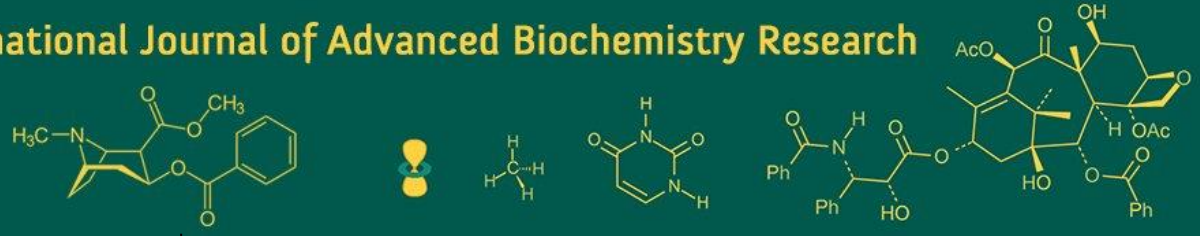
International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(8): 348-352
www.biochemjournal.com
Received: 26-05-2024
Accepted: 29-06-2024

Mohanad Fadhl Hussain Al Musodi
Department of Animal Production. Agriculture College, Karbala University, Karbala, Iraq

Corresponding Author:
Mohanad Fadhl Hussain Al.Musodi
Department of Animal Production. Agriculture College, Karbala University, Karbala, Iraq

The biological role of pomegranate seed by-product on performance and some physiological properties in Iraqi Awassi lambs

Mohanad Fadhl Hussain Al Musodi

DOI: https://doi.org/10.33545/26174693.2024.v8.i8e.1753

Abstract
Using of 15 local Awassi lambs, 3 to 4 months old, to study the effect of adding pomegranate juice waste to the concentrated diet. The lambs were divided into three groups equally at random. The first group was considered a control group and was fed a concentrated diet with an amount of 3% of body weight in addition to green fodder, and the second group (2% PSbP). They were fed the same diet as before, with the addition of 2% of pomegranate seed by product (PSbP) with concentration diet, while 5% of pomegranate seed by product (5% PSbP) was added to the concentration diet of the third group, and the following criteria were studied (final body weight, lamb growth curve, economic feasibility of fattening, troponin concentration, and liver function), and the results showed that there was a significant positive effect of the additives on body weight, the growth curve, the economic feasibility of fattening and cardiac troponin I (cTnI) Concentration in the 2^{ed} and 3rd groups compared to the control group, while there was no significant effect of the treatment on liver function.

Keywords: Biological role, pomegranate seed by-product, physiological properties, Iraqi Awassi lambs

Introduction
In order to maximise feed nutrient absorption and boost animal productivity, new materials have been discovered as a result of th**e** expansion of animal feed production and the usage of a variety of feed materials (Jadoh, 2020) [1], As long as the nutritious content of the alternative feed—such as fruit by-products and certain yeast—is understood, it may be profitable to substitute locally produced feed for imported feed in order to reduce the costs associated with feeding animals (Alhomidy *et al*., 2011) [2]. Feed additives play a critical role in maintaining animal health and optimising overall nutrition while also ensuring a specific level of product quality. To find out how beneficial they are for enhancing nutritional status, metabolic balance, and production efficiency in animals, many organizations are researching some vitamins, vitamin-like materials, essential fatty acids, nutraceuticals and probiotics (Ameen and Rabea, 2006) [3]. Fruit waste and farm debris are increasingly being used as supplements to ruminant feed because they contain nutritive compounds that are important for biochemical processes and physiological changes in the bodies of animals, and because secondary metabolism products found in plant waste products can protect livestock from certain agents that cause degenerative diseases. (Achilonu *et al*., 2018) [6].
The pomegranate (*Punica granatum* L.), which has gained popularity recently for its nutritional and medicinal qualities as well as its appealing appearance, is a fruit by-products utilised in animal feeding, Because of its therapeutic properties, it is also utilised in pharmaceutical and cosmetic applications. (Bellini *et al*., 2010) [4].
In traditional medicine, pomegranate seeds, leaves, roots, and rind are used to treat a wide range of conditions, such as healing of wound, cancer risk reduction, antioxidants, anti-inflammatory agent, anti-arthritics, and anti-proliferative factor. The significant pharmacological activity of pomegranates is especially noteworthy in terms of potential therapeutic benefits. Among the many types of phytochemicals include alkaloids, lavonoids, saponin, CHO, steroids, triterpenoids, carotenoids, amino acids, tannins, coumarins and phenolics. (Anjali, *et al*., 2020) [5], Pretreatment with pomegranate extract demonstrated significant cardio protection against daunorubicin-induced cardiovascular damage; it decreased cardiac injury by significantly reducing blood IL17, LDH, MDA, and troponin I

(cTnI) levels (Al-Kuraishy and Al-Gareeb, 2016) [7]. Pomegranate seed pomace have a variety of contents, such as bioactive, preservative, oxidative, and various fatty acids, which aid in the digestion and absorption of essential substances engaged in or contributing to the process of growth and building (Jadoh, 2020) [1]. When fed pomegranate peel powder-containing meals, Awassi lambs' rate of consumption and final weight rose by 1.5 & 3%, respectively (Saeed *et al*., 2017) [8], The weight, amount of feed consumed, final weight, and blood albumin content of fat-tail lambs increased when pomegranate byproducts were added to their ration by 5 and 10% (Kazemi and Valizadeh, 2021) [9]. Barki lambs fed on powdered dried pomegranate byproducts experienced increases in body weight, at the rate of digestibility of the feed components, the level of total blood protein and albumin, and the level of cholesterol in the blood at a rate of 10, 20, and 30 grammes (El-Elamie, 2021). Therefore, The aim of this study is to study the effect of pomegranate juice by products (seeds, bark, and peel) to the feed of male Awassi lambs on final weight, growth, troponin I, the economic feasibility of the lambs feed and liver enzymes.

Materials and Methods

This experiment was conducted to detection the effects of adding pomegranate waste to the diet of Iraqi male Awassi lambs, and it continued from March 16 to June 17, 2022. 15 Awassi lambs, 3–4 months old, were selected and randomly divided into equal groups. The first was fed a concentrated diet at 3% of their body weight, with additional green fodder and free grazing. This group was considered the control group, while the 2nd group was fed the same previous diet with addition of pomegranate seed by- product (PSbP) at 2% of the weight of the concentrated feed, and the 3rd group was fed the previous diet of the control group with addition of pomegranate seed by -product (PSbP) at 5% of the weight of the concentrated feed**.** The following criteria were measured: final body weight, growth curve, economic feasibility of fattening lambs, liver enzymes, and cardiac troponin I.

Table 1: The chemical composition of pomegranate seed by product (PSbP)

Sequence	Chemical substance	Percentage
1	Fiber	21.192
2	oil	6.560
3	Carbohydrate	51.381
4	moisture	7.718
5	Ash	3.305
6	protein	9.844

*Chemical analysis of the samples was conducted in the central laboratory at the College of Agriculture, University of Baghdad.

The final body weight of the lambs was measured at the end of the experiment period on the last day of the three months, while the weights of the lambs were measured continuously once every month for the purpose of calculating the growth curve of the lambs, and the economic feasibility of fattening among the groups was measured at the end of the experiment period according to

Economic feasibility = Net revenue/Total cost (El-Elaime, 2021) [10]

Blood samples: From the start of the project to its conclusion, plus zero time collection, blood samples were drawn every month. Using disposable, syringes, blood samples were obtained from The neck from the jugular vein following site sterilization. The samples were placed in sterile 10-milliliter tubes and after 15 minutes of centrifuging at 3000 rpm to separate the serum, Alanine Aminotransferase and was estimated.

Determination of liver (function) enzymes U/L activity.
The method of Reitman and Frankel (1957) [11] was used to determine the colorimetric AST and ALT activities for liver (function) enzymes (ALT and AST) and cardiac troponin cTnI.

Determination of cardiac troponin I
A sheep Troponin I (TN-I) Elisa kit used from China (SunLong Biotech Co., LTD), quantify cardiac protein troponin I (cTnI) according to the manufacturer's instructions (Basbugan *et al*., 2010) [12].

Statistical Analytical
SAS (Statistical Analysis System - version 9.1) was used to do the statistical analysis of the data. ANOVA in one manner and two ways (SAS, 2010) [13]. To evaluate significant differences between means, the Least Significant Differences (LSD) used when (p<0.05).

Results and discussion

Final body weight of lambs Kg
Table 2 notes a significant increase (p<0.05) in the final weight of lambs in the two treatment groups compared with control group p<0.05

Table 2: Effects of treatment on final body weight of lambs.Kg.

Groups/Period	G_1 (control)	G_2 2% PSbp	G_3 5%PSbp	LSD
Initial weight	18.50±0.48a	17.95±0.63a	18.64±0.31a	2.04
Final weight	29.48±0.65b	33.11±0.76a	32.838±1.12a	

Lambs growth curve
It is noted from Figure 1 that the growth of the first and second treatment groups was significantly higher (p<0.05) compared to the control group.

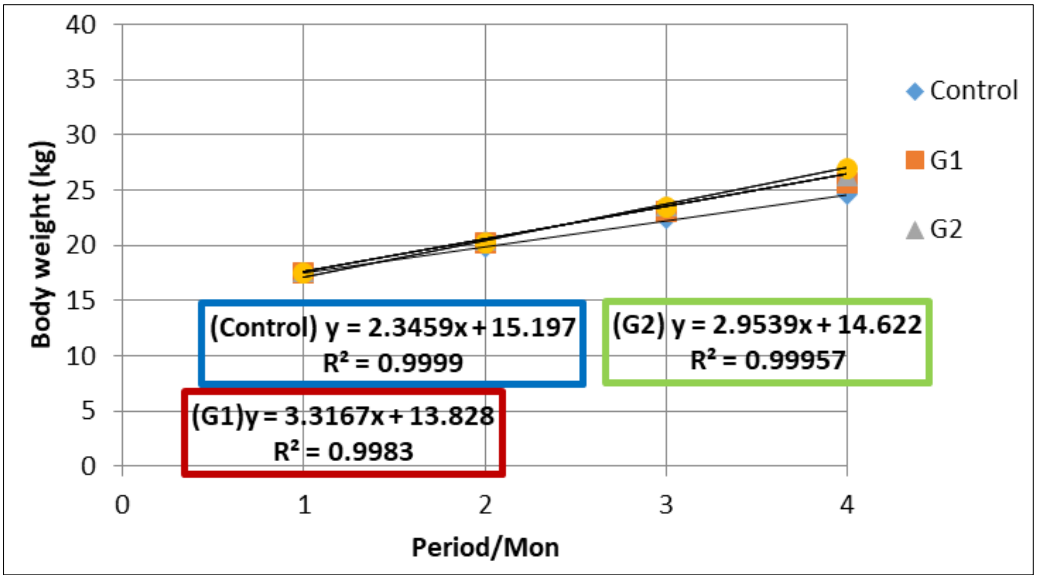


Fig 1: The growth curve of lambs.

X= fatting period
Y= Weight prediction equation
R= Expectancy coefficient

The chemical composition of the remnants of the pomegranate sticks varies in its content of fiber and other foodstuffs, as indicated by (Kotsampasi *et al*., 2021) [23] and this difference can be attributed to the use and manufacture of pomegranate as well as the method of extracting pomegranate juice (Taher-Maddah *et al*., 2012) [24], in current study. The Pomegranate seed by product was contain a good amounts of nutrients as in (table 1) for the composition of pomegranate by product.

The significant superiority in the final weight and the growth curve of the lambs, Table2 and Figure 1, It could be the result of effect of (PSbP) according to its chemical composition(table 1), which contains many important substances for growth and weight gain, such as CHO, protein, and fats (El-hamamsy,S.and El-khamissi H, 2020) [14] Moreover, vitamins and minerals such as riboflavin, thymine, and ascorbic acid. Tocopherol and vitamin E (Rowayshed *et al*., 2013) [15]. Also, pomegranate seed by product contains significant amounts of tannins (Sadq *et al*., 2016) [16]. Tannins are effective compounds that protect food protein from decomposition in the rumen under the influence of micro organisms. Thus, the food protein reaches the true stomach and small intestine to be digested by enzymes. There is the effect of digestive enzymes and amino acids are absorbed with high efficiency, lead to increasing in the growth and weights of the animal (Saeed *et al*., 2017) [17]. Moreover, reasons may be due to the effect of pomegranate sedd by product in adjusting the nitrogen balance in the rumen in terms of reducing the loss of non-protein nitrogenous substances in the formular of ammonia (Hussein., 2012) [18] or by improving environment conditions of rumen., which leads to an increase in the formation of microbial protein by the natural flora, is considered an good source of proteins in ruminants. it is made in the rumen and digested in the stomach and small intestine, this increases the growth and weight of the lambs (Abarghuei *et al*., 2013) [19] in addition, because of the increase in blood proteins (Ali and Al-Okaily, 2016) [20] and thus increases the growth rate and weight of the lambs. These results are consistent. With both (Jadoh, 2020) [1] (Jandel, 2021) [21] and (El-Elaime, 2021) [10]

Economic feasibility/ratio

Lambs of the first and second treatment groups is significantly superior (p<0.05) with control group.

Table 3: The effect of treatment on the Economic feasibility/ratio

Animal groups	Economic feasibility/ratio
Control	0.18±0.11c
2nd group (2% PSbp)	0.26±0.05ab
3rd group 5% PSbp	0.28±0.015a
LSD`	0.03

The reason for the superiority of the lambs of the two treatment group the expense of the control group in the ratio of economic feasibility table 3 could be attributed to the superior weight of the lambs, as in Table 2, which leads to an increase in the amount of meat produced from the lambs due to their increased weight and an increase in the size of the skeletal muscles due to the influence of pomegranate sedd by product (PSbP) by 2 and 5% with concentrate diet. Which lead to increase the growth and weight of lambs (Jadoh, 2020 and Moradi *et al*., 2020) [1]. and ultimately improve the resulting economic income,and these results correspond to (Sultana *et al*., 2010) [22]

Troponin/ng/ml

It is noted from Table 4 that there was an insignificant decrease (p<0.05) in the concentration of cardiac troponin in the 2ed and 3rd treatment groups compared with control group.

Table 4: Effect of treatment on Troponin I, ng/ml in lambs (M±SE).

Months/ Animals/group	Zero time	1st Mon.	2nd Mon.	3rd Mon.
G1(control)	0.13±0.028a	0.13±0.05a	0.13±0.09a	0.16±0.11a
G2(2%pSb)	0.12±0.11a	0.11±0.013a	0.09±0.014a	0.11±0.018b
G3 (5%PSb)	0.11±0.14a	0.13±0.018a	0.09±0.019a	0.10±0.017b
LSD	0.05			

The significant decline (p<0.05) and within normal limits level of troponin in the treatment groups compared to the control group may be due to the effect of the feed treatment with pomegranate juice residues, which contain several

important substances such as tannins that act as antioxidants and keep heart cells from breaking down, thereby reducing the level of troponin (Ismail *et al*. 2012) [25], as they have a significant role in protecting and treating cardiac cell wounds, thus decreasing the concentration of cTn I (Stoner and Gupta 2001) [26], These results correspond to by (Taskin and Deger (2021) [27]. The reason for the low cTn I may be due to the abundant content of vitamin(C and E) (Rowayshed *et al*., 2013) [15], Which act as antioxidants that protect heart cells from free radicals and break down, which leads to a decrease in the level of cTn I in the blood (Khan *et al*., 2020). The results of our study agree with Aloutaibi *et al*., (2017) [28]

5. ALT enzyme

There was insignificant difference between the experimental groups in the concentration of the ALT enzyme, despite the numerical decrease in the second and third groups, but the difference did not reach significance

Table 5: Effect of dietary pomegranate seed by product on ALT enzyme UI/L in lambs, (M±SE).

Months/Group	Zero Time	1st Mon.	2nd Mon.	3rd Mon.
G1 (control)	11.6±4.28	11.8±3.05	11.9±3.1909	11.8±4.11
G2 (2% pSbp)	10.8±3.11	10.9±4.12	10.2±5. 14	10.0±3. 28
G3 (5% PSbp)	11.3±2.14	11.5±3. 18	10.8±4. 29	10.1±3.37
LSD	5.13			

6-AST enzyme UI/L.

There was insignificant difference between the experimental groups in the concentration of the AST enzyme, despite the numerical decrease in the second and third groups, but the difference did not reach significance

Table 6: Effect of treatment on AST enzyme UI/L in lambs (M±SE).

Months/Group	zero time	1st Mon.	2nd Mon.	3rd Mon.
G1(control)	60.6±4. 08	61.8±5.35	60.9±7.19	61.3±5.71
G2(2%pSbp)	60.8±7.10	59.8±6.02	58.2±5. 44	58.30±7. 08
G3 (5%PSbp)	61.3±6.03	61.5±7. 18	57.8±6. 09	57.81±5.67
LSD	4.05			

Many studies have indicated the effects of pomegranates and their products on reducing the harmful effects on liver cells and protecting them from being destroyed by free radicals. Pomegranates and their products contain highly effective antioxidant compounds found in the seeds, peels, bark, and juice of pomegranate and one of the most important substances is that they contain multiple phenols (Agha *et al*., 2013 and Seeram *et al*., 2005) [29, 30]. These compounds work to protect cells from the effects of free radicals (Salim *et al*., 2014) [31], and therefore the decrease in liver enzymes can be attributed mathematically, as the decrease did not reach the level of significance and is within the normal limits of their levels. This is consistent with the (Obeidat, 2023) [32], who indicated that there is no significant difference in the level of liver enzymes in Awassi lambs after adding 10% pomegranate juice manufacturing waste to the diet.

References

1. Jadoh SK. Role of dietary ground *Punica granatum* seeds with/without Gastrocell probiotic in some productive and reproductive aspects of local male rabbits. MSc thesis, College of Veterinary Medicine, Baghdad University; c2020.
2. NAlhomidy S, Basmaeil S, Al-Owaimer AN, El-Waziry AM, Koohmaraie M. Effect of feeding different amounts of discarded dates on growth and efficiency of digestion in sheep. Australian Journal of Basic and Applied Sciences. 2011;5(3):636-640.
3. Amen AF, Rabea A. Rabbit rearing under many conditions in modern methods. Department of Agriculture Extension, Morocco. An update on the use and investigation of probiotics in health and disease. Gut. 2006;62:787-796.
4. Bellini E, Giordan E, Malfa LA. Minor fruit tree species in Italy: traditional and innovative fruit culture. Persimmon and *Punica granatum* as study cases. Italus Hortus. 2010;17:75-90.
5. Anjali A, Dixit K, Sahoo J. Pharmacognostical and pharmacological activity of *Punica granatum* (pomegranate). International Journal of Research in Pharmaceutical Sciences. 2020;11(3):4695-704.
6. Achilonu M, Shale K, Arthur G, Naidoo K, Michael M. Phytochemical benefits of agroresidues as alternative nutritive dietary resources for pig and poultry farming. Journal of Chemistry. 2018;6:1-15.
7. Al-Kuraishy HM, Al-Gareeb AI. Potential effects of *Punica granatum* on lipid peroxidation and pro-inflammatory changes in daunorubicin-induced cardiotoxicity in rats. International Journal of Preventive Medicine. 2016;7(1):85-89.
8. Saeed A, Abdulridha F, Hussien Z, Mohsen M, Fathel M. Effect of addition of different levels of *Punica granatum* peel powder to concentrate diet on productive performance of Awassi lambs. Al-Qadisiyah Journal of Veterinary Medicine Sciences. 2017;16(1):27-28.
9. Kazemi M, Valizadeh R. The effect of dietary supplementation of ensiled *Punica granatum* by-products on growth performance, nutrient digestibility, haematology parameters, and meat characteristics of fat-tail lambs. Italian Journal of Animal Science. 2021;20(1):1532-1543.
10. El-Elaime R. Impact of using different levels of *Punica granatum* by-product as a feed additive on performance of growing Barki lambs. Egyptian Journal of Nutrition and Feeds. 2021;24(1):25-34.
11. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Analytical Journal of Clinical Pathology. 1957;28:56-60.
12. Basbugan Y, Agaoglu Z, Yuksek N. An investigation on serum troponin concentration in healthy ruminants. Kafkas University Veterinary Faculty Journal. 2010;16(4):641-645.
13. Statistical Analysis System (SAS). User guide for personal computers. Cam, NC: SAS Institute Inc.; 2010.

14. El-Hamamsy S, El-Khamissi H. Phytochemicals, antioxidant activity, and identification of phenolic compounds by HPLC of *Punica granatum*. Journal of Agricultural Chemistry and Biotechnology. 2020;11(4):79-84.
15. Rowayshed G, Salama A, Abul-Fadi A, Akila-Hamza S, Mohamed E. Nutritional and chemical evaluation for *Punica granatum* fruit peel and seeds powders by-products. Middle East Journal of Applied Sciences. 2013;3(4):169-179.
16. Sadq S, Ramzi D, Hamasalim H, Ahmed K. Growth performance and digestibility in Karadi lambs receiving different levels of *Punica granatum* peels. Journal of Animal Sciences. 2016;6(1):16-23.
17. Saeed A, Abdulridha E, Hussien Z, Mohsen M, Fathel M. Effect of addition of different levels of *Punica granatum* peel powder to concentrate diet on productive performance of Awassi lambs. Al-Qadisiyah Journal of Veterinary Medicine Sciences. 2017;16(3):27-28.
18. Hussein SAM. The effect of using different ratios of *Punica granatum* peels with two ratios of concentrate on digestion coefficients and performance of Awassi lambs. MSc thesis, Tikrit University; c2012.
19. Abarghuei MJ, Rouzbehan Y, Salem M, Zamiri MJ. Nutrient digestion, ruminal fermentation, and performance of dairy cows fed *Punica granatum* peel extract. Livestock Science. 2013;157:452-461.
20. Ali EH, Al-Okaily BM. The protective role of *Punica granatum* seed oil (Pometone) on serum protein in sodium fluoride-treated female rats. Iraqi Journal of Veterinary Medicine. 2016;40(1):61-68.
21. Jandel MM. The nutritional importance and biological efficiency of *Punica granatum* peels and its effect on some biochemical blood features of experimental diabetic rats. PhD thesis, College of Agriculture, Tikrit University; c2021.
22. Sultana N, Hossain S, Chowdhury S, Hassan M, Ershaduzzaman M. Effects of age on intake, growth, nutrient utilization, and carcass characteristics of castrated native sheep. Bangladesh Veterinarian. 2010;27(2):62-73.
23. Kotsampasi B, Christodoulou C, Mavrommatis A, Mitsiopoulou C, Bampidis V, Christodoulou V, *et al*. Effects of dietary *Punica granatum* seed cake supplementation on performance, carcass characteristics, and meat quality of growing lambs. Animal Feed Science and Technology. 2021;273:114815.
24. Taher-Maddah M, Maheri-Sis N, Salamatdoustnobar R, Ahmadzadeh A. Estimating fermentation characteristics and nutritive value of ensiled and dried *Punica granatum* seeds for ruminants using *in vitro* gas production technique. Open Veterinary Journal. 2012;2(1):40-45.
25. Ismail T, Sestili P, Akhtar S. *Punica granatum* peel and fruit extracts: a review of potential anti-inflammatory and anti-infective effects. Journal of Ethnopharmacology. 2012;143:397-405.
26. Stoner GD, Gupta A. Ellagic acid. Carcinogenesis. 2001;22:1737-1746.
27. Taskin E, Deger Y. Effects of ellagic acid on levels of sialic acid and cardiac markers in rats with myocardial infarction. Fresenius Environmental Bulletin. 2021;30(7):9011-9017.
28. Aloutaibi G, Gashlan H, Moselhy S, Al-Malki A, Khan J. Possible cardioprotective action of *Punica granatum* juice and propolis against myocardial infarction induced in rats. African Journal of Traditional, Complementary, and Alternative Medicines. 2017;14(5):138-146.
29. Agha F, Hassannane M, Omara E, Hasan A, El-Toumy S. Protective effect of *Punica granatum* peel extract against pentachlorophenol-induced oxidative stress, cytogenetic toxicity, and hepatic damage in rats. Australian Journal of Basic and Applied Sciences. 2013;7(2):853-864.
30. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D. *In vitro* antiproliferative, apoptotic, and antioxidant activities of punicalagin, ellagic acid, and a total *Punica granatum* tannin extract are enhanced in combination with other polyphenols as found in *Punica granatum* juice. Journal of Nutritional Biochemistry. 2005;16(1):1-23.
31. Salim A, Abou-Arab A, Mohamed S, Eldesouky T. Influence of *Punica granatum* on dimethoate-induced hepatotoxicity in rats. International Journal of Veterinary and Agricultural Food Sciences. 2014;8(8):871-876.
32. Obeidat BS. Effect of feeding *Punica granatum* seed pulp on Awassi lambs' nutrient digestibility, growth performance, and carcass quality. Veterinary World; c2023. p. 588-594.