



## Effect of using pomegranate peels on some biochemical and hormonal parameters of Arabi Ramas

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### Abstract:

This study was conducted in the animal field of Agriculture College / Basra University from 10/11/2019 to 10/2/2020, A total of 12 Arabi rams aged 1.5 and > 3.00 years (six rams each) were bought from the local markets (weighed 45 and 65 kg). Rams divided randomly into four groups (three rams each) and were placed in a semi-shaded pens dimensions (4 x 2) M. Before the start of the experiment, rams were dosed pomegranate peels two weeks as introductory period by a gelatin capsule with a high ability to not be analyzed orally, according to the following experimental treatments. Rams aged 1.5 years were divided into two groups (3 rams each), one group with no pomegranate peels and the other was dosed with 100 gm / day. Rams aged > 3 years were divided and dosed as the rams aged 1.5 years. Ten days after the experiment began, blood samples were taken, as well as semen was collected from rams and repeated every ten days until the end of the experiment. The results showed that pomegranate peels significantly increased the concentrations of FSH, LH and Testosterone hormones in rams aged 1.5 and > 3 years. Cholesterol and triglycerides were significantly lowered by the dose of pomegranate peels.

**Keywords:** pomegranate peels, Arabi rams, parameters, biochemical, hormonal

### تأثير استخدام قشور الرمان على بعض المعايير الكيموحيوية والهرمونية لذكور الكباش العربية

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### الخلاصة

أجريت هذه الدراسة في الحقل الحيواني لكلية الزراعة / جامعة البصرة خلال الفترة من 10/11/2019 ولغاية 10/2/2020، حيث تم شراء 12 كبشاً عربياً بعمر 1.5 و < 3.00 سنة (ستة كباش لكل منهما) من الأسواق المحلية بوزن يتراوح بين (45-65 كجم ± 3.00 كجم). قسمت الكباش عشوائياً إلى أربع مجموعات (ثلاثة كباش في كل مجموعة) وتوضع في أقفاص أبعادها (2×4) م. قبل البدء بالتجربة تم تجريع الكباش بقشور الرمان كفترة تمهيدية لمدة أسبوعين بواسطة كبسولة جيلاتينية ذات قدرة عالية على عدم التحليل عن طريق الفم وذلك حسب المعاملات التجريبية التالية. تم تقسيم الكباش بعمر 1.5 سنة إلى مجموعتين (3 كباش لكل منهما)، مجموعة بدون قشور الرمان وجرعت الأخرى بجرعة 100 غم. الكباش بعمر أكبر من 3 سنوات مقسمة

وجرعات على حسب عمر الكباش بعمر 1.5 سنة. وبعد عشرة أيام من بدء التجربة تم أخذ عينات الدم من الكباش وتكرر كل عشرة أيام حتى نهاية التجربة. أظهرت النتائج أن قشور الرمان أدت إلى زيادة معنوية في تركيز هرموني LH و FSH والتستسترون في الكباش بعمر 1.5 وأكبر من 3 سنوات. انخفض مستوى الكوليسترول والدهون الثلاثية بشكل ملحوظ في المعاملات التي جرعت قشر الرمان

الكلمات المفتاحية: قشور الرمان، الكباش العربية، المعايير، الكيموحيوية، الهرمونية.

## Introduction

Most studies have reported that pomegranate peels contain many active chemical compounds such as tannins, alkaloids, polyphenols, anthocyanin, terpenes, flavonoids, saponins, steroids, glycosides and amino acids (Hamid *et al.*, 2009). Omer *et al.* (2019) indicated that the total value of essential and nonessential amino acids in pomegranate peels are 51.30 and 48.37 g/ 100 g of total protein respectively. Pomegranate peels contain all the major minerals such as calcium (Ca), phosphorous (P), potassium (K), sodium (Na), and magnesium (Mg), (342, 120, 150, 68, and 56 mg / 100 g respectively). It also contains many unique elements such as zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), and selenium, (1.08, 0.86, 0.65, 6.11 and 1.07 mg / 100 g, respectively). Moreover, pomegranate peel contains some vitamins (B1, B2, C, E, A) and the dried pomegranate peel has high levels of unsaturated fatty acids. Oxidative stress is one of the many causes of male infertility by causing weak sperms and increased cellular damage by free radicals derived from ROS reactive oxygen species (ROS) (Agarwal *et al.*, 2014). Hussein and Shuja (2013) indicated that lambs consuming diet with pomegranate peel powder at different levels 2, 4, 6% for 84 days, caused a significant increase in the concentration of serum cholesterol. Khan *et al.*, (2013) noted a significant effect of adding different levels of pomegranate peels 1, 2, 4% to the diet of Arabi lambs in the level of cholesterol, which reached, respectively, 1.55, 1.41, 1.61 mmol / liter. Abbas *et al.*, (2019) reported that the addition of different levels of pomegranate peels (2, 3, 4%) to dairy cows' feed led a significant decrease in the level of cholesterol in blood plasma in all treatment groups compared to the control treatment. Rajabi *et al.*, (2017) indicated that there were no effects on the concentration of triglycerides in the serum of Kurdish lambs fed on different levels of pomegranate peel powder in the diet. Modarresi *et al.*, (2010) found that there was no significant effect on the concentration of triglycerides in the blood serum of goats, to diet containing the pomegranate pulp compared with the control group. However, Hundal *et al.*, (2019) found that the buffalo serum triglycerides concentration was not significantly influenced when fed pomegranate peels compared with the control group. Dkhil *et al.*, (2013) observed a significant increase in the levels of testosterone hormone in the serum of the groups that were dosed with pomegranate peel extract or pomegranate juice. Ramzi, (2016) showed that added pomegranate peel powder at different levels to the diet (1, 2, 4) %, respectively, a significant increase in the level of serum luteinizing hormone (LH) in all treated groups of Karadi lambs compared with a control. The present study aims to determine the effect of dosing Arabi rams with pomegranate peels on the level of hormones (FSH, LH and Testosterone) and the blood serum biochemical parameters (cholesterol and triglycerides)

## Materials and methods

This study was conducted in the animal field of Agriculture College/ Basra University from 10/11/2019 to 10/2/2020. A total of 12 Arabi rams were bought from the local markets (ranged between 45-65 kg) and divided randomly into four groups (three rams each) and were placed in a semi-shaded pens (4 x 2) m. Water was provided freely during the period of the experiment. The rams were fed a concentrated diet at a rate of 3% of their body weight at two times, in the morning and evening. The diet was adjusted based on the weight of rams every two weeks. Before the start of the experiment, rams were dosed pomegranate peels with a two-week introductory period in the form of a gelatin capsule with a high ability to not be analyzed orally, according to the following experimental treatments:

T1 - The first treatment (3) Rams aged 1.5 years, a control treatment without dosing the pomegranate peel.

T2 -The second treatment (3) Rams aged 1.5 years; 100 gm/day of pomegranate peels were dosed.

T3-The third treatment (3) Rams aged 3< years, a control treatment without dosing the pomegranate peels.

T4-The fourth treatment (3) Rams aged 3< year, 100 gm/day of pomegranate peels were dosed.

Ten days after the experiment began, blood samples from rams were collected and continued every ten days until the end of the experiment. Some of biochemical parameters: cholesterol, triglyceride and hormones such as FSH, LH, and testosterone concentrations were determined. The levels of FSH, LH, and testosterone estimated in the Physiology Laboratory of the Faculty of Agriculture department of Animal Production, using a ready-made kit produced by monobin -U.S.A and employing the ELISA technique at a wavelength of 450 nanometers (Odell et al., 1981). triglyceride and cholesterol were determined by using biochemical kits (Biolabo Maizy, France) by spectrophotometer (JENWAY 6300).

## Results and discussion

### The effect of pomegranate peel on sex hormones

Table (1) indicates the effect of dosing on the period of collection in the level of FSH hormone ng/ml in the blood serum of the Arabi rams. There is a significant ( $p \leq 0.05$ ) difference between the treatments during the collection periods, which the first and second collection had no significant difference among the treatments, but in the third and fourth collection, the second treatment was significantly higher than T1, T3, and T4 while, at the fifth collection, there were no significant differences noticed between different treatments. At the sixth, seventh and eighth groups of treatment T2 were significantly higher than T1, T3, T4 treatments. The reason for the high concentration of FSH is due to the active substrate of pomegranate peels which are considered high antioxidants such as tannins, phenols and flavonoids that can directly or indirectly reduce oxidative damage resulting from the excessive production of free radicals (AL-Olayan *et al*, 2014).

**Table (1): The effect of pomegranate peel dosage and the collection period on the level of FSH hormone (ng/ml) in the blood serum of Arabi rams (Mean  $\pm$  SD)**

Collection of blood every at	Treatment				The level of significance
	T1	T2	T3	T4	
1 <sup>st</sup>	0.020 $\pm$ 0.34	0.0152 $\pm$ 0.34	0.0152 $\pm$ 0.32	0.010 $\pm$ 0.36	N.S
2 <sup>nd</sup>	0.02 $\pm$ 0.33	0.01 $\pm$ 0.36	0.05 $\pm$ 0.32	0.02 $\pm$ 0.35	N.S
3 <sup>rd</sup>	0.01 $\pm$ 0.32c	0.01 $\pm$ 0.46a	0.03 $\pm$ 0.29c	0.02 $\pm$ 0.39b	*
4 <sup>th</sup>	0.05 $\pm$ 0.34c	0.13 $\pm$ 0.51a	0.16 $\pm$ 0.31c	0.09 $\pm$ 0.43b	*
5 <sup>th</sup>	0.05 $\pm$ 0.44	0.13 $\pm$ 0.49	0.16 $\pm$ 0.35	0.09 $\pm$ 0.42	N.S
6 <sup>th</sup>	0.020 $\pm$ 0.33c	0.030 $\pm$ 0.64a	0.049 $\pm$ 0.31c	0.040 $\pm$ 0.54b	*
7 <sup>th</sup>	0.05 $\pm$ 0.33c	0.01 $\pm$ 0.70a	0.01 $\pm$ 0.35c	0.02 $\pm$ 0.59b	*
8 <sup>th</sup>	0.01 $\pm$ 0.33c	0.02 $\pm$ 0.75a	0.06 $\pm$ 0.31c	0.04 $\pm$ 0.63b	*

\* Different letters within a column means significant differences at the level ( $p \leq 0.05$ ) between treatments, N.S: There were no significant differences between the experimental treatments, T1 - The first treatment (3) Rams aged 1.5 years, a control treatment without dosing the pomegranate peel.T2 -The second treatment (3) Rams aged 1.5 years , 100 gm of pomegranate peels were dosed. T3-The third treatment (3) Rams aged 3< year , a control treatment without dosing the pomegranate peels. T4-The fourth treatment (3) Rams aged 3< years , 100 gm of pomegranate peels were dosed.

Table (2) indicates the effect of dosing on the level of LH (ng / ml) in the serum of the Arab rams. There was a significant ( $p \leq 0.05$ ) difference between the experimental treatments during the collection periods. In the first, second and fifth collections, there was no significant effect was found between the treatments. While, for groups from other collection, T2 exceeds T1, T3, and T4. The reason for the

increase in the concentration of LH is due to the ability of the active of components in pomegranate to reduce stress hormones such as cortisol, as its rise causes a decrease in the concentration of releasing hormones (GnRH). which play in important role in the production of FSH and LH hormones (Azeem et al, 2019).

**Table (2). The effect of pomegranate peel dosage and the collection period on the level of LH hormone (ng/ml) in the blood serum of Arabi rams (Mean  $\pm$  SD)**

Collection of blood every at	Treatment				The level of significance
	T1	T2	T3	T4	
1 <sup>st</sup>	0.05 $\pm$ 0.23	0.02 $\pm$ 0.23	0.02 $\pm$ 0.26	0.02 $\pm$ 0.22	N.S
2 <sup>nd</sup>	0.01 $\pm$ 0.24	0.03 $\pm$ 0.24	0.01 $\pm$ 0.24	0.01 $\pm$ 0.24	N.S
3 <sup>rd</sup>	0.01 $\pm$ 0.22c	0.03 $\pm$ 0.33a	0.01 $\pm$ 0.22c	0.02 $\pm$ 0.27b	*
4 <sup>th</sup>	0.01 $\pm$ 0.26c	0.03 $\pm$ 0.42a	0.01 $\pm$ 0.23d	0.03 $\pm$ 0.33b	*
5 <sup>th</sup>	0.01 $\pm$ 0.23c	0.03 $\pm$ 0.53a	0.01 $\pm$ 0.21c	0.02 $\pm$ 0.43b	*
6 <sup>th</sup>	0.01 $\pm$ 0.24c	0.01 $\pm$ 0.64a	0.01 $\pm$ 0.23c	0.03 $\pm$ 0.53b	*
7 <sup>th</sup>	0.01 $\pm$ 0.22c	0.03 $\pm$ 0.71a	0.01 $\pm$ 0.21d	0.01 $\pm$ 0.67b	*
8 <sup>th</sup>	0.02 $\pm$ 0.23c	0.06 $\pm$ 0.78a	0.01 $\pm$ 0.23c	0.03 $\pm$ 0.73b	*

\* Different letters within a column means significant differences at the level ( $p \leq 0.05$ ) between treatments, N.S: There were no significant differences between the experimental treatments, T1 - The first treatment (3) Rams aged 1.5 year, a control treatment without dosing the pomegranate peel. T2 -The second treatment (3) Rams aged 1.5 year , 100 gm of pomegranate peels were dosed. T3-The third treatment (3) Rams aged 3< year , a control treatment without dosing the pomegranate peels. T4-The fourth treatment (3) Rams aged 3< year , 100 gm of pomegranate peels were dosed.

Table (3) indicates the effect of pomegranate peel dosing at different periods of collection on the level of testosterone hormone in the blood serum of Arabi rams. There is a significant difference ( $p \leq 0.05$ ) between the experimental treatments during the collection periods. in the first collection there is no significant difference between the treatments, while in the second collection treatment exceeded the first treatment on T2 and T4 and it did not differ significantly with T3, and no significant difference was observed between the two treatments (T2, T3). in the third collection, no significant difference was observed between the experimental treatments, but in the fourth collection, the T2 exceeded T1, T3, T4, on the fifth

collection, there were no significant differences between the experimental treatments, while the sixth and seventh collection the treatment T2 exceed T1, T3, T4 treatments, in the eighth collection the treatment T2 exceed on T4, T3, T1 treatments and there was no a difference between the two treatments . The reason for the high testosterone concentration maybe due to that is flavonoid extract of pomegranate peels activates the mechanism responsible of hormone synthesis in Leydig cells (AL-Saeed and Hadi, 2015). Atilgan et al. (2014) suggest and uint the increase in testosterone maybe due to the bioactive compounds in pomegranate extract, which increases the production of natural antioxidants in the testicles, which can increase not only the production of sperm, but the concentration of testosterone.

**Table (3). The effect of pomegranate peel dosage and the collection period on the level of testosterone hormone (ng/ml) in the blood serum of Arabi rams (Mean  $\pm$  SD)**

Collection of blood every at	Treatment				The level of significance
	T1	T2	T3	T4	
1 <sup>st</sup>	0.17 $\pm$ 1.19	0.11 $\pm$ 1.06	0.14 $\pm$ 1.18	0.13 $\pm$ 1.08	N.S
2 <sup>nd</sup>	0.10 $\pm$ 1.31a	0.11 $\pm$ 1.12b	0.05 $\pm$ 1.25ab	0.01 $\pm$ 1.00c	*
3 <sup>rd</sup>	0.10 $\pm$ 1.22	0.08 $\pm$ 1.31	0.13 $\pm$ 1.22	0.05 $\pm$ 1.15	N.S
4 <sup>th</sup>	0.03 $\pm$ 1.29b	0.07 $\pm$ 1.35a	0.03 $\pm$ 1.25b	0.03 $\pm$ 1.21c	*
5 <sup>th</sup>	0.03 $\pm$ 1.27	0.07 $\pm$ 1.38	0.16 $\pm$ 1.16	0.01 $\pm$ 1.35	N.S
6 <sup>th</sup>	0.03 $\pm$ 1.26c	0.04 $\pm$ 1.47a	0.02 $\pm$ 1.23c	0.01 $\pm$ 1.38b	*
7 <sup>th</sup>	0.20 $\pm$ 1.32bc	0.06 $\pm$ 1.72a	0.04 $\pm$ 1.27c	0.03 $\pm$ 1.44b	*
8 <sup>th</sup>	0.18 $\pm$ 1.43b	0.08 $\pm$ 1.92a	0.03 $\pm$ 1.23c	0.06 $\pm$ 1.48b	*

\* Different letters within a column means significant differences at the level ( $p \leq 0.05$ ) between treatments, N.S: There were no significant differences between the experimental treatments, T1 - The first treatment (3) Rams aged 1.5 year, a control treatment without dosing the pomegranate peel. T2 -The second treatment (3) Rams aged 1.5 year , 100 gm of pomegranate peels were dosed. T3-The third treatment (3) Rams aged 3< years , a control treatment without dosing the pomegranate peels. T4-The fourth treatment (3) Rams aged 3< years , 100 gm of pomegranate peels were dosed.

Table (4) shows the effect of pomegranate peel dosage and the collection period on the cholesterol concentration in the blood serum of the Arab rams. A significant difference ( $p \leq 0.05$ ) between the collection was observed periods, on the first collection the treatment T3 significant exceed the T1, T2 and T4 treatments. In the second collection there was significantly increase the in two treatments( T1 and T3), in which no significant difference was noticed between them and T2 and T4 treatments. As for the third collection, there was no significant difference between the experimental treatments, while the fourth collection was significantly higher than the T1, T2, and T3 treatments. In the fifth collection the treatment T3 showed a significant increase compared to T1, T2 and T4 treatments and no significant difference was observed between the treatments T1, T2, and T4. T3 treatment was significantly higher compand to other treatments in the seventh collection, In the eighth collection no significant differences were noticed between the experimental treatments. The reason of the low cholesterol concentration in the groups treated with pomegranate peel may be due to the high concentration of sex hormones such as testosterone, which cholesterol is one of the major components involved in the synthesis of this hormone . (Anoosh et al., 2010).

**Table (4) : The effect of pomegranate peel dosage and the collection period on the level of cholesterol concentration (mg/100 ml) in the blood serum of Arabi rams (mean  $\pm$  standard deviation)**

Collection of blood every at	Treatment				The level of significance
	T1	T2	T3	T4	
1 <sup>st</sup>	0.98 $\pm$ 66.13b	1.91 $\pm$ 64.00c	1.75 $\pm$ 68.40a	1.92 $\pm$ 63.51c	*
2 <sup>nd</sup>	0.96 $\pm$ 69.12a	2.62 $\pm$ 65.46b	0.44 $\pm$ 70.69a	3.44 $\pm$ 63.50b	*
3 <sup>rd</sup>	0.76 $\pm$ 65.51	0.41 $\pm$ 65.92	2.82 $\pm$ 65.10	2.80 $\pm$ 65.10	N.S
4 <sup>th</sup>	1.40 $\pm$ 68.84a	2.73 $\pm$ 65.74a	0.97 $\pm$ 70.61a	2.54 $\pm$ 64.23b	*
5 <sup>th</sup>	1.61 $\pm$ 66.80b	2.02 $\pm$ 64.49b	0.55 $\pm$ 69.53a	1.89 $\pm$ 64.88b	*
6 <sup>th</sup>	1.04 $\pm$ 66.13a	1.59 $\pm$ 63.60b	2.54 $\pm$ 68.61a	1.94 $\pm$ 63.60b	*
7 <sup>th</sup>	1.28 $\pm$ 68.63b	0.92 $\pm$ 63.70c	1.47 $\pm$ 70.86a	0.52 $\pm$ 64.97c	*
8 <sup>th</sup>	0.73 $\pm$ 68.23	4.16 $\pm$ 65.25	0.68 $\pm$ 68.17	1.27 $\pm$ 66.26	N.S

\* Different letters within a column means significant differences at the level ( $p \leq 0.05$ ) between treatments, N.S: There were no significant differences between the experimental treatments, T1 - The first treatment (3) Rams aged 1.5 year, a control treatment without dosing the pomegranate peel. T2 -The second treatment (3) Rams aged 1.5 year , 100 gm of pomegranate peels were dosed. T3-The third treatment (3) Rams aged 3< year , a control treatment without dosing the pomegranate peels. T4-The fourth treatment (3) Rams aged 3< year , 100 gm of pomegranate peels were dosed.

Table (5) indicates the effect of pomegranate peel dosage and the collection period on the concentration of triglycerides in the blood serum of the Arabi rams. A significant ( $p \leq 0.05$ ) difference between the collection periods was noted. in the first collection , a significant decrease in the concentration of triglycerides for the two treatments T4 and T2 was shown compared to T1 and T3 treatments , In which no significant difference was noticed between them, while in the second and third collecting no significant difference was observed between the experimental treatments, and in the fourth and fifth collection , a significant decrease was found in the T4 and T2 in the concentration of triglycerides compared with T1 and T3 treatments . The sixth and seventh collection, no significant difference was observed between the experimental treatments, while the eighth collection, a significant decrease was found in T4 and T2 treatments, compared with T3 and T1 treatments. The reason for the decrease in triglycerides in the groups treated with pomegranate peels is due to the tannins (tannic and ellagic acid), which have inhibitory effect on the activity of the lipase enzyme and the absorption of fats in the

intestine and increase the exit of fats in the farces, since the fats are not absorbed by the intestine unless they are exposed to pancreatic lipase (Lei et al., 2007).

**Table (5) : The effect of pomegranate peel dosage and the collection period on the level of triglyceride concentration (mg/100 ml) in the blood serum of Arabi rams (Mean  $\pm$  SD)**

Collection of blood every at	Treatment				The level of significance
	T1	T2	T3	T4	
1 <sup>st</sup>	0.38 $\pm$ 33.06a	1.45 $\pm$ 29.90b	1.01 $\pm$ 33.82a	1.05 $\pm$ 31.63b	*
2 <sup>nd</sup>	0.47 $\pm$ 33.79	1.56 $\pm$ 31.04	0.48 $\pm$ 32.95	1.44 $\pm$ 31.89	N.S
3 <sup>rd</sup>	2.53 $\pm$ 34.44	0.85 $\pm$ 31.58	1.42 $\pm$ 34.50	0.81 $\pm$ 32.69	N.S
4 <sup>th</sup>	0.93 $\pm$ 33.53a	1.35 $\pm$ 29.87b	0.38 $\pm$ 33.40a	0.72 $\pm$ 31.99b	*
5 <sup>th</sup>	0.91 $\pm$ 33.52a	0.83 $\pm$ 31.18b	0.74 $\pm$ 34.73a	2.26 $\pm$ 31.13b	*
6 <sup>th</sup>	2.48 $\pm$ 34.39	1.46 $\pm$ 31.24	1.38 $\pm$ 34.32	0.18 $\pm$ 32.63	N.S
7 <sup>th</sup>	0.63 $\pm$ 33.74	1.64 $\pm$ 31.17	0.62 $\pm$ 33.12	1.71 $\pm$ 31.88	N.S
8 <sup>th</sup>	0.38 $\pm$ 33.23b	1.05 $\pm$ 30.11c	0.54 $\pm$ 34.33a	0.46 $\pm$ 31.24c	*

\* Different letters within a column means significant differences at the level ( $p \leq 0.05$ ) between treatments, N.S: There were no significant differences between the experimental treatments, T1 - The first treatment (3) Rams aged 1.5 year, a control treatment without dosing the pomegranate peel. T2 -The second treatment (3) Rams aged 1.5 year , 100 gm of pomegranate peels were dosed. T3-The third treatment (3) Rams aged 3< year , a control treatment without dosing the pomegranate peels. T4-The fourth treatment (3) Rams aged 3< year , 100 gm of pomegranate peels were dosed.

## Conclusions

The results showed a significant ( $p \leq 0.05$ ) increase in the concentration of sex hormones (FSH, LH and testosterone) and some biochemical parameters (cholesterol and triglycerides) in the blood serum when dosing Arabi rams with pomegranate peels.

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