red arrow), and muscular mucosa (mm); H&E stain.100x

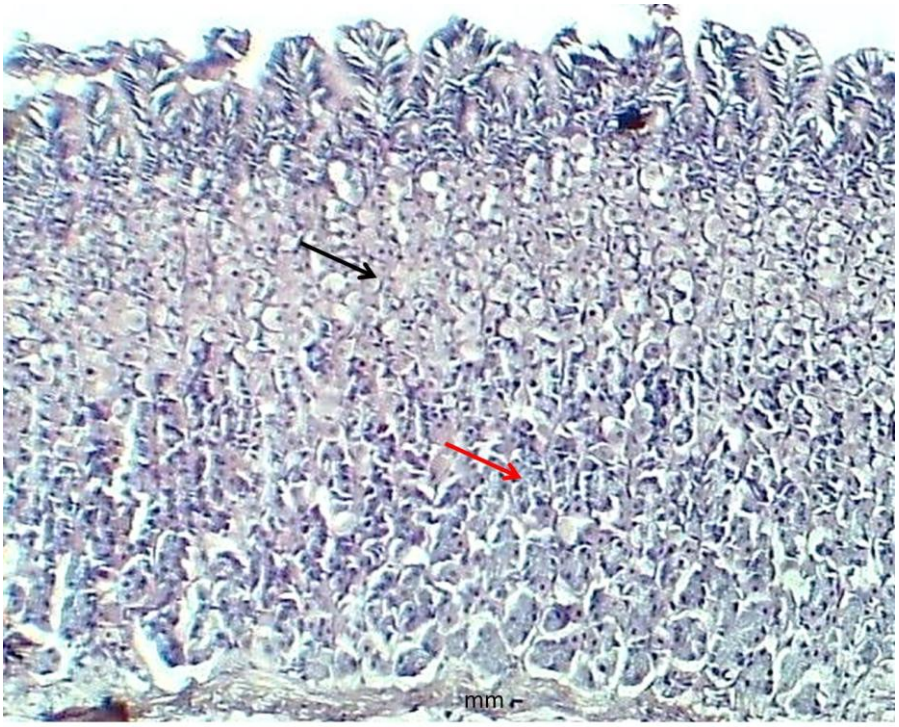

Figure 4: The section of stomach tissue in treated T1 shows parietal cells (Black arrow), chief cells (Red arrow), and muscular mucosa (mm); H&E stain.100x

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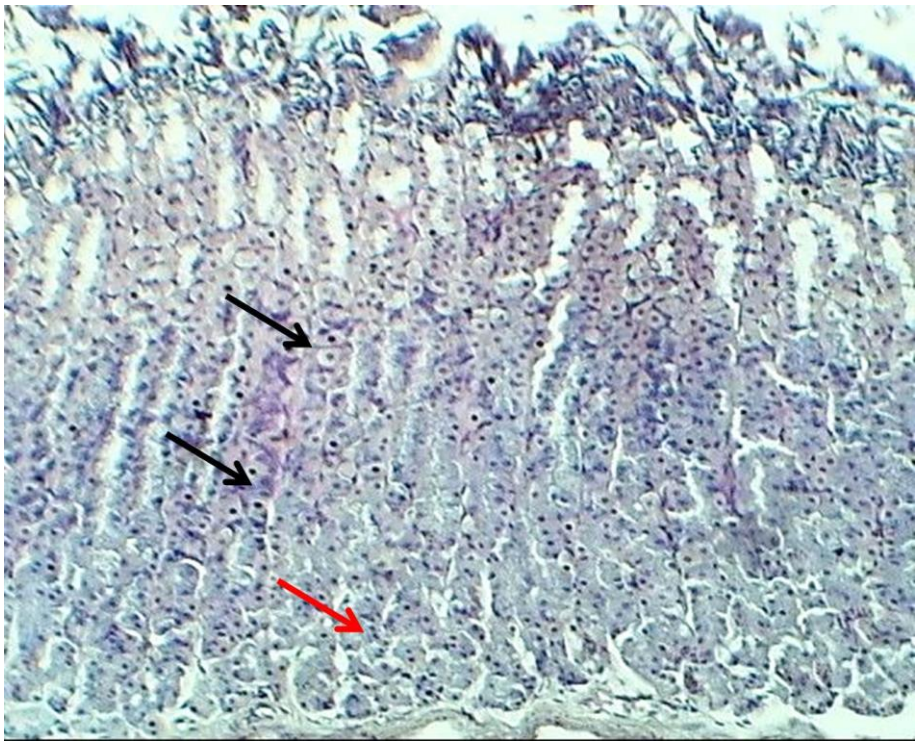

Figure 5: Magnified section of stomach tissue in treated T2 shows parietal cells (Black arrow) chief cells (Red arrows). H&E stain.400x

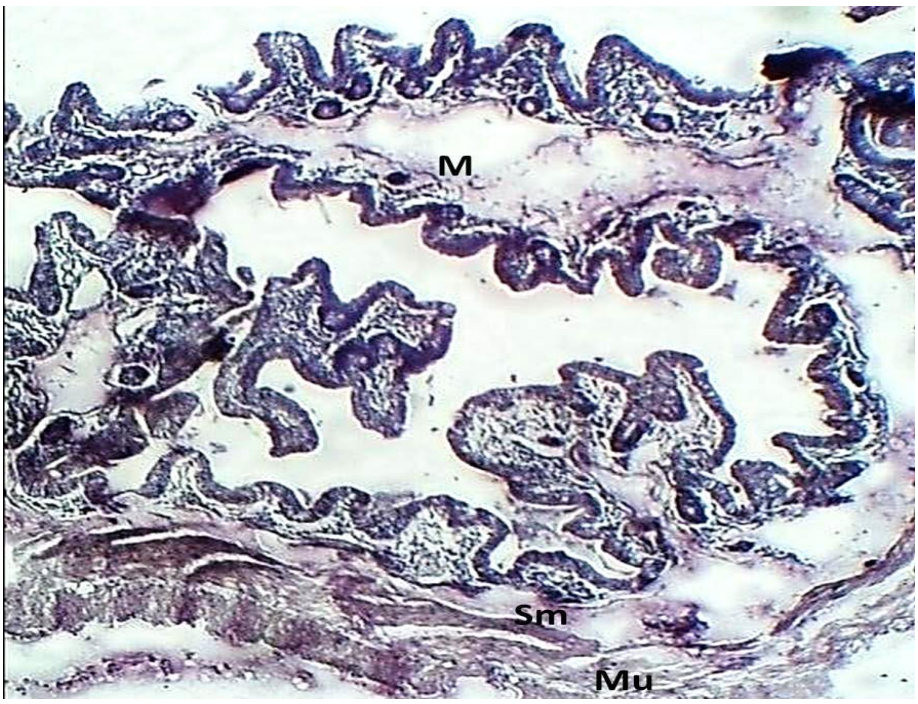

Figure 6: The normal caeca tissue in thecontrol shows the mucosa, sub-mucosa and muscularis; H&E stain.100x

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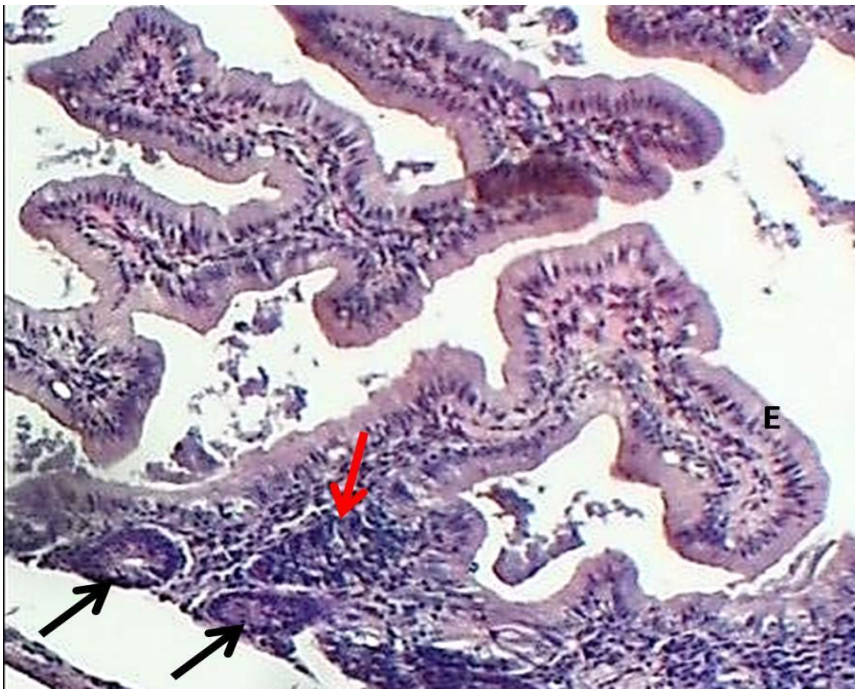

Figure 7: The treated caecal tissue with T1 group shows the normal epithelial mucosa, Lymphatic tissue (Red arrow) and the epithelial crypts (Black arrows); H&E stain.100x

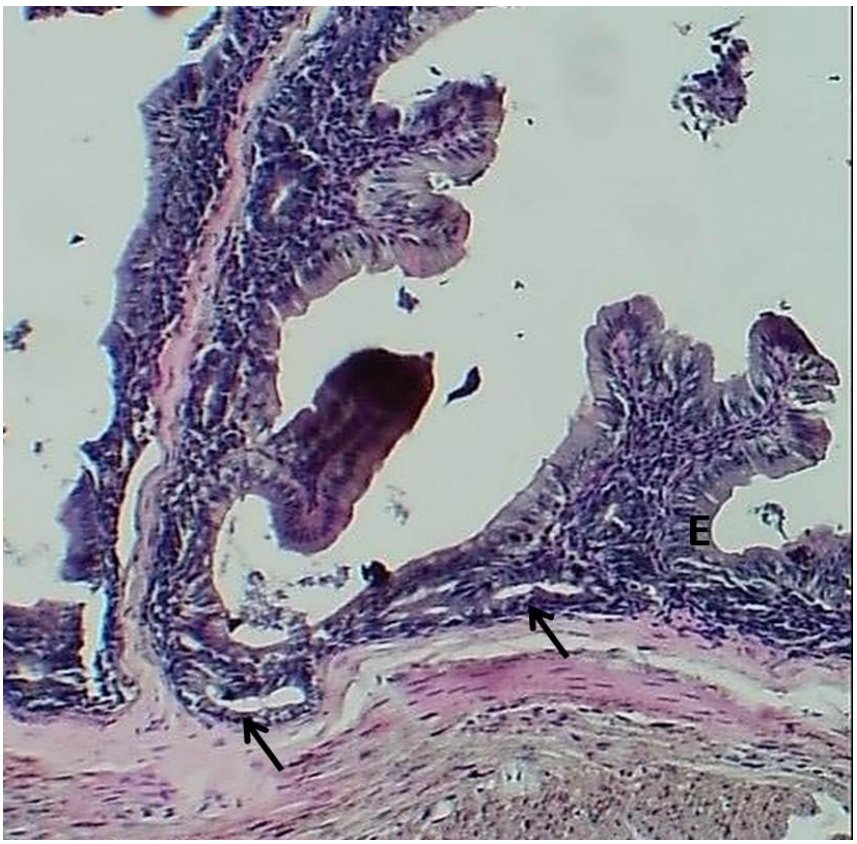

Figure 8: The treated caecal tissue with T2 group shows the normal epithelial mucosa and the epithelial crypts (Arrows); H&E stain.100x

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Discussion

Productive values and performance findings

The findings of the performance traits indicated that admirations of different doses of PPE showed a positive impact on production values and performance of female local rabbits, especially when the low dose was used. The enhancement of the production and performance values, including an increase of body weight and gain,could be referred to bio-active compounds such as polyphenols, including flavonoids, phenolic acids and tannins, which exist in this extract (Ibrahim et al., 2017; Dakheel et al., 2021). These compounds showed their abilities to be anti-inflammatory, antimicrobial, anthelmintic and anticancer effects in different researches (Caietal., 2004; Li et al., 2006; Williams et al., 2014; Dakheel et al., 2020; Al-Bedhawi and Dakheel, 2021). Furthermore, the bio-active components played a critical role to reduce the effects of free radical bounds that could induce stresses, which can cause the cellular damage (Mushtaqet al., 2015).On the other hand, the improvement of FCR and FI of treated groups compared to control as a result of enhancing body weight in general. The positive results on growth performance in treated groups indicated thatPPE enricheswith important nutrients for growth traits (Celia et al., 2016).Similarly, Amri et al., (2017) reported that final body weightand weight gain recorded a significant increase in supplemented pomegranate for rabbits compared to control without supplement. Moreover, Hassan et al. (2020) findings revealed that treated rabbits with pomegranate supplement of improved significantly ($P < 0.05$) the average of body weight, FI and FCR at different diet levels.However, these findings disagreed with results of Amri et al. (2017) who reported that daily administration diets of pomegranate extracts led to decline in the bodyweight of lab animal (rats) by the level of 250 mg extract per kg body weight.

The pH values of the stomach and caecum

This study found that the stomach and caecum content pH were significant differences among treated groups and control, especially T2. The current results agreed with Mady et al. (2016) who recorded decreasing pH values of caecal contents that lead to an improvement in the caecal eco-system through dropping in the number of pathogens, but this study did not measure the stomach pH as our study did it. However, the present study showed no significant comparisons amongthe treatment of T1 and controlon the stomach and caecal pH compared to the rabbits in T2 groups. This finding confirms the pH values of stomach and caecum content had affected by the compositions of the high concentration polyphenols in

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this treatment. On the other hand, low doses of PPE led to no effects on the pH of stomach and caecum content compared to control; this result agreed to Cerisuelo et al. (2014) who used other polyphenols and essential oil compositions at different concentrations on broilers.

Bacterial counting of the caecum

Interestingly, this study revealed that orally administratedPPE reduced significantly ($P<0.05$) the total counting of some harmful bacteria such as*E. coli*and Salmonella spp. These findings agreed with Mancini et al. (2019) who revealed that tannins could play an important role in intestinal mucosa as a protective factor in the presence of digestive problems.Similarly,Fathi et al., (2019) reported that different concentrations of plant extract (0.1% or 0.2% of *Eucalyptus camaldulensis*), which contain high tannins,hada significant reduction on total bacterial counting, including *E. coli* and Salmonella. This result was associated with shortening in caecum length as well.

However, the mechanicalaction of PPEneeds for further research. It is interesting to determine how the PPE shortens caecumlength and reduces pathogenic microbes. Nonetheless, this actioncouldbe donethe antimicrobial activities ofPPEthatimpacted on these pathogens. It could be also concluded that PPE(either 100 mg/ml or 200 mg/ml) improvedtheimmune-system of rabbitsplus enhance the antioxidant stressbyreduction the pathogens in the caecum.

Histological findings

In this study, the control group of the rabbits showed the normal stomach and caecal tissues and structures, histopathologically; no changes were detected in the control. These findings confirmed that these rabbits were in health status during the experimental period. The normal tissues both in the gastric epithelium or chief cells and in muscular mucosa were demonstrated. However, the histological sections of treated groups (T1 and T2) illustrated slightly differences in the epithelial cells of the stomach and more secretory activities in the chief cells; this might be due to the antioxidant effect of PPE(Ibrahim et al., 2017). The current findings were in agreement with the findings of some studies (Negi et al., 2003; Afaq et al., 2005; Zahin et al., 2010) that proved a combination of antioxidant compositions of different plant materials, which showed a significant enhancement over the pomegranate treatment.

Conversely, the presentstudyevaluated epithelial cells of the caecum in these rabbits histopathologically. The findings were slightly different among groups; so the treated T1 and T2 showed improvement in the structures of the caecum, especially in the epithelial mucosa and epithelial crypts.It can be concluded that PPE might enhance the fermentation processes in the caecum, which could lead to being improved the intestinal function. This result agreed with Mancini et al. (2019) and Fathi et al. (2019) who reported that plants contain tannin compositions could improve the intestinal mucosa and protect the digestive system, in general, from the different problems such as nutritional factors e.g. pH.

Conclusions:

The current results indicated that administratedorally of PPE at 100 mg/ml led to be significantly efficient than 200 mg/ml on the female rabbits regarding the improvement of the body weight and gain, FCR values and feed intake at a low dose; however, increasing these doses had no significant difference on the trials of growth performance. Moreover, the low dose of PPE showed an interesting effect on the pH values of the stomach and caecum. Conversely, this study interestingly reported that orally administrated PPE decreased significantly ($P<0.05$) the total counting of two harmful bacteria including *E. coli* and Salmonella spp.Therefore, PPE with 100 mg/ml may provide a promising novel of enhancement agent to promoting the health and performance of the female rabbit.

In general, this study did not determine evidence on the microbial counts of the caecal and faecal content, whereas it impaired nutrient digestibility. Thus, more researches need to be done in this field.

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Conflict of Interest

The authors have no conflict of interest to declare.

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