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Potential Role Of Dietary Pomegranate Seed Powder ((Punica Granatum L) And / Or Saccharomyces Cerevisiae In Some Physiological And Immune Aspects In Local Male Lambs

By

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Abstract

This study was conducted to find out the effect of adding pomegranate seeds pomace (PSP) and saccharomyces cerevisiae (SC) to the diet of local lambs on some productive traits.(Hb ,RBCs ,WBCs ,PCV ,macrophages activity ,cTnI ,and IgG) It inducted in animal field of veterinary medicine college Baghdad university and lasted for the period between 12/1/2022 and 12/6/2022, used 20 lambs at 2–3 months of age grouped in four groups G1 as control group fed concentration diets 2.5% of body weight with wheat straw and alfalfa ,G2 fed same ration and 3% dried pomegranate seed pomace powder (PSP),G3 fed same ration and 4% PSP with 3g of saccharomyces cerevisiae (SC),G4 fed same ration and 6% PSP with3g of saccharomyces cerevisiae (SC), water supply at libdum. Blood samples were drawn from all lambs monthly to measure some haematological and immunological characteristics, including haemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBCs), white blood cell count (WBCs), immunoglobulin (IgG), Troponin I(cTnI), and macrophage activity. The results of administration of PSP, SC with diets of lambs showed statistic significant differences ($P \leq 0.05$) and remarkable effect between treated and un treated groups for all physiological and immune aspects, in some period of experiment compared with control group, therefore it could be concluded from this study that using of PSP and or SC to treated groups it could contribute in improve some of some physiological and immune aspects traits of male lambs.

Keywords: pomegranate seed powder (PSP); saccharomyces cerevisiae(SC); haemoglobin (Hb); packed cell volume (PCV); red blood cell count (RBCs).

Introduction

Animal nutrition is concerned with the dietary requirements of animals used in agriculture and food production, as well as zoos, aquariums, and wildlife management. Carbohydrates, proteins, lipids, minerals, vitamins, and water are the major types of nutrition. (Berg et al., 2002). The growth in the production of animal feed and the use of diverse feed materials has led to the discovery of new materials that may be used in feed components of animal feed to increase animal productivity and optimize feed nutrient consumption. (Babeker, 2011; Lam et al., 2019; Jadoh, 2020).

To lower animal production feed costs, it may be advantageous to replace imported feedstuffs with locally produced feedstuffs, as long as the nutritive value of the

alternative feedstuff is understood, Like fruit by- products and some yeast (Alhomidy et al., 2011). Nowadays, we can observe that the treatment of plants and medicinal herbs has taken on a prominent role in medical science, with herbal remedies being found in almost every pharmacy in our modern world (Al-Khalili, 2010; Kaya et al., 2019).

Although pomegranate seeds are an industry byproduct, current study has highlighted their potential use as source of seed oil having health advantages.. (Habibnia et al., 2012),. Pomegranate seeds are high in antioxidants (Jing et al., 2012). Fiber and lipids make up the majority of their composition (Eikani et al., 2012). with an oil content of 12 to 20% (Lansky & Newman, 2007). Pomegranate seed oils are high in polyunsaturated fatty acids, particularly linoleic and punicic acid, according to several studies (Eikani et al., 2012; Liu et al., 2012) with tocopherols (Jing et al., 2012).

The pomegranate seeds were used to improve the blood picture and protect against free radicals in local male rabbits (Jadoh, 2020).and elevated the concemtration of blood protein in rats **(Ali and Al-okaily ,2016)** and correct the oxidative effect on body tissues **(Al-okaily et al ,2015)** The concentration of IgG and the efficiency of immune cell phagocytosis increased in rabbits fed a ration supplemented with pomegranate peel powder. (EL-sissi et al ,2018),

Saccharomyces cerevisiae (SC) supplementation was found to positively stimulate changes in blood parameters. (Milewski and Sobiech,2009), In ewes, food supplementation with dry yeast resulted in an increase in blood glucose (BG) levels (Kawas et al. 2007).

Sc supplementation had a significant effect on hematological parameters such as Hb, PCV, and RBC counts in weaned Najdi ram lambs. It appears that adding Sc to some of the diets increased iron and salt uptake from the small intestine. (Hussein, 2014).

Live saccharomyces cerevisiae(SC) increases propionic acid production, which is the primary substrate for glucose synthesis in ruminants, according to these researchers. Increased mannan-oligosaccharide (MOS) concentrations, which are VFA substrates and promote an increase in the energy metabolism parameters, could explain the higher BG level in sheep fed with dried yeast. Additionally, The processes involved in hemoglobin (Hb) synthesis were improved by SC supplementation in the small intestine by increasing iron salt absorption. (Dobicki et al., 2005).

Saccharomyces yeast cell wall component mannan oligosaccharides (MOS) influence on immunological regulation, in direct effect to decrease pathogenic bacteria and harmful metabolites, (Newman and Newman ,2001).

the goal of this study was to tind out how dietary pomegranate seed pomace (PSP)mixed with SC affected several physiological and immune features in local male lambs.

Materials And Methods

This experiment was carried out in the animal farm of the University of Baghdad's College of Veterinary Medicine. The experiment lasted from January 12, 2022, through June 12, 2022. A reputable and well-known source provided twenty local-bred male lambs weighing 17.50 kg and aged 2 to 3 months. Lambs were placed into four groups,

taking into account the live weight of lambs each with a single pen measure 2.5 x 4 m. All pens were furnished with cans to use for concentrate and forage diets at the same farm. Before the experiment began, clean, fresh water was always available .

Animal feeding:

1- The first group (G1), which is the control group, was fed concentrated feed containing wheat straw and green alfalfa at a ratio of 2.5percent of body weight..
2- The second group (G2) was administered the same diet as the first1st group, with the addition of 3% of dried, crushed, and powdered pomegranate seeds (PSPP) to the concentrated diet..
3- The third group (G3) was fed the same food as the control group, but with the addition of 3 gm of saccharomyces cerevisiae (SC) and 4% of dried and crushed pomegranate seeds pomace powder (PSPP) per head daily..
4- The fourth group (G4) fed on the ration of the control group, in addition to 6% of dried and crushed pomegranate seeds pomace powder (PSPP) and 3 gm of saccharomyces cerevisiae (SC) bread yeast for each head daily added to it with the concentrated ration, With the concentrated ration.

The PSP were obtained at a local market as aby-product, and SC as Baker's yeast powder are used.

Blood samples:

From the beginning through the end of the investigation, blood samples were obtained at monthly intervals plus a zero-time collection. After sterilizing the site of blood collection with disposable sterilized syringes, blood samples were obtained from the jugular vein. The blood samples were divided into two parts, with the first kept in a 5 mL test tube containing anti-coagulant EDTA to evaluate RBCs count, WBCS count, Hb concentration, PCV %, , and macrophage activity (phagocytosis)%.while the remaining blood samples were stored in sterilized (10 ml) tubes and centrifuged (3000 rpm) for 15 minutes to estimate IgG concentration, and cardiac troponin I(cTnI).

Blood analysis:

The Veterinary Automated Hemoanalyzer (Genex) was used to obtain the blood image parameters (RBC count, WBC count, blood hemoglobin Hb concentration, and PCV%, For cell counting, use the impedance method; for hemoglobin determination, use the cyanide-free colorimetric approach according to Okada and Schwan,(1960).

macrophages activity %

A differential leukocyte count was performed by fixing a thin, homogeneous smear of blood. Then the smear was stained with Leishman's Stain (1-2 minutes), then the dye was diluted using a phosphate buffer solution (PBS)and left for 8 minutes, then the slide was washed with normal water and left to dry, and (100 white cells) were accounted for each sample. (Mckenzie, 1996).

The phagocytic activity of neutrophils was tested by adding 0.1 cm of Nitro blue tetrazolium (NBT) at a concentration of 0.1% to a similar volume of venous blood treated with EDTA, taking into account the mixing well. The incubation process was carried out for thirty minutes, and after the incubation period, the mixture was mixed well, a homogeneous smear was prepared on a clean slide and then dyed with Lishman's stain. The macrophages were examined under the oil lens using a light microscope, and a

calculation was made. (Park et al. ,1968) As follows, the phagocytic cells that showed a positive result of the process of phagocytosis were identified by observing the formazan granules formed inside these cells, which indicate the positive result of the process:

Macrophages activity %= number of NBT reduced macrophages /total number of macrophages x 100 (Simmons and Statland, 1997).

Determination of cardiac troponin I(cTnI)

Cardiac protein troponin I (cTnI) measured by using a sheep Troponin I (cTnI) Elisa kit, from china (SunLong Biotech Co.,LTD), And Follow the manufacturer instructions.

The immunoassay sandwich approach according to Basbugan, et al (2010).

Determination of serum IgG

The concentration of G-globulin was measured using a sheep immunoglobulin G(IgG)Elisa kit from china (SunLong Biotech Co., LTD), according to Killingsworth and savory (1972) and Follow the manufacturer instructions.

Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA, two-way ANOVA (SAS, 2010.) and Least significant differences (LSD) post hoc test were performed to assess significant differences among means. $P < 0.05$ is considered statistically significant. (Sorli,D. 1995)

Results

1.Haemoglobin concentration g/dl:

The level of haemoglobin in the blood showed a significant ($P<0.05$) superiority in the lambs of the 2^{nd}, 3^{rd}, and 4^{th} treatment groups at the expense of the lambs of the control group, starting from the third month until the experiment's conclusion. . Also, the haemoglobin concentration in the 2nd, 3rd, and 4th treatment groups increased significantly ($P<0.05$) with the progression of the experiment period. Table 1

2.packed cell volume (PCV) %:

A significant ($P<0.05$) increase in the percentage of packed cell volume (PCV) % in the 2nd, 3rd, and 4rth treatment groups compared to the control group in the last month of the experiment. Also, The PCV % in the third and fourth treatment groups increased significantly with the progression of the experiment period. Table 2

3.Red blood cells count cell x 106/mcL:

The number of red blood cells, in which it is seen that the 3rd and 4th treatment groups were significantly ($P<0.05$) superior to the control group in the second month to the end of the experiment period while, The number of red blood cells increased significantly($P<0.05$) with the progression of the experiment in all treatments. Table 3

4.white blood cells count(WBCs) cell x10^3:

The total white blood cells (WBCs) count, it is noted that the WBCs count in the third and fourth treatment groups was significantly($P<0.05$) superior than control group in

the fourth and five month of the experiment, while The number of white blood cells increased significantly(P<0.05) with the progression of the experiment in the 2nd ,3rd and 4th groups. Table 4

5.macrophages activity %

The macrophage activity percentage as showed, significant(p<0.05) differences between the experimental animals, as the 3rd and 4th groups outperformed the control group from 3rd months to the end of the experiment period) While the effectiveness of the phagocytes increased significantly (p<0.05) with the progression of the experiment time in the 2nd , 3rd , and 4th groups, Table 5

6.Immunoglobulin g (IgG) g/L

Immunoglobulin G (IgG) levels differed between the experimental groups, as the 2^{nd} ,3^{rd} and 4^{th} groups were significantly (P<0.05) superior than control group in the last third month of experiment while, IgG concentration increased significantly(P<0.05) in 2nd ,3rd and 4th groups with the progression of the trial period. Table 6

7.Troponin I (cTn I) ng/ml

The level of cardiac troponin I (cTn I) protein decreased significantly (P<0.05) in the 2^{nd} ,3^{rd}, and 4^{th} treatment groups, respectively, compared to its level in the control group during the last three months of the experiment, And with the progression of the trial period. Table 7

Discussion

1.Haemoglobin concentration g/dl:

The concentration of haemoglobin is increases in 2^{nd} ,3^{rd} ,and 4^{th} groups ,with the progression of the experiment, may be reflect of the increase in red blood cells, which increases with the age of the lambs due to the increase in body weight. These results are consistent with (El-Gaafarawy et al ,2003), which indicated a significant increase in the concentration of haemoglobin with the advancing age of the animal,

Pomegranate seeds pomace powder (PSPP) contain important nutrients for the manufacture of haemoglobin because they contain iron, zinc, and copper (Rowayshed et al., 2013). In addition to containing flavonoids (Tehranifar et al., 2010), vitamin C (Ramadan et al., 2009),tannins (Wang et al., 2010), , In addition to this, the antioxidants are abundant in pomegranate seeds, which work to preserve blood cells and protect them from the effects of free radicals and the rest of the cellular toxins that destroy them (Halliwell and Gutteridge, 1999), thus prolonging the life of cells and maintaining hemoglobin, as reports indicate an increase in the absorption of iron from the small intestine in the presence of pomegranates fruit , It in turn causes the concentration of hemoglobin to rise. and its agree with (Abdul-Zahra and Jwad 2020). Or it could be because of the role of polyphenols in preserving red blood cells and haemoglobin against lipid peroxidation. Alternatively, by using a polyphenol iron-binding complex to remove excess iron (Perron and Brumaghim, (2009). Or, may be attributed to the role of pomegranate seeds in activating the secretion of the hormone erythropoietin from the kidneys, which responsible of the formation of RBCs in bone marrow and thus increases haemoglobin (Banihani et al ,2019). This result was consolidated by Jadoh, (2020)

Or it may be due to the role of SC in increasing the absorption of iron from food in the small intestine with the availability of quantities of vitamin B that have important role

and increase the synthesis of haemoglobin (Kander 2004). This result was consolidated by Milewski and Sobiech (2009), Or, haemoglobin may rise due to the synergistic action of yeast and pomegranate seeds because the two contain important nutrients that enter the composition of hemoglobin, such as iron and zinc, (Rowayshed et al., 2013The results of the current study supported the findings of Al Dairi (2014), **Abed and AL-zwean, (2018)** and Mahdi (2020).

2.packed cell volume (PCV) %:

The significantly increase recorded in the percentage of PCV % indicates an improvement in the health status of lambs treated with both PSP and SC and an improvement in the rumen environment, especially the integrity of the small intestine, which increases the absorption of nutrients, the elements necessary for the formation of red blood cells. According to Al-Hussaini, (2017), the superiority of the second group may be attributed to the role of pomegranate seeds pomace (PSP). That have numerous beneficial nutrients found in pomegranate seed pomace and also their synergistic and boosting impacts on various action processes. These processes include an impact on the erythropoiesis in the bone marrow, which affects red blood cell count and causes an increase in PCV% and this corresponds to Manthou et al. (2017). It agrees with the result of the current study, Jadoh (2020).

Or it could be linked to SC's function in increasing the metabolic activity in the rumen by increasing the number of carbohydrate-digesting bacteria in food and the rest of the elements available in the PSP, which enter directly into the manufacture of erythrocytes and their numbers increase, and consequently the blood accumulation increases finally increase the PCV% in blood (Shwayel and Rasheed,2016). The results of the current study supported the findings of Mahdi, (2020),

3.Red blood cells count cell x 10^6/mL:

Or it can be attributed to the pomegranate seed pomace powder being a good source of iron and zinc, which are important elements in the manufacture of RBCs in the bone marrow. (Rowayshed et al., 2013). Or the role of pomegranate seeds in increasing the absorption of iron from the digested food in the intestines which ultimately results in an increase in the amount of hemoglobin, which leads finally to an increase in RBC count (Abdul-Zahra and Jwad, 2020) Or may by the role of polyphenols in preserving red blood cells against lipid peroxidation. Alternatively, by using a polyphenol iron-binding complex to remove excess iron , This conclusion corresponds with Perron and Brumaghim (2009). As a result, the antioxidant properties of polyphenolic compounds against RBCS peroxidative damage caused a concentration-dependent inhibition activity on OH product. (Anghileri and Thouvenot, 2000). Or, the reason for the high numbers of red blood cells may be the effect of pomegranate seed on raising the levels of the blood hormone that wins from the kidneys and controls the rates of red blood cell manufacturing and adjusts its level, which leads to an increase in the production of RBCs in the bone marrow Manthou et al., (2017). and Jadoh ,(2020)

Or it may be retained to the effect of SC on iron absorption rates from the alimentary canal. With its ability to produce B-vitamins, SC acts as a probiotic, increasing the rate of iron absorption from the small intestine. As a result, the production rates of red blood cells increase and their numbers also increase. (Kander, 2004), The role of SC in improving the environment of the rumen and increasing the number microflora count (Dobicki et al. 2005) and increasing the efficiency of the rumen and thus increasing the digestion of nutrients, including PS, which contain good amounts of carbohydrates,

fats, proteins, and minerals elements, and release nutrients in it, may explain the moral rise in blood cells. These results were supported by the findings of**,(Mahdi and Yousif ,2019).**

4.white blood cells count(WBCs) cell x10^3/ml

A significant increase in the number of white blood cells is observed with a high percentage of PSP in the diet, and this may be due to the therapeutic effects of pomegranates components (Toutou et al. 2019), as pomegranate seeds improve animal health and immunity (Ramzi, 2015). This demonstrates the pomegranate seed pomace powder (PSPP) nutritional components' overall impact, This could be explained by the biological and therapeutic properties of PS , which contain a variety of polyphenols, minerals, vitamins, and phytochemicals that have anti-oxidant effects. The results of our current study agree with both Abatenh et al. (2018), and Jadoh (2020.

Or it could be linked to SC's nutritional function as it contains vitamin B, which plays an important role in the manufacture of blood cells (Kander, 2004), and this conclusion is consistent with that mentioned by Milewski and Sobiech (2009), or it related to the production of additional numbers of immune cells (antibodies) (Lafleur, 2008),The immune capacity of SC is centered on the complex B-1,3/1,6 D-glucans and manna-oilgosacchrides, which are found in the cell wall of the SC, and this mechanism involves the activation and formation of immune complexes such as anti-bacterial antibodies. These outcomes are consistent with Al-Jassim et al. (2018)

5.macrophages activity %

It is noticed from table 5 above that the 3rd and 4th groups outperformed the control group in the percentage of macrophage activity, This may be attributed to the pomegranate seed powder containing good amounts of a group of vitamins, especially vitamins B and C (Rowayshed et al., 2013) and antioxidants (Albrecht et al., 2004), which have a stimulating role in the activity of macrophages (Ehmedah et al., 2020)., which agrees with the findings of Hikosaka et al,2007).

The ellagic, gallic, and punicalagen isolated from pomegranate seeds increased the activity of macrophages (Bensaad et al ,2017), which helps in raising the efficiency of killing of phagocytes and inflammation, and polyphenols such as gallic and ellagic acids affect Available in abundance in pomegranate seed powder, they are substances with great biological activity in activating dormant macrophages by increasing their response to released cytoxins (interferon and interleukins)(Shapouri-Moghaddam et al. 2018) ,. This conclusion agrees with Alves et al, (2013), Or, the reason for the increase in the effectiveness of phagocytic cells may be attributed to the presence of tannin, (Gerson et al., 1982). The results of the current study are consistent with Mossalem et al. (2016), and Siraj et al. (2018) .

Or it may be retained to the role of SC in enhancing immunity by increasing the activity of neutrophil phagocytosis in lambs (Malaczewska and Milewski , 2010) and ewes (Zabek et al., 2014), and this is consistent with what was reported by Wojcik ,(2010) and Milewski et al., (2010), who concluded that adding SC to the diet led to an increase in the activity of phagocytic cells and an improvement in the animal's health condition. It may also be due to the ability of SC to synthesize vitamin B in the rumen (Kander, 2004) , which leads to an improvement in the effectiveness of macrophages with the effect of the immune-boosting vitamin (Ehmedah, et al., 2020

Or, the reason for the increase in the activity of phagocytes may be due to the presence of β-glucans compounds in the cell wall of the SC (Wilewski et al., 2007), β-glucans activate the mechanism of killing germs by neutrophil cells (Demir et al. 2007), The results of our current study agree with Ortuo et al. (2002) and Asmaa et al. (2020).

6.Immunoglobulin g (IgG) g/L

The effect of the nutritional components of PSPP may be due to the presence of an abundance of vitamins and minerals like A, E, C, and selenium (Rowayshed et al., 2013) that activate lymphocytes and increase the production of IgG"(James et al,2002). Or, the cause of the rise in IgG may be the improvement in the level of blood proteins, including immunoglobulin (Worku et al. 2016) As well as the presence of polysaccharides in abundance in pomegranate seeds, which have an immune role in activating lymphocytes and leading to increased production of IgG (Joseph et al 2012), This theory is consistent with what was mentioned (Yamasaki et al., 2006).

Also, the polyphenols available in PS, especially ellagic acid, raise the concentration of IgG in the serum (Laily et al. 2011), which perform several functions, the most important of which is anti-oxidation, as it preserves lymphocytes from damage by free radicals and increases their effectiveness and thus increases the production of IgG, It increases its level in the blood (Laily et al., 2016 our results of our current study agree with Abbas et al. (2019),

Saccharomyces cerevisiae(SC) may increase the digestibility of nutrients in PS, including vitamins and minerals (Rowayshed et al., 2013). and fatty acids. (Shabbir et al., 2017). On ways to increase the effectiveness of microorganisms in the rumen, and the products of the digestion of PS interfere in the manufacture of antibodies, which leads to an increase in their concentration in the blood (Milewski and Sobiech 2009), SC is one of the feed additives that acts as a vital booster that maintains animal health and the safety of its immune system, as scientific reports indicate an increase in IgG in the serum of cows after providing their diet with SC by a10 g /per animal per day. (Cakiroglu et al., 2010).

Or it could be attributed to the SC action it activates the animal's immune system and the increase in IgG to the fact that some components of the SC cell membrane contain β-glucans **(Al- Obaidi et al,(2010), Mahdi and Naser ,(2014)** and Samuelsen et al., 2014), mannose, and oligosaccharides (Muthusamy et al., 2011), which are stimulants for lymphocytes, stimulating them to increase the production of IgG and increase its level in the blood (Silva et al., 2009). Our conclusion also corresponds to that of Jensen et al. (2008).

The high IgG concentration in lambs may be brought on by a rise in net globulins, which is brought on by a gain in gamma globulin brought on by the multiplication of kopffer cells as well as an elevation in plasma cells. as a result of adding SC to the lamb ration. (Raghebian et al., 2016). The results of our study agree with both Milewski and Sobiech. (2009), and Kewan et al. (2021).

7.Troponin I (cTn I) ng/ml

The significant decrease (P<0.05) in the concentration of cTn I with the progress of the experience of the experiment may be due to the cumulative effect of pomegranate seed pomace (PSP) and saccharomyces cerevisiae(SC) , which act as strong antioxidants that protect the cardiac cells from the harmful effects of free radicals , which increases its

effect with the increased time of the experiment to improve heart health(Aloutaibi et al., 2017).

The significant decrease($P<0.05$) in the level of cTn I as shown in the table above in all treatment groups compared with the control group was attributed to the effect of PS on protecting the heart because they contain strong antioxidant compounds such as multiple phenols, including ellagic acid, punicalagin, and tannins, which have strong antioxidant activity (Lanny 2000, Pountsi et al. 2005, Ismail et al 2012), as it has a significant role in protecting and treating cardiac cell wounds, thus decreasing the concentration of cTn I (Stoner and Gupta 2001), The conclusion is consistent with what was indicated by Taskin and Deger (2021), The reason for the low cTn I may be due to the abundant content of vitamin C and vitamin E (Rowayshed et al., 2013), which are powerful antioxidants that protect heart cells from the effects of free radicals and prevent cell destruction, 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), and El-Wakf et al., (2018),

the role of SC in lowering cTn I may be indirect by increasing the efficiency of the rumen and improving the processes of digestion (Milewski and Sobiech, 2009). This leads to the good digestion of PS and the release of large amounts of active substances in the PS as antioxidants from phenols (Charalampia and Koutelidakis, 2017), which plays an important role in protecting heart cells from damage and lowering cTn I levels. It can also be due to the effect of SC directly through its role as an antioxidant (Du et al. 2020) by reducing oxidative stress on heart cells, which can lower values of LDL-c and total cholesterol, and protect the heart cells. β-glucan supplements are frequently used to improve heart health (Rhoads et al., 2013), The reason for the effect of SC on lowering cTn I may be due to its content of vitamins A, B, and C (Lzah et al., 2019), which express powerful antioxidants that protect heart muscle cells from destruction, thus reducing the level of cTn I in the blood (Khan et al., 2020). Because of SC abundant magnesium content, it is one of the minerals that helps stimulate blood circulation, also works to strengthen the muscles of the body, especially the heart muscle, and improve cardiac muscle health. cTn I concentration is reduced as a result (Cot et al., 2016).

Conclusion

The addition of pomegranate seeds pomace, and yeast led to an improvement in the blood profile of the lambs in terms of increasing the concentration of hemoglobin, red, and white blood cells, in addition to increasing the ability of flagellated cells and increasing the concentration of immunoglobulin G (IgG). and cardiac troponin I (cTnI).

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Ethics

The authors are responsible for addressing any ethical issues that may arise after

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Table (1) *Effect of dietary (PSP) with or without (SC)on haemoglobin (g/dl) of local male lambs (M±SE)*

months / 4 animals / group	zero time	1st month	2nd month	3rd month	4thmonth	5thmonth
G1 (control)	9.78±0.39 A a	10.12±0.16 A a	9.89±0.09 A a	9.98±0.12 A b	9.84±0.21 A b	9.90±0.25 A b
G2 (3%PSP)	9.94±0.77 B a	10.14±0.16 AB a	10.94±0.27 A a	10.96±0.21 A a	11.24±0.24 A a	11.32±0.24 A a
G3 (3g SC+4%PSP)	10.08±0.38 B a	10.32±0.30 AB a	10.12±0.56 A a	10.66±0.41 A a	11.08±0.46 A a	11.26±0.35 A a
G4 (3g SC+6%PSP)	9.94±0.30 B a	10.52±0.13 AB a	10.16±0.32 AB a	10.94±0.28 A a	11.26±0.18 A a	11.40±0.17 A a
LSD	0.93					

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

Table (2) *Effect of dietary (PSP) with or without (SC) on packed cell volume (PCV) % of local male lambs (M±SE)*

months / animal/ group	zero time	1st month	2nd month	3rd month	4thmonth	5thmonth
G1 (control)	30.68±1.78 A a	30.74±1.15 A a	30.80±0.80 A a	31.06±0.64 A a	31.40±0.55 A a	31.54±0.53 A b
G2 (3%PSP)	31.70±1.97 A a	32.08±1.47 A a	32.28±1.43 A a	A32.84±1.34 A a	A33.36±1.04 A a	33.84±0.88 A a
G3 (3g SC+4%PSP)	30.64±1.87 B a	32.48±1.08 AB a	32.84±1.03 AB a	33.06±0.95 A a	AB33.40±0.75 A a	33.82±0.71 A a
G4 (3g SC+6%PSP)	30.02±1.21 B a	31.72±0.39 AB a	32.22±0.17 AB a	32.24±0.16 A a	A33.10±0.20 A a	33.78±0.09 A a

Lsd	3.02

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

Table (3) *Effect of dietary pomegranate seed powder (PSP) with or without bread yeast (Saccharomyces cerevisiae on Red blood cells count cell x 10^6/mL of local male lambs (M±SE) .*

months / animals/ group	zero time	1st month	2nd month	3rd month	4thmonth	5thmonth
G1 (control)	4.74±0.11 C a	4.85±0.10 C a	4.85±0.07 C b	4.97±0.04 BC b	5.36±0.21 B b	5.80±0.04 A b
G2 (3%PSP)	4.62±0.19 D a	4.91±0.17 CD a	5.06±0.19 Ca b	5.16±0.25 BC ab	5.55±0.16 B ab	5.97±0.02 A ab
G3 (3g SC+4%PSP)	4.62±0.14 B a	4.86±0.16 B a	5.22±0.06 AB a	5.96±0.08 A a	5.98±0.02 A a	6.13±0.05 A a
G4 (3g SC+6%PSP)	4.54±0.18 D a	4.91±0.13 CD a	5.15±0.16 BC a	5.66±0.15 B a	6.27±0.16 A a	6.52±0.14 A a
LSD	0.40					

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

Table (4) *Effect of dietary (PSP) with or without SC on WBC count cell x $10^{3/}$/ml of local male lambs (M±SE)*

months / animals/ group	zero time	1st month	2nd month	3rd month	4thmonth	5thmonth
G1 (control)	8.76±0.46 A a	8.62±0.44 A a	8.44±0.12 A a	8.56±0.13 A a	8.74±0.14 A b	8.78±0.15 A b
G2 (3%PSP)	8.74±0.40 AB a	8.72±0.56 B a	8.96±0.57 AB a	8.54±0.60 B a	9.02±0.30 AB ab	9.70±0.15 A ab
G3 (5g SC+4%PSP)	8.62±0.60 B a	8.92±0.35 AB a	8.84±0.16 AB a	8.68±0.34 B a	9.74±0.09 A a	10.42±0.16 A a
G4 (3g SC+6%DRGP)	8.76±0.44 C a	8.98±0.20 C a	8.96±0.19 C a	8.66±0.12 C a	9.98±0.14 B a	11.28±0.22 A a
LSD			0.96			

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

Table (5) *Effect of dietary (PSP) with or without (SC)on macrophages activity % of local male lambs (M±SE).*

months / animals/group	zero time	1st month	2nd month	3rd month	4thmonth	5thmonth
G1 (control)	20.80±2.47 A a	20.80±2.39 A a	21.40±2.37 A a	22.20±2.22 A b	24.00±1.92 A b	25.00±1.67 A b
G2 (3%PSP)	20.00±2.30 D a	21.20±2.17 CD a	22.80±1.88 C a	25.60±2.01 B ab	27.20±1.39 AB ab	28.40±1.43 A ab
G3 (3g SC+4%PSP)	21.00±2.79 C a	22.00±2.54 C a	24.40±0.81 B a	27.40±0.67 AB a	28.60±0.67 A a	A30.40±1.20 A a
G4 (3g SC+6%PSP)	21.20±2.05 C a	21.80±1.46 C a	26.00±1.26 B a	28.60±0.92 AB a	32.60±0.40 A a	A31.20±0.86 A a
LSD`	5.05					

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

Table (6) *Effect of dietary (PSP) with or without (SC) immunoglobulin g(IgG) g/L of local male lambs (M±SE).*

months / animals/group	zero time	1st month	2nd month	3rd month	4th month	5th month
G1 (control)	8.36±0.28 A a	9.16±0.23 A a	9.65±0.15 A a	9.78±0.16 A b	9.77±0.20 A b	10.24±0.23 A b
G2 (3%PSP)	8.22±0.27 B a	9.26±0.13 B a	AB10.78±0.37 ABa	11.60±0.35 A a	11.94±0.06 A a	12.26±0.15 A a
G3 (3g SC + 4% PS)	8.26±0.56 D a	9.30±0.20 C a	10.90±0.34 B a	12.32±0.45 AB a	12.72±0.25 A a	13.14±0.18 A a
G4 (3g SC + 6%PS)	8.40±0.41 C a	9.20±0.63 B a	10.82±0.52 AB a	11.88±0.35 A a	12.42±0.19 A a	13.60±0.032 A a
LSD	1.62					

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

Table (7) *Effect of dietary (PSP) with or without(SC) Troponin I (cTn I) ng/ml of local male lambs (M±SE).*

months / animals/group	zero time	1st month	2nd month	3rd month	4th month	5th month
G1(control)	0.14±0.008 A a	0.15±0.03 A a	0.14±0.01 A a	0.13±0.01 A a	0.14±0.007 A a	0.14±0.006 A a
G2(3%pSp)	0.14±0.01 A a	0.10±0.003 B a	0.10±0.004 B b	0.10±0.008 B b	0.08±0.007 B b	0.08±0.008 B b
G3 (3g SC +4%PSP)	0.13±0.01 A a	0.11±0.008 AB a	0.10±0.009 B b	0.09±0.007 BC b	0.07±0.006 C b	0.07±0.007 C b
G4 (3g SC+6%PSP)	0.14±0.04 A a	0.10±0.01 B a	0.10±0.01 B b	0.09±0.002 B b	0.07±0.009 B b	0.04±0.003 C b
LSD	0.03					

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