

ROLE OF DIETARY GROUND POMEGRANATE SEED POMACE AND / OR GASTRO CELL PROBIOTIC IN SOME HEALTH ASPECTS OF LOCAL MALE RABBITS

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Abstract

His study aimed to find out the role of dietary ground pomegranate seeds pomace and/or Gasto Cell probiotic in some health aspect of local male rabbits, this study was conducted in the animal house/ Vet. Med. Collage-Baghdad University.

Twenty-eight local male rabbits aged (2.5-3) months were used and divided randomly into four groups (7 each) as follows:

The first group (control), second(Probiotic CFU10/rabbit), third(ground pomegranate seeds pomace 5%) and fourth (Probiotic+ground pomegranate seeds pomace 5%), all groups were daily fed on concentrate diet (pellet) (75gm/head), alfalfa offered with water freely. Blood samples were taken from the heart directly to find out the hemoglobin concentration(Hb), packed cell volume (PCV%) hematocrit, Red, and White blood cells count. The results of this study revealed significant differences in some health traits among groups of different periods. In conclusion, this study documented the beneficial effect and concluded that the use of ground pomegranate seed pomace 5% and/or GastoCell Probiotic had improved some health characteristics of local male rabbits.

Key words: Pomegranate Seed Pomace, Rabbits, Health, Probiotic, GastoCell.

Introduction

The feeding costs have long been known as the largest expense and the greatest cash investment in animal producing, this has a significant effect on the animals' growth rate and health status. (*Berg et al.*, 2002), feed additives are extremely important, generally contribute to nutrition optimization of the animals and provide by this a certain standard of products quality. Vitamins, vitamin-like compounds, essential fatty acids, as well as probiotics, nutraceuticals, and food by-products are under investigation by different groups, in order to evaluate their effectiveness in the animal in improving nutritional status, metabolic balance, and production efficiency (Amen and Rabea, 2006; Gropp, 2010).

Pomegranate is one of the most ancient cultivated trees traditionally used in human nutrition and folkloric medicine for the treatment of different diseases also has

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been used as a symbol of life, fertility, immortality, and human-related activities, also has idiosyncratic features and has gained attention in complementary and alternative medicine (Hussen and Arrack, 2014; Karimi *et al.*, 2017; Al-Okailyand Ali, 2019). This fruit is either consumed fresh or used in the juice industry, also Increasing agroindustrial units for producing pomegranate juice led to increased processing of by-products including peels and seeds (Shabtay *et al.*, 2008), that have a wide range of phytochemicals like flavonoids, polyunsaturated fatty acid, alkaloids, vitamins and sterols (Viuda-Martos *et al.*, 2010; Jing *et al.*, 2012).

Probiotics are live micro-organisms or cultures of bacteria that have been used as growth promoters to replace the widely used antibiotic and synthetic chemical feed supplements (Salvatore and Vandenplas, 2010; Avadhani and Miley, 2011).

Accordingly, this study was aimed to investigate the

role of dietary ground pomegranate seeds pomace in diet and/or GastoCell probiotics in some health aspects of local male rabbits.

Materials and Methods

Animals and Experimental Design

This experiment was accomplished in the animal house of Vet. Med. Collage, Baghdad University from 22 October-2019 to 5 January-2020. Twenty-eight local male rabbits at the age of (2.5-3) months. Experimental groups were checked to ensure their health and preventive treated against the internal and external parasites. Animals left two weeks for acclimation. The animals fed on diet pellet (75gm/head), also alfalfa and water offered freely, animals organized as four groups (7each) as following:

The first group (control) (G_1) feed concentrate diet of pellet and clear water, second (G_2) feed concentrate diet of pellet and supplied water with Probiotic as a 106 CFU/Rabbit daily, third (G_3) feed with concentrate diet of pellet with ground pomegranate seed pomace 5% and clear water, fourth (G_4) feed with concentrate diet of pellet with ground pomegranate seed pomace 5% and supplied Probiotic as a 10^6 CFU/Rabbit with water. Blood samples were taken biweekly from heart directly for analysis blood characters (RBC and WBC count, Hemoglobin (Hb), PCV%).

Biochemical assay of blood

Red blood cells counts (RBC) (Broukus, and Andreasen, 2003). Total white blood cells counts (WBC) (John and Lewis, 1984). Hemoglobin (Hb) concentration and packed cell volume (PCV%) (Close, 1986; Dacie and Lewis 1991).

Statistical analysis

Collected hematological data were analyzed as a complete Randomized Design (CRD) by using SA Sprogram (ANOVA) Least significant differences (LSD) was applied to detect the significant differences among different groups means at (p>0.05) level (SAS, 2010).

Results and Discussion

RBC count (106/ml)

The results of table 1 and Fig. 1 that related with means of red blood cells count of treated groups in experiment, were observed all groups participated among them in the significant (p<0.05) superiority of red blood cell count and it turned out at baseline and $2^{\rm nd}$ week of experiment, while the G_3 continued in significant (p<0.05) superiority more than groups during $4^{\rm th}$, $6^{\rm th}$ and $8^{\rm th}$ week of experiment in values (7.13±0.19), (7.46±0.15), (7.87±0.13) respectively. At the same time the G_4 participate the G_3 especially at $6^{\rm th}$ week of experiment, also all treated groups continued the superiority than their means compare with baseline and it was clear in $8^{\rm th}$ week

Table 1: Effect of dietary ground pomegranate seed Pomace and /or Probiotic on RBC count (10⁶/ml) of local male rabbits M±SE.

Period	Baseline	W2	W4	W6	W8
Groups					
G_{l}	A a 6.23±0.23	A a 6.27±0.22	Ab 6.31±0.18	Ab 6.33±0.21	Ac 6.57±0.19
G ₂	Ba 6.20±0.30	AB a 6.37±0.30	AB ab 6.56±0.31	AB b 6.76±0.30	A bc 6.90±0.28
G ₃	Ca 6.36±0.25	BCa6.71±0.22	AB a 7.13±0.19	A a 7.46±0.15	Aa 7.87±0.13
G_{4}	Ca 6.24±0.29	BCa6.61±0.27	ABC ab 6.84±0.32	AB a 7.16±0.25	A ab 7.51±0.24
LSD	0.6973				

Means with a different small letter in the same column are significantly different ($P \le 0.05$). Means with a different capital letter in the same row are significantly different ($P \le 0.05$).

Table 2: Effect of dietary ground pomegranate seed Pomace and /or Probiotic on hemoglobin level g/dl of local male rabbits Mean±SE.

Period Groups	Baseline	W2	W4	W6	W8
G_{1}	Ab 11.47±0.33	Ab 11.70±0.33	A c11.89±0.36	Ac 12.04±0.36	A c 12.20±0.43
G_2	Bb11.56±0.43	ABb12.00±0.43	AB c 12.37±0.41	Ac 12.74±0.41	Ac 13.10±0.41
G ₃	Ca13.29±0.39	BC a 14.27±0.43	Ba 15.17±0.54	Aa 16.64±0.60	A a 17.26±0.45
G_{4}	Cab 12.57±0.37	BCa13.34±0.32	B b 13.91±0.32	AB b 14.44±0.35	Ab 15.12±0.30
LSD	1.1471				

Means with a different small letter in the same column significantly different ($P \le 0.05$). Means with a different capital letter in the same row significantly different ($P \le 0.05$).

percentage (1 e v 70) of focal male rabbits wear = 51.					
Period Groups	Baseline	W2	W4	W6	W8
G_1	Ba37.29±0.64	ABb38.00±0.37	ABb39.00±0.37	ABc39.71±0.47	Ab 41.00±0.30
G_2	Ba39.71±0.68	AB ab 40.43±0.64	AB ab 41.43±0.52	AB bc 42.00±0.69	Ab 43.00±0.61
G_3	Ca40.00±1.82	BCa41.43±1.78	$Ba43.71\pm1.72$	A a 46.29±1.30	$Aa48.00\pm1.02$
G_{4}	Da 38.00±1.09a	CD ab 39.29±1.06	BC ab 41.43±1.32	AB ab 44.29±1.35	A a 46.29±1.28
LSD	2.9938				

Table 3: Effect of dietary ground pomegranate seed Pomace and /or Probiotic on packed cell volume percentage (PCV %) of local male rabbits Mean±SE.

Means with a different small letter in the same column significantly different ($P \le 0.05$).

Means with a different capital letter in the same row significantly different (P < 0.05).

Table 4: Effect of dietary ground pomegranate seed Pomace and /or Probiotic on WBC count (10³/ml) of local male rabbits M±SE.

Period Groups	Baseline	W2	W4	W6	W8
G_{l}	Bc 4.77±0.19	Bc4.94±0.21	AB c 5.20±0.24	A c 5.40±0.33	Ab 5.64±0.33
G_{2}	B bc 4.96±0.21	AB bc 5.21±0.24	AB c5.23±0.19	A c 5.56±0.22	Ab 5.70±0.18
G_3	Ba 5.87±0.23	AB a 6.34±0.16	A a 6.79±0.12	A a 7.26±0.10	A a 7.61±0.10
G_{4}	Bab 5.46±0.14	AB b 5.63±0.14	AB b 5.83±0.11	A b 6.04±0.10	A b 6.07±0.20
LSD	0.5664				

Means with a different small letter in the same column significantly different ($P \le 0.05$). Means with a different capital letter in the same row significantly different ($P \le 0.05$).

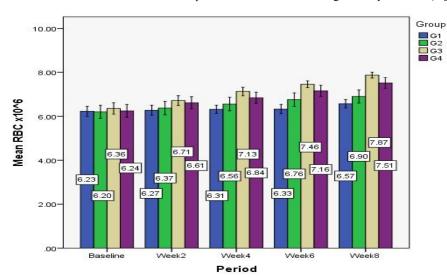


Fig. 1: Effect of dietary ground pomegranate seed Pomace and /or Probiotic on RBC count (106/ml) of local male rabbits.

except the G₁ which lasted for the duration of the experiment.

(control) (G_1) feed concentrate diet of pellet and clear water, second (G_2) feed concentrate diet of pellet and supplied water with Probiotic as a 106 CFU/Rabbit daily, third (G_3) feed with concentrate diet of pellet with ground pomegranate seed pomace 5% and clear water, fourth (G_4) feed with concentrate diet of pellet with ground pomegranate seed pomace 5% and supplied Probiotic as a 10^6 CFU/Rabbit with water

Hemoglobin (Hb) g/dl

The results of table 2 and Fig. 2 that related with hemoglobin values levels showed that the G₃ recorded significantly (p<0.05) higher than all groups during 2nd, 4th, 6th and 8th week of experiment in values (14.27±0.43), $(15.17\pm0.54),$ $(16.64\pm0.60),$ (17.26 ± 0.45) respectively, also the group G, revealed significantly (p<0.05) higher in estimates from baseline until the end of experiment, at the same time all treated groups including the control group G, recorded significantly (p<0.05) higher especially during 6th and 8th week of experiment respectively, that is, over

time there was improvement in the blood properties or characteristics, that is, there is a cumulative effect of treatment over the course of the experiment.

PCV%

We can observed the results of table 3 and Fig. 3 that related with packed cell volume (PCV) percentage of treated groups in experiment, where it is observed that G_3 group recorded significantly (p<0.05) higher compare with all groups. At experiment from beginning

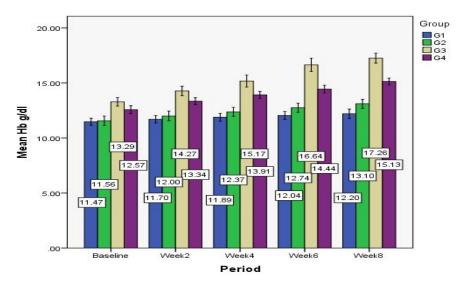


Fig. 2: Effect of dietary ground pomegranate seed Pomace and /or Probiotic on hemoglobin level g/dl of local male rabbits.

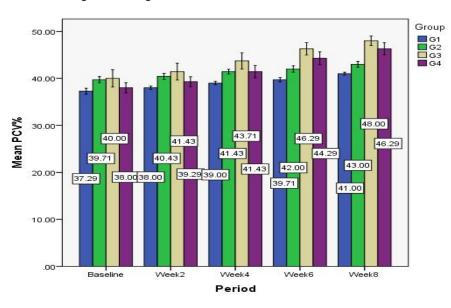


Fig. 3: Effect of dietary ground pomegranate seed Pomace and /or Probiotic on packed cell volume percentage (PCV %) of local male rabbits.

until the end of experiment in values (41.43 \pm 1.78), (43.71 \pm 1.72), (46.29 \pm 1.3), (48.00 \pm 1.02) for 2nd, 4th, 6th and 8th week of experiment respectively, whereas the G₄ participate the G₃ at the end of experiment in 8th week in values (46.29 \pm 1.28), at the same time the G₃ showed significantly (p<0.05) higher than their assessments compare with baseline at 6th and 8th week in values (46.29 \pm 1.30), (48.00 \pm 1.02) respectively, also at the same time all groups revealed significantly (p<0.05) higher than their assessments especially at 8th week of experiment.

Hemoglobin is the main functional unit of red blood cells, and it's impact on any cause is negatively reflected in the function of the cells (RBC), especially that hemoglobin is the only contributor to performance of this

function (oxygen transport), and the hematocrit (PCV) is a measure of the real blood components, which are the relative sizes of white and red blood cells, as well as the change in its value is an induction of the occurrence of a disorder in one or both of them, and the decrease in (PCV) depend mainly on the number and size of red blood cells (RBC), this confirmed by (Melillo, 2007).

The increase in the values of hemoglobin, hematocrit (PCV), and erythrocyte (RBC) count tables 1, 2 and 3 respectively, for the treated groups in the experiment and for the periods from the baseline and 2nd week of experiment, which can be explained briefly that the animals of these groups may have under good management and there for good health and were feeding on good quality diets, where a healthy environment was available in the gut (GIT) especially in intestine, and thus lead to an increase in the absorption of necessary nutrients that stimulated the manufacturing of erythrocyte. Also the role of Probiotic in improving the absorption rate through intestine this confirmed and agreement with several studies (Opera et al., 2012; Ozkan et al., 2012; Kadhim, 2013). At the same time group (G₂) continued to significantly exceed the above criteria until the end of the trial period, which was participate by

group (G_4) with superiority and for some periods, that is over time there was an improvement in the blood picture, this means there is accumulative effect of the type of treatment over the course of experiment and in the same path, and the possible explanation for this may be due to the many effective nutritional components in the pomegranate seed power as well as their synergistic and stimulating effects in action events through its effect in the erythrocyte manufacturing system (erythropoisis) in the blood-forming tissue of the bone marrow and consequently the effect on red blood cell count which is followed by an increase in hematocrit and hemoglobin and this agreement withAheman *et al.*, (2013), or perhaps it may be due to the effect on responsible synthetic

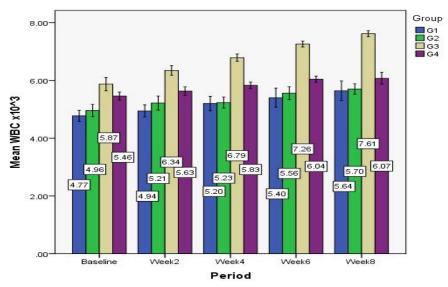


Fig. 4: Effect of dietary ground pomegranate seed Pomace and /or Probiotic on WBC count (10³/ml) of local male rabbits.

enzymes on manufacture of (RBC) especially the hormone (erythropoietin) which is excreted from the kidney and this is confirmed and accordance with Khan et al., (2017). Also the increase can be attributed to the effective and anti-oxidant components that the pomegranate seed powder contains like (Polyphenols, flavonides, vitamins) as a oxidizing agents and their potential synergistic effect and through their effects on some enzymes for (RBC) and responsibility for hemolysis and prevent degradation and their fragility and thus the effect on the lifetime of the erythrocytes like (G₂PD Glucose-6-phosphate dehydrogenase), and this interference with the action of these enzymes by preventing oxidative stress and the large amount of free radicals to which the erythrocytes are more exposed to, and this confirmed and is identical with Phuoc and Jamikorn, (2017).

Total white blood cells count WBC (10³/ml)

The results of table 4 and Fig. 4 that regarded with white blood cells count that showed the same trend of hemoglobin estimates, were it showed the (G_3) recorded significantly (p <0.05) higher than all treated groups in experiment in all periods and in values (6.34 ± 0.16) , (6.79 ± 0.12) , (7.26 ± 0.10) , (7.61 ± 0.10) respectively, whilst all groups revealed significantly (p<0.05) higher in white blood cell count compared with baseline were it was obvious during 6^{th} and 8^{th} week of experiment.

The immune system of the body is the first line of defense to repel any kind of pathogenic and other challenge that is prepared to produce antibodies and thus eliminate any external antigen, and the nutrition materials, their quality and components have an effective role in stimulating the immune system of any living organism (Opera *et al.*, 2012; Phuocand Jamikorn, 2017).

The incidence of significantly increase in means of white blood cells table 4 and Fig. 4, the same tendency or pathway for blood parameters like hemoglobin and red blood cells, especially group (G₃) which continue to excel throughout the experiment period. This shows the cumulative effect of the nutritional materials found in the pomegranate seed powder. A possible explanation for this is that seeds have biological and therapeutic prosperities, as they contain many Polyphenols, minerals, vitamins and phytochemicals with

anti-oxidant effects and inhibiting free radicals, thus treating many diseases as oxidative stress, metabolic and infectious diseases, and these substances are able to stimulate the immune system and thus increase the number of white blood cells (WBC) in all their types, as well as it is not possible to over lock the role of Probiotic as a vital booster in stimulation events of immune system, this confirmed and agreement with Khan *et al.*, (2017).

It could be concluded from this study that the use of ground pomegranate seed pomace 5% and/or Gasto Cell Probiotic had improved some health status parameters of local male rabbits.

References

Berg, J., J.L. Tymoczko and L. Stryer (2002). Biochemistry (5th ed.). San Francisco: W.H. Freeman, p. 603. ISBN 0-7167-4684-0.

Gropp, J.M. (2010). Influence of additives used in animal nutrition on the quality of food. *In: Proceedings of the 2nd COST International Feed for Health Conference*.

Ameen, A.F. and A. Rabea (2006). Rabbit rearing under many condition in modern methods. Department of Agriculture Extension.Moraco.

Karimi, M., R. Sadeghi and J. Kokini (2017). Pomegranate as a promising opportunity in medicine and nanotechnology. *Trends Food Sci. Technol.*, **69:** 59–73.

Hussen, W.M. and J.K. Arrack (2014). Protective Role of Pomegranate Peel Extract on Testis in Adult Male Rabbits Treated with carbon Tetrachloride. *The Iraqi Journal of Veterinary Medicine*, **38(1)**: 74-82.

Al-Okaily, B.N. and E.H. Ali (2019). Effect of pomegranate seed oil against Hepatotoxicity- induced by sodium fluoride in Adult Female Rats (part II). *Iraqi Journal of Veterinary*

- Medicine, 43(1): 102-112.
- Shabtay, A., H. Eitam, Y. Tadmor, A. Orlov, A. Meir, P. Weinberg, Z.G. Weinberg, Y. Chen, A. Brosh, I. Izhaki and Z. Kerem (2008). Nutritive and antioxidative potential of fresh and stored pomegranate industrial byproduct as a novel beefcattle feed. *J. Agric. Food Chem.*, **56:** 10063-10070.
- Jing, P., T. Ye, H. Shi, Y. Sheng, M. Slavin, B. Gao, L. Liu and I. Yu (2012). Antioxidant prosperities and phytochemical composition of pomegranate seeds. *Food Chemistry*, 132(3): 1457-1464.
- Viuda-Martos, M., J. Fernández-Lopéz and J.A. Pérez-Álvarez (2010). Pomegranate and its many functional components as related to human health: A review. *Compr. Rev. Food Sci. Food Saf.*, **9:** 635–643.
- Salvatore, S. and Y. Vandenplas (2010). Prebiotics and Probiotics in Therapyand Prevention of Gastrointestinal Diseases in Children. In Bioactive Foods in Promoting Health, ed. RRWR Preedy, Boston: Academic Press, 181-203.
- Avadhani, A. and H. Miley (2011). Probiotics for prevention of antibioticassociated diarrhea and Clostridium difficile-associated disease in hospitalized adults-a meta-analysis. *J. Am. Acad. Nurse Pract.*, **23:** 269-274.
- Broukus, C.W. and C.B. Andreasen (2003). Erythrocytesn.In Duncan and Prasses. Veterinary Laboratory Medicine. Clinical Pathology 4th edition. Latimer, K.S. Mahaffy.E.A. and Prasse. K.W. Blackwell publishing. Chapter 1, 11-45.
- John, S.V. and S.M. Lewis (1984). Basic Hematology Techniques Practical Hematology, 6th (ed), 22-45.
- Coles, E.N. (1986). Veterinary clinical pathology 4th (ed) W.B. Saunders Co. Philadelphia, USA.
- Dacie, J.V. and S.M. Lewis (1991). Practical Hematology 7th (ed). London: Churchill Livingstone, 37-85.
- SAS (2010). Statistical Analysis System Users Guide. Version

- 9.1, SAS Institute Inc., Cary, NC., USA.
- Melillo, A. (2007). Rabbit clinical pathology. *J. Exot. Pet. Med.* **16:** 135-145.
- Dacie, J.V. and S.M. Lewis (1991). Practical Hematology 7th ed. London: Churchill Livigstone, 37-85.
- Opera, M., T. Iwuji, I. Igwa, I. Etuk and I. Maxwell (2012). Hematological and biochemical responses of adult rabbits to aqueous extract of OcmiumGratissimum leaves. *J. Phys. Pharm. Adv.*, **2(9):** 301-306.
- Ozkan, C., A. Kaya and Y. Akgul (2012). Normal Values of Hematology and some biochemical parameters in serum and urine of New Zealand White rabbits. *World Rabbit. Sci.*, **20**: 253-259.
- Kadhim, Q.M. (2013). Effect of replacement of ground CoriandrumSativum and or TrigonellafoenumGraecum seeds in the diet on growth and some productive, reproductive and biochemical traits of male local rabbits. M.S.c Thesis. Vet. Med. Collage. Baghdad University.
- Aheman, T., A.H. Abu and V. Gbor (2013). Hematological and serum biochemical parameters of rabbit fed varying dietary levels of water spinach (Ipomoea Aquatic) leaf meal. Pelagia research library. Advance In Applied Science Research, 4(2): 370-373.
- Khan, N.H., A.L. Ying, C.G. Tian and O.W. Shantini (2017). Screening of Punuicagranatum seeds for antibacterial and antioxidant activity with various extracts. *J. Bioteccho. and Phyto.*, **1(1):** 1-7.
- Phuoc, T. and U. Jamikorn (2017). Effects of Probiotic supplement (Bacillus subtilis and lactobacillus acidophilus) on feed efficiency, growth performance, and microbial population of weaning rabbits. Asian—Australas. *J. Anim. Sci.*, **30(2):** 198-205.