ISSN: 2356-7767

Assessment of Dietary Supplementation with Pomegranate Peel Powder or Its Extract on Productive Performance and Immune Status of Rabbits

Ashgan, F. El-Sissi[*]; Bahakim, A.S.A.[**] Fatma, E. Saba[**] and Osman, A.O.[**]

*Department of Immunology, Animal Health Research Institute, Dokki, Giza, Egypt (*Corresponding authors).
**Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt

Received in 9/5/2018
Accepted in 13/6/2018

Abstract

Pomegranate peels were subjected for extracting using a mixture of ethanol, methanol and water, the nutritive value of extract was evaluated by photochemical analysis which revealed high level of total phenolic, tannins, flavonoid and antioxidant activity. To study the effects of dietary supplementation of pomegranate peel powder (PPP) or pomegranate peel extract (PPE) on growth performance and immune status of rabbits, forty-five male V-Line weaned rabbits were allocated into five groups. Rabbits in first group (G1) were fed basal diet and served as control and rabbits in 2nd and 3rd groups (G2&G3) were fed the basal diet supplemented with PPP at 1% and 1.5% respectively, and rabbits in 4th and 5th groups (G4&G5) were administered PPE at 0.14 % and 0.21% respectively for two months experimental period. All rabbits were vaccinated with rabbit viral hemorrhagic disease (RVHD) vaccine at 7th week of age. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated. Blood samples were collected to measure some immunological parameters, protein profile, serum malondialdehyde (MDA) and catalase activity. The results showed that, low ratio of PPP or PPE significantly increased BWG and improved FCR and could enhance both innate and humeral immunity of rabbits as they significantly increased ($p<0.05$) phagocytic activity of peripheral blood mononuclear cell, serum lysozyme activity, total protein (TP), antibody titer against RVHD, immunoglobulin G (IgG) values, as well as improve oxidant /antioxidant balance. However, these effects were diminished in rabbit's feed high ratio of PPP or PPE, so it could be necessary to adjust the dosage for growing rabbits. In conclusion, Pomegranate peels powder (1%) or its extracts (0.14%) could strengthen the immune system which provides health benefits to V-line growing rabbits and may be employed as dietary supplement to growing rabbits.

Key world: *Rabbit- Pomegranate peels- feed conversion ratio- phagocytic activity– RVHD antibody.*

Introduction

Weaning is the most cretical period for young animals that associated with high incidence of gastrointestinal problems which represent the major causes of collapses in rabbits breeding farms. Mortality rate was about half-higher during two weeks after weaning compared to the day 49th to slaughtering period **(Gidenne and Garcia 2006).** Mortality was always caused by acute digestive disorders, without any identification of a specific pathology at autopsy, which varied between 1.7 to 19.4% **(Gidenne *et al.,* 2005).** It additionally reported that, mortality is reduced by using phytogenic flavors because of optimization of the immune system **(Fortun and Boullier, 2007).**

Pomegranate peels are considered inedible elements or by-product obtained through juice process. It is important source of phenolics, flavonoid, minerals, complex polysaccharides

and hydrolysable tannins **(Seeram *et al.,* 2005).** In addition, different parts of the pomegranate fruit, especially the peel, could act as potential antimicrobial agent and so could be proposed as a safe natural alternative to synthetic antimicrobial agents. The high tannin content, particularly punicalagin, found in pomegranate extracts, has been reported as the main compound responsible for such antimicrobial activity **(Rosas-Burgos *et al.,* 2017).** The studies of **Li *et al.,* (2006)** demonstrated higher antioxidant capability of the peels relative to the juice, mainly because of water-soluble polyphenols, and hydrolysable tannins. The antioxidant of pomegranate extract might improve immune perform **(Shabtay *et al.,* 2008).**

There is limited research on the utilization of pomegranates in animal feeding. Even so, it's been shown that, once pomegranate fruits rind powder fed to rabbits at 100 mg /kg body weight, stimulation in the cell-mediated and humeral immune response was ascertained **(Gracious *et al.,* 2001). Shabtay *et al.,* (2008)** recorded that, dietary supplementation of wet fresh pomegranate peels (PP) increased weight gain in bull calves and suggested that, antioxidant and immunomodulatory properties of PP might improve immune function. Moreover, supplementing pomegranate extracts at 5 or 10 g /day to preweaned calves improve immune response and health due to their polyphenols content **(Oliveira *et al.* 2010).** Also, dietary pomegranate by-products improved immunity and the intestinal microbial ecosystem of broilers **(Ahmed and Yang, 2017).**

Nowadays researchers give special attention to the utilization of food industry-derived residues, either for the reduction of the environmental harm and consequent reduction of costs to the treatment process or for their potential use in the development of high value-added product **(Garau *et al.* 2007).** The immune system provides a biological marker to evaluate the potential health benefits of dietary supplements in food on animal production **(Gracious *et al.,* 2001).**

The present experiment was conducted to prepare pomegranate peel extracts using mixture of solvent and evaluate their nutritive value by photochemical analysis. As well as examine the effects of dietary supplementation of pomegranate peel in two different application forms (powder or extract) in low & high doses on growth performance and immune status of V-line growing rabbits.

Materials and Methods

This experiment was conducted in the rabbitry of El-Gemeza research station, El-Gharbia Governorate, Animal Production Research Institute, Agricultural Research Center, Ministry of Agricultural, Egypt.

1-Preparation of pomegranate peel extracts

Pomegranates were obtained from local market; the peels were manually removed; dried at 45°C for 3 days and then powdered. The powder was extracted with mixture of ethanol, methanol and water in equal ratio for 24 h at 25°C according to **Li *et al.,* (2006).** The crude extract was sieved through What man No.1 paper. The filtrates were concentrated under reduced pressure with a rotary evaporator at 50°C, and then kept at 4°C until use.

2- Photochemical analysis of pomegranate peel extracts:

2.1- Total phenol content (TPC): The concentrations of total phenolics (TP) in PPE were determined by the Folin-Ciocalteu colorimetric method of **Singh *et al.,* (2002),** the TP content expressed as mg gallic acid equivalents (GAE) per g of extract weight.

2.2- Estimation of Total Tannins: The concentrations of total tannin (TT) in PPE were determined by the vanillin assay of **Sun *et al.,* (1998).** The results were expressed as mg catechin equivalent (CE) per g of extract weight.

2.3- Estimation of Total Flavonoid Content: The concentrations of Flavonoid content in PPE were determined by the aluminum chloride colorimetric method of **Woisky and Salatino (1998)**.

2.4- Antioxidant activity: The free radical scavenging properties of pomegranate peel extract were evaluated by spectrophotometric

method using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH) according to **Zhao *et al.*, (2006).** DPPH radical scavenging effect was calculated according to following equation:

% scavenging rate = [1-(A1 -A2)/ A0] x 100

(Where A0 represents the absorbance of the control (DPPH solution) and A1 represents the absorbance of extract, A2 represents the absorbance without DPPH).

3-Experimental design: Forty-five weaned V-line male rabbits, aged 5 weeks and averaged 850gm body weight were allocated randomly into five groups each of nine rabbits with three replicates. The rabbits of 1st group (G1) fed a basal diet and served as control. The rabbits in G2 and G3 were fed the basal diet supplemented with 1.0 and 1.5% PPP respectively, and those of G4 and G5 were administered PPE at 0.14 or 0.21% of diet respectively, spray over basal diet (corresponding 1% & 1.5% of powder). Feed and water were provided ad-libitum during two months experimental period. The basal diet was formulated to satisfy **NRC (1977)** recommend (Table 1), all rabbits were vaccinated with RVHD vaccine at 7th week of age.

4-Samples:

-Serum samples were collected from ear vein,9 samples/ group, for detection of lysozyme activity, nitric oxide (NO), IgG, T. protein, albumen, MDA value and catalase activity at 9th, 11th and 13th weeks of age. Also, for detection of antibodies titer against RVHD vaccine at 0 , 2nd, 4th, 6th weeks post vaccination (7th, 9th, 11th and 13th weeks of age).

-Heparinized blood was collected (6 samples/ group) for phagocytosis assay one-week post vaccination with RVHD (8th weeks of age).

5-Performance traits:

Individual live body weight (LBW) was recorded at 5weeks of age and at the end of experiment. As well as total feed intake (TFI) was recorded, then body weight gain (BWG) and FCR (gm of feed consumed / gm weight gain) were calculated.

Table (1). Composition and chemical analysis (%) of the experimental diets for growth rabbits.

Ingredients	Pomegranate in the experimental diet (%)				
	Basal diet	**PPP 1%**	**PPP 1.5%**	**PPE 0.14%**	**PPE 0.21%**
Clover hay (12% CP)	32.75	32.00	31.75	32.75	32.75
Yellow corn	17.80	17.80	17.80	17.80	17.80
Soybean meal (44%)	15.20	15.20	15.20	15.20	15.20
Wheat bran	11.00	10.75	10.50	11.00	11.00
Barley grain	17.30	17.20	17.15	17.30	17.30
Pomegranate peel	00.00	1.00	1.50	00.00	00.00
Molasses	3.00	3.00	3.00	3.00	3.00
Limestone	0.4	0.4	0.4	0.4	0.4
Salt	0.35	0.35	0.35	0.35	0.35
Dl- Methionine	0.20	0.20	0.20	0.20	0.20
L. Lysin	0.10	0.10	0.10	0.10	0.10
Di-calcium-Phosphate	1.60	1.75	1.75	1.60	1.60
Premix (vit. +Min.Mix	0.30	0.30	0.30	0.30	0.30
Total	100.00	**100.00**	100.00	100.00	100.00
Chemical analysis: Dry Matter	90.05	90.08	90.09	90.05	90.05
Organic Matter	91.52	91.84	91.84	91.52	91.52
Crude protein	16.07	16.02	16.00	16.07	16.07
Ether extract	3.47	3.44	3.44	3.47	3.47
Crude fiber	13.49	13.51	13.52	13.49	13.49
Nitrogen Free Extract	58.53	58.51	58.49	58.53	58.53
NDF	37.76	37.81	37.81	37.76	37.76
Ash	8.48	8.50	8.50	8.48	8.48
DE (kcal/kg)	2516	2509	2509	2516	2516

PPP = Pomegranate Peel powder **PPE** = Pomegranate Peel extracted

6-Evaluation of innate immune response:

6.1- Phagocytic activity:

Phagocytic activity of peripheral blood mononuclear cell was determined using *Candida Albicans*, according to **Chu and Dietert, (1989).** The phagocytic percent (number of phagocytic macrophages /total no of macrophages) and phagocytic index (number of macrophages engulf ≥3 Candida spores/total no of macrophages) were calculated.

6.2- Lysozyme Assay:

Lysozyme activity was measured by agarose gel plate lyses assay. Briefly, lysoplates were prepared by dissolving 1% agarose in PBS (0.067mol, pH6.3) in which *Micrococcus lysodeikticus* (50 mg/100 ml agarose) had been dispersed. Then 25 µl of serum samples and standard lysozyme were added into each well. After 18 hours, the cleared zones diameter was measured. The concentration of lysozyme obtained from logarithmic curve of standard lysozyme **(Peeters and Vantrappen 1977).**

6.3- Nitric oxide assay:

It carried out according to **Yang *et al.* (2010)** by Griess reagent after deproteinization of serum and reduction of nitrate to nitrite using Cu plated Cd. The optical density was determined at 545 nm with an ELISA plate reader. Nitric oxide concentration was calculated from standard curve using NaNO2.

7-Evaluation of humeral immune response:

7.1- Detection of Antibodies to RHDV:

Antibodies to RHDV vaccine were measured by using Hemagglutination inhibition test (HI) according to **Park *et al.,* (1991)** used 4HA units of RHDV and 0.5% human type O red blood cell. The HI titer of the serum is the highest dilution causing complete inhibition of Hemagglutination of 4 units of RHDV.

7.2- Detection of rabbit immunoglobulin:

Serum IgG were measure using ELISA kit of Glory Science CO., Ltd, Del Rio., TX 78810, USA according to the manufacturer`s instructions

8- Estimation of protein profile:

Total protein and albumin were measured using commercial diagnostic kits of Bio-Analytics kit, USA following the manufacturer's instructions according to **Bakerman (1984) and Doumas and Biggs (1972)** respectively. While serum globulins were determined by subtracting the value of albumin from the serum total proteins.

9– Catalase and Malondialdehyde assay:

Catalase activity was measured according to **Aebi (1983),** where the rate of 10 Mm hydrogen peroxide (H_2O_2) decomposition was followed by monitoring absorption at 240 nm in 50 Mm PBS pH 7 at a decrease of 0.05 in optical density corresponds to 3.45µ mole of H_2O_2 and one unit of catalase will decompose 1µ mole H_2O_2/min. Malondialdehyde was measured using commercial diagnostic kits according to **Ohkawa *et al.,* (1979)** following the manufacturer`s instructions.

10- Statistical analysis:

Data obtained were statistically analyzed using analysis of variance and comparing between groups were performed using least significant difference (LSD) at $P<0.05$ according to **Petrie and Watson (1999)** and using SPSS.

Results

The pomegranate peels were extracted with mixture of solvents. Yield % and photochemical analysis of pomegranate peel extracts were shown in (Table 2).

Regarding to growth performance of growing rabbits, there were significant decrease of TFI with rabbits fed on high ratio of PPP (G3) & PPE (5) comparing with control group (G1). Rabbits fed on low ratio of PPP (G2) & PPE (G5) showed significant increase of BWG and significant decrease of FCR comparing with control group (G1) (Table3).

Table (2). Yield % and photochemical analysis of pomegranate peel extracts:

Yield %	DPPH			Total Phenolics mg GAE/gm	Total tannin mg CE/gm	Total flavoniod mg /gm
	25 µg/ml	50 µg/ml	75 µg/ml			
14.3 %	65.4%	73.4%	79.1%	278.5	235	28.8

Table (3). Effect of dietary supplementation of pomegranate peel powder or pomegranate peels extract (high and low ratio) on growth performance of growing rabbits:

Parameters / Group	Initial Body Weight (IBW)(gm)	Final Body Weight (FBW) (gm)	Body Weight Gain (BWG) (gm))	Total Feed Intake (TFI)(gm)	Feed/ Conversion Ratio (FCR)
(G1) Control	847±56.66	2118±57	1217±21A	4420±67.67A	3.63±.04A
(G2) PPP 1%	854±37.42	2135±15	1281±33.31aB	4305±15.87B	3.36±.07Ba
(G3) PPP 1.5%	855±27.41	2024±26A	1169±22.94Cb	4120±59.00Cba	3.52±.07C
(G4) PPE 0.14%	864±64.96	2183±54Ba	1319±20.01aDc	4314±33.32Dbc	3.27±.03ac
(G5) PPE 0.21%	846±23.86	2057±29b	1211±37.47d	4144±41.0dba	3.42±.12

-Values represent means (n =9) ± SE
- Mean with small litters indicate significantly different against the capital of the same litter in the same column (P<0.05)

Regarding phagocytic activity (Photo1 & Table 4), there were significant increase ($P < 0.05$) in phagocytic % of peripheral blood mononuclear cell of rabbits feed low concentration of PPE (G4), also there were significant increase ($P < 0.05$) in phagocytic index in rabbit of G2 and G4 at 8th weeks of age comparing with control group. The lysozyme activity recorded significant decrease in serum of rabbits in (G2) at 9th week, then lysozyme activity tended to increase toward the end of experiment in all groups, with significant increase in rabbits of G3 and G4 at 11th week and in rabbits of G2, G3 and G4 at 13th week of age. There was no significant change in Nitric oxide values in all tested groups comparing with control group, while the mortality rate have been decrease in all treated groups comparing with control group.

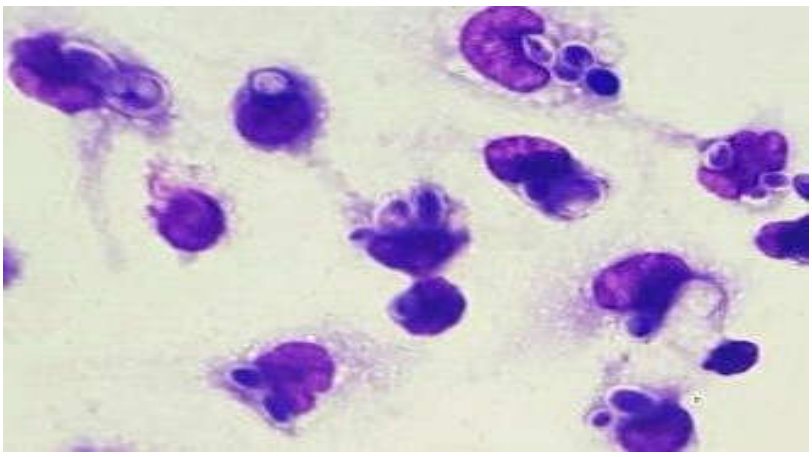

Photo (1). Phagocytic activity of Peripheral blood mononuclear cell showing phagocytic cells engulf Candida spores. (Giemsa stain×100)

Table (4). Effects of dietary supplementation of pomegranate peel powder (PPP) or pomegranate peel extract (PPE) on mortality rate and innate immune response .

Parameters	Age	G1 (control)	G2 PPP (1 %)	G3 PPP (1.5 %)	(G4) PPE (0.14%)	(G5) PPE (0.21%)
Mortality rate		33.3%	11.1%	22.2%	0%	11.1
Phagocytic %	**8thweek**	54±3.45A	56.6±2.29	53.8±1.98	59.4±2.11a	55.4±3.01
Phagocytic index	**8thweek**	0.31±0.05A	0.37±0.03a	0.34±0.04	0.42±0.06a	0.33±0.03
Lysozyme (µg/ml)	**9thweek**	275±16A	212±17aB	251±46	269±27b	231±49
	11thweek	297±26 A	260±31 B	349±33 ab	396±29 abD	270± 28 d
	13thweek	352±28 A	426±33 a	446±17 aC	458±24 abd	345±14 cD
Nitric oxide (µmol/ml)	**9thweek**	11.98±0.15	11.62±0.11	11.31±0.36	9.42±0.67	9.74±0.88
	11thweek	10.10±0.48	10.53±0.44	12.12±0.83	9.34±1.16	11.66±0.56
	13thweek	10.39±0.53	9.14±0.92	7.76±0.73	7.32±0.93	9.29±0.52

-Values represent means (n =9) ± SE
- Mean with small litters indicate significantly different against the capital of the same litter in the same row (P<0.05).

Concerning proteins profile (Table 5), rabbit in G2&G4 recoded significant increases (P< 0.05) in T. proteins which contributed to increase in albumin at 9th week in G2 and at 9th and 11th weeks in G4 comparing with control group. In contrast, rabbit in G3&G5 showed significant decrease in T. proteins that contributed to significant decrease in albumin and globulin at 13th week of age comparing with control group.

Table (5). Effects of dietary supplementation of pomegranate peel powder (PPP) or pomegranate peel extract (PPE) on protein profile.

Parameters	Age	G1 (control)	G2 PPP (1 %)	G3 PPP (1.5 %)	G2 PPP (0.14 %)	(G5) PPE (0.21%)
Total protein (gm/dL)	**9th week**	6.20±0.13 A	6.83±0.14 aB	6.12±0.22 bC	6.98±0.11 acD	6.34±0.10 bd
	11th week	6.39±0.34 A	6.43±0.12 B	6.22±0.23 C	7.12±0.14abcD	5.90±0.16 abd
	13th week	6.45±0.08 A	6.30±0.18 B	5.67±0.06 abC	6.64±0.07 cD	5.52±0.23 abd
Albumen (gm/dL)	**9th week**	3.55±0.05 A	3.96±0.16 aB	3.42±0.05 bC	3.93±0.11 ac	3.67±0.24 b
	11th week	3.57±0.19 A	3.63±0.05 B	3.47±0.09 C	3.75±0.08 cD	3.28±0.10 abd
	13th week	3.63±0.10 A	3.45±0.08 B	3.30±0.08 aC	3.77±0.07 bcD	3.31±0.09 ad
Globulin (gm/dl)	**9th week**	2.66±0.10 A	2.87±0.06	2.70±0.21 B	3.06±0.11	2.67±0.11
	11th week	2.81±0.30	2.80±0.12	2.75±0.24	3.37±0.19 A	2.62±0.20
	13th week	2.83±0.14 A	2.84±0.22 B	2.37±0.10 abC	2.87±0.07 c	2.21±0.17a
A/G ratio	**9th week**	1.34±0.05	1.38±0.07	1.30±0.10	1.29±0.07	1.38±0.07
	11th week	1.34±0.17	1.30±.06	1.31±0.12	1.15±0.10 A	1.56±0.14 a
	13th week	1.31±0.10	1.25±0.12	1.41±0.09	1.31±0.05	1.27±0.07

Values represent means (n =9) ± SE
- Mean with small litters indicate significantly different against the capital of the same litter in the same row (P<0.05).

Concerning the humeral immune response (figures 1&2) there were significant increase in HI antibody titers against RHDV vaccine at 4th and 6th weeks post vaccination for rabbit feed low concentration of PPP (G2) and PPE (G4) comparing with those of control group (G1), the antibody titer was diminished in rabbit's groups feed high concentration of PPP (G3)& PPE(G5), although it was better comparing to control group (G1).

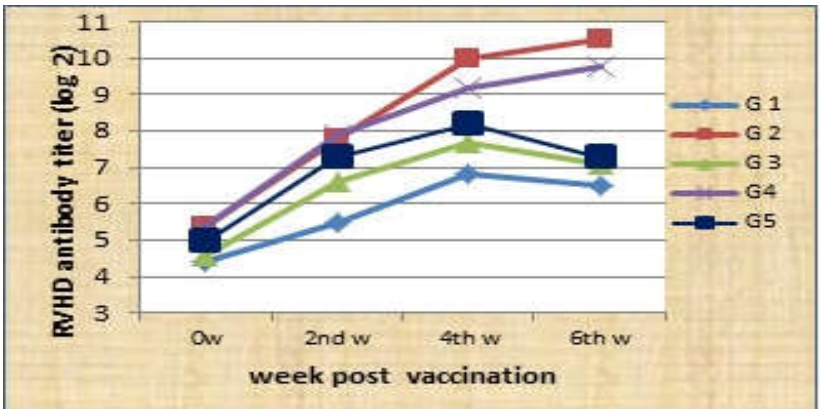

Figure (1). Effect of dietary supplementation of pomegranate peel powder (PPP) or pomegranate peel extract (PPE) on antibody titer against RVHD vaccine.

Regarding to IgG values, there were significant increase ($P < 0.05$) in IgG of rabbit feed low concentration of PPP at 10th& 12th weeks of age and in rabbit feed low concentration of PPE at 8th and 10th weeks of age. On the contrary, rabbits feed high concentration of PPP and PPE showed significant decrease of IgG at 12th weeks of age

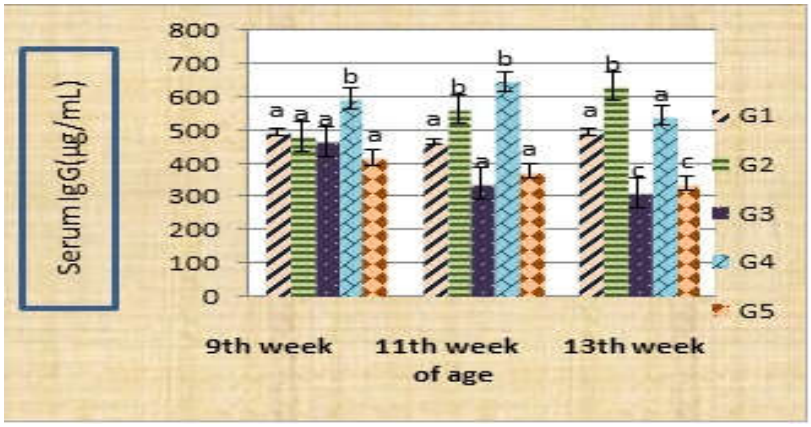

Figure (2). Effect of dietary supplementation of pomegranate peel powder (PPP) or pomegranate peel extract (PPE) on serum IgG (µg/ml).

As shown in Table (6), there was significant decrease ($P < 0.05$) in serum MDA in all treated groups at 9th week of age and significant increase in catalase activity in all treated groups at 9th week of age and in G2, G3 and G5 at 10th week of age.

Table (6). Effect of dietary supplementation of pomegranate peel powder or pomegranate peel extract on Malondialdehyde and Catalase activity of growing rabbits.

Parameters	Age	G1 (control)	G2 PPP (1 %)	G3 PPP (1.5 %)	(G4) PPE (0.14)	(G5) PPE(0.21%)
MDA (nmol/dl)	9th week	5.97±0.59 A	4.36±.11a	4.18±.23a	3.79±.05a	3.42±.28a
	11th week	5.18±0.31A	4.35±.24	4.42±.11	4.39±.95	4.31±.35
	13th week	5.13±0.24A	4.52±.18	4.89±.59	4.26±.46	4.57±.39
Cataleas (nmol/dl)	9th week	6.56 ± 0.19 A	8.97±0.71aB	7.90 ± 0.39 a bC	8.71±0.75ac	8.80 ± 0.20 ac
	11th week	7.39±0.87 A	8.52±0.70 a	9.68 ± 0.87a	8.15 ± 0.54	9.10±0.52a
	13th week	9.38 ± 1.16	10.04±1.01	11.04 ± 0.30	11.14 ± 0.98	10.97 ± 0.79

-Values represent means (n =9) ± SE
- Mean with small litters indicate significantly different against the capital of the same litter in the same row ($P<0.05$)

Discussion

The pomegranate peels were extracted with mixture of solvents, the yield % was 14.3 %. An approximate value, 16.3, had been found by **Malviya *et al.* (2014).** However, **Li *et al.,* (2006)** obtained 31.5%.

Photochemical analysis showed that, PPE contain high ratio of phenolic compounds, mainly tannins and much less of flavoniod as well as excessive antioxidant activity. These results are supported with previous studies recorded that, the major phenolics in PPE were tannins **(Madrigal-Carballo *et al.,* 2009)** and the minor was flavoniod **(Shiban *et al.,* 2012).** Also, **Khan *et al.,* (2017)** recorded that, aqueous suspension of PPP has shown strong anti-oxidant activity. In general, the potent antioxidant activity of pomegranate is attributed to its polyphenols includes flavonoids as well as condensed and hydrolysable tannin **(Jayaprakash, 2017).**

Regarding the growth performance, high PPP and PPE levels in diet of rabbit have significant negative effect on feed intake in comparing with control group. These results agree with those of **Oliveira *et al.,* (2010)** who recorded that feeding pomegranate extract reduced feed intake. It was known that tannins present at high concentration in pomegranate peel that may inhibited feed intake, attributed to the astringency sensation caused by the formation of complexes between tannins and salivary glycoproteins **(Oliveira, *et al.,* 2010).** In the same regard, **Makled *et al.* (2003)** reported that bucks fed 0.50 and 0. 25% tannic acid consumed less ($P<0.01$) amount of feed than the control group. Rabbits fed on diet supplemented with low ratio of PPP & PPE showed significant increase in BWG resulting in significantly improve of FCR. The obtained results agree with **Nassralla *et al.,* (2016).** In the same regard, Kara **(2016)** recorded that; condensed tannins (CT) have both positive (in low levels) and negative (in high level) effects on rabbit's performance.

Reduction of mortality rate among treated groups in this study, may be due to the antibacterial effects of pomegranate **(Rosas-Burgos *et al.,* 2017)** as the limit on bacterial infections mainly E. coli, have an important role in reducing mortality or may be due to optimization of immune system **(Fortun and Boullier, 2007).**

Related to innate immune responses, low ratio of PPP (G2) and PPE (G4) exhibited significant increase of phagocytic activity of peripheral blood mononuclear cell and in serum lysozyme activity, which reflected improvement of immune system. The increase of lysozyme toward the end of experiment in all groups may be because of pathogen detection by innate system, which has prompted phagocytic cell to safeguard rabbit against invading microorganisms or also because of intensive housing condition that cause environmental stress with the consequence of continuous inflammatory response **(Moscati *et al.,* 2008).** The obtained results are supported by **Harikrishnan *et al.,* (2010)** who suggest that intraperitoneal administration of the leaf extracts of P. granatum clearly enhance the innate immune responses and disease resistance in Paralichthysolivaceus. The increased values of lysozyme have been previously recorded after activation of the immune system with immunomodulants **(Saurabh & Sahoo, 2008).** Also, it was recorded that PPE supplementation increase of lysozymes values on broilers **(Kishawy *et al.,* 2016).** While the decrease of lysozyme activity at starting of the experiment, may be due to suppress growth of pathogenic bacteria by antibacterial effects of PP **(Benossi, *et al.,* 2015).**

Serum TP is an important immunological and health indicator. However low PPP and PPE levels in diet of rabbit resulted in significant increase in TP, rabbit feed high ratio of PPP and PPE for 2 months showed significant decrease ($P < 0.05$) in TP at 13th week of age. In the same regard, **Worku *et al.,* (2016)** determine a slight decrease in TP in goats whereas **Oni *et al.,* (2012)** recorded increase in TP in sheep feed leaves of tannin containing plants. The decrease of TP might attribute partially to the presence of tannins in pomegranate peel more than tolerable limits, as tannins could bind with dietary protein by multiple phenolic hydroxyl groups, preventing its absorption and utilization **(Schofield *et al.,* 2001).**

Regarding to humeral immune responses of rabbits under the treatment of low ratio of PPP (G2) and PPE (G4) exhibited significant increase in antibody titer against RVHD vaccine and IgG values, the antibody titer was diminished in rabbit's groups feed high ratio of PPP and PPE, however still higher than that of control, whereas IgG values were significantly decrease comparing to control group. The obtained results are supported by Gracious et al. (2001), who found that pomegranate fruit rind powder orally recorded a rise in antibody titer to typhoid-H antigen. Also, **Oliveira *et al.,* (2010)** recorded that, pomegranate polyphenols improved total IgG responses to ovalbumin vaccination. In addition, **Laily *et al.,* (2016)** recorded that, pomegranate could improve immunity status of mice associate with an emergency situation. The reverse results of immune responds with high ratio of PPP and PPE is perhaps due to high levels of tannin that cause decrease of TP recorded in present study. A deficiency of protein or amino acids has long been known to impair immune function, as amino acids like arginine, glutamine and cysteine have important role in immune response by activation of lymphocytes and macrophages, control gene expression and synthesis of specific proteins as cytokines, antibodies and cytotoxic substances **(Li *et al.,* 2007).**

Weaning is the most critical period of rabbit's life (**Gidenne and Garcia, 2006),** as rabbits subject to a lot of stress post weaning, which can disturb oxidant /antioxidant balance. In present study, all treated groups showed significant decrease of MDA along with significant increase in catalase activity comparing with control group, but only at first growth period. This was explained by the fact that, feeding rabbits with diet supplemented with PPP or PPE proven to have high antioxidant activity might maintain oxidant /antioxidant balance. In the same regard, **Aboonabi *et al.* (2014)** showed that pomegranate reduced lipid peroxidation and improved antioxidant enzymatic status. The inhibition of lipid peroxidation was related to the ability of phenolic and flavonoid compounds, of P. *granatum,* to inhibit oxida-

tive enzymes **(Chidambara-Murthy *et al.*, 2002).**

In conclusion: PPE has shown good antioxidant activity, attributed to presence of huge quantity of polyphenolic compounds (flavenoid & tannin). Dietary supplementation of PP in two different form (powder or extract) in low dose to V-line growing rabbits maintain health status by reducing digestive disorders and related mortality and improve growth performance characteristics (BWG & FCR). Also clearly enhance both innate and humeral immunity (phagocytic activity of peripheral blood mononuclear cell, lysozyme activity , IgG value and the antibody titer against RVHDV), as well as maintain oxidant/ antioxidant balance, this effect were diminish with high dose, thus probably, it could be necessary to adjust the indefinite quantity for growing rabbits by considering the tannin content .Also it was notice that rabbits feed on PPE in low concentration gave the best results.

References

Aboonabi, A. and Rahmat, A.F. (2014). Antioxidant effect of pomegranate against streptozotocin-nicotinamide generated oxidative stress induced diabetic rats. Troxicol Rep.; 1: 915–22.

Aebi, H. (1983). Catalase In : Bermeyer, H. (Ed), "methods of Enzymatic Analysis "Verlag-Chemie, Weiunhein, pp. 273-286.

Ahmed S.T. and Yang, C. (2017). Effects of Dietary Punicagranatum L. By-products on Performance, Immunity, Intestinal and Fecal Microbiology, and Odorous Gas Emissions from Excreta in Broilers J. Poult. Sci., 54: 157-166.

Bakerman, A.S. (1984). "A colorimetric method of the determination of serum total protein. "ABCs of interpretive laboratory DATA, 2nd ED., Griffen and Tilghman, 374.

Benossi-Marchi, L.; Monteiro, A.R.; Mikcha, J.; Santos, A.; Chinelatto, M.; Marques, D. and Costa, S.C. (2015). Evaluation of antioxidant and antimicrobial capacity of pomegranate peel extract (punicagranatuml.) under different drying temperatures, Chemical Engineering Transactions, 44, 121-126.

Chidambara-Murthy, K.N.; Jayaprakasha, G.K. and Singh, R.P. (2002). Studies on antioxidant activity of pomegranate peel extract using in vivo models. J. Agric. Food Chem., 50: 4791-4795.

Chu, Y. and Dietert, R.R. (1989). Monocyte function in chicken with hereditary dystrophy.Poultry Science,68:226-232

Doumas, B.T. and Biggs, H.G. (1972). "A Colorimetric Method of the Determination of Serum Albumin."Standard Methods of Clinical Chemistry, 7, Academic press, New York.

Fortun, L. and S. Boullier (2007). A review on the interactions between gut microflora and digestive mucosal immunity. Possible ways to improve the health of rabbits. Live stoke Science; 107: 1-18.

Garau, M.C.; Simal, S.; Rosselló, C. and Femenia, A. (2007). Effect of air-drying temperature on physicochemical properties of dietary fibre and antioxidant capacity of orange (Citrus aurantiumv. Canoneta) by-products. Food Chemistry, 104, 1014-1024

Gidenne, T. and Garcia, J. (2006). Nutritional strategies improving the digestive health of the weaned rabbit. In: Maertens L., Coudert P (eds). Recent Advances in Rabbit Sciences. ILVO, Animal Science United, Melle- Belgium; 229-238.

Gidenne, T.; Jehl, N.; Perez, J.M.; Arveux, P.; Bourdillon, A.; Mousset, J.L.; Duperray, J.; Stephan, S. and Lamboley, B. (2005). Effect of cereal sources and processing in diets for the growing rabbit. II. Effects on performances and mortality by enteropathy. Animal Research. 54: 65-72.

Gracious, R.R.; Selvasubramanian, S. and Jayasundar, S. (2001). Immunomodulatory activity of Punicagranatum in rabbits A preliminary study. J. Ethnopharmacol. 78, 85–87.

Harikrishnan, R.; Heo, J.; Balasundaram, C.; Kim, M.C.; Kim, J.S.; Han, Y. and Heo, J. (2010). Effect of Punicagranatum

solvent extracts on immune system and disease resistance in Paralichthysolivaceus against lymphocystis disease virus (LDV) Fish and Shellfish Immunology 29, 668–673

Jayaprakash, A. (2017). Punicagranatum: A Review on Phytochemicals, Antioxidant and Antimicrobial Properties. Journal of Academia and Industrial Research V 59 Feb. 132-138.

Kara, K. (2016). Effect of dietary fiber and condensed tannins concentration from various fibrous feedstuffs on vitro gas production kinetics with rabbit faecal inoculum Journal of Animal and Feed Sciences, 25: 266–272.

Khan, S.; Patel, A. and Bhise, K.S. (2017). peel Antioxidant activity of pomegranate powder Journal of Drug Delivery & Therapeutics.; 7(2): 81-84.

Kishawy, T.A.; Omar, A.E. and Gomaa, A.M. (2016). Growth performance and immunity of broilers fed rancid oil diets that supplemented with pomegranate peel extract and sage oil Japanese Journal of Veterinary Research 64 (Supplement 2): S31-38.

Laily, N.; Harahap, A.R.; Aji, G.K.; Sukarti, I.; Ascobat, P. and Wijayanti, R.D.E. (2016). Potential Use of Pomegranate (Punicagranatum) Extract as an Immune -Stimulant Based on in vitro and in vivo Models Mal J Nutr, 22(2): 279- 287.

Li, Y.; Guo, C.; Yang, J.; Wei, J.; Xu, J. and Cheng, S. (2006). Evaluation of antioxidant properties of PPE in comparison with pomegranate pulp extract. Food Chemistry. 96: 254-260.

Li, P.; Yin, Y.L.; Li, D.; Kim, S.W. and Guoyao, W.U. (2007). Review Article Amino acids and immune function. British Journal of Nutrition, 98: 237–252

Madrigal-Carballo, Rodriguez, G.; Krueger, C.; Dreher, M. and Reed, J. (2009). "Pomegranate (Punicagranatum) Sup- plements: Authenticity, Antioxidant and Polyphenols Com- position," Journal of Functional Foods, Vol. 1, No. 3, pp. 324-329.

Makled, M.N.; Sharara, H.H. and Abd El-Fattah, H. (2003). Tolerance of California rabbits to different levels of dietary tannic acid. Egyptian Journal of Poultry Science. 23 (III): 641-654.

Malviya, S.; Arvind, AlokJha and Navam-Hettiarachchy (2014). Antioxidant and antibacterial potential of pomegranate peel extracts. J Food Sci. Technol, 51(12): 4132–4137.

Moscati, L.; Dal, Bosco A.; Battistacci, L.; Cardinali, R.; Mugnai, C. and Castellini, C. (2008). Native immunity and oxidative traits of growing rabbits World Rabbit Sci., 16: 213 - 220.

Nassralla, M.M.; Fatma, E.S. and Abo-Wardah (2016). Effect of Feeding Pomegranate (Punicagranatum L.) Peels and It's Extract on growth performance and carcass characteristics of growing v-line male rabbits. Egyptian J. Nutrition and feeds, 19(3): 463-472.

NRC (1977). The Nutrient requirements of rabbits. National Research Council, second revised edition Washington DC., US

Ohkawa, H.; Ohishui, N. and Yagi, K. (1979). Assay for lipid peroxides in animals' tissues by thiobarbituric acid reaction. Anal Biochem, 95: 351-358.

Oliveira, R.A.; Narciso, C.D.; Bisinotto, R.S.; Perdomo, M.C.; Ballou, M.A.; Dreher, M. and Santos, J.E.P. (2010). Effects of feeding polyphenols from pomegranate extract on health, growth, nutrient digestion, and immune-competence of calves. Dairy Science, 93: 4280-4291.

Oni, A.O.; Arigbede, O.M.; Sowande, O.S.; Anele, U.Y.; Onwuka, C.I. and Aderinboye, R.Y. (2012). Haematological and serum biochemical parameters of West African Dwarf goats fed dried cassava leaves-based concentrate diets. Tropical Animal Health and Production Vol. 44: 483–490.

Park, J.H.; Kida, H.; Ueda, K. and Gorgo, M. (1991). "Etiology of rabbit haemorrhagic disease spontaneously occurring in Korea. J. Vet. Med., B, 38(10): 749-754.

Peeters, L. and Vantrapen, R. (1977). "Factors influencing lysozyme determination by lysoplate method." Clin. Chim.

Acta., 74: 217-255.

Petrie, A. and P. Watson (1999). Statistics for Veterinary and Animal Science. 1st Ed., PP. 90-99, the Blackwell science Ltd, United Kingdom.

Rosas-Burgos, E.C. Burgos-Hernández, A.; Noguera-Artiaga, l.; Kačaiová, hernández-Garcia, F.; Cárdenas– lópez, J.L. and Carbonell-Barrachina, Á.A. (2017). Antimicrobial activity of pomegranate peel extracts as affected by cultivar. J Sci Food Agric. Feb; 97(3): 802-810.

Saurabh, S. and Sahoo P.K. (2008). Lysozyme: an important defense molecule of fish innates immune system Aquaculture Research, 39: 223-239.

Schofield, P.; Mbugua, D.M. and Pell, A.N. (2001). Analysis of condensed tannins: a review. Anim. Feed Sci. Tech. 91, 21–40.

Seeram, N.P.; Adams, L.S.; Henning, S.M.; Niu, Y.; Zhang, Y.; Nair, M.G. and D. Heber (2005). In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin,ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J. Nutr. Biochem., 16: 360-367.

Shabtay, A.; Eitam, H.; Tadmor, Y.; Orlov, A.; Meir, A.; Weinberg, P.; Weinberg, Z.G.; Chen, Y.; Brosh, A. Izhakil and Kerem, Z. (2008). Nutritive and antioxidative potential of fresh and stored pomegranate industrial byproduct as a novel beef cattle feed. J Agric Food Chem. 12; 56(21): 10063-70.

Shiban, M.S.; Al-Otaibi, M.M. and Al-Zoreky, N.S. (2012). Antioxidant Activity of Pomegranate (Punicagranatum L.) Fruit Peels. Food and Nutrition Sciences, 3, 991-996.

Singh, R.P.; Murthy, K.N.C. and Jayaprakasha, G.K. (2002). Studies on antioxidant activity of pomegranate (Punicagranatum) peel and seed extracts using in vitro models. Journal of Agricultural and Food Chemistry, 50, 81–86.

Sun, B.; Ricardo-da-Silva, J.M. and Spranger, I. (1998). Critical factors of vanillin assay for catechins and proanthocyanidins. Journal of Agricultural and Food Chemistry, 46, 4267–4274.

Woisky, R. and Salatino, A. (1998). Analysis of propolis: some parameters and procedures for chemical quality control. J. Apic. Res. 37: 99-105.

Worku, M.; Ahmed Abdalla, Sarah Adjei-Fremah and Hamid Ismail (2016). The Impact of Diet on Expression of Genes Involved in Innate Immunity in Goat Blood. Journal of Agricultural Science; Vol. 8, No. 3; 1-9.

Yang, M.B.; Raza, Asim; Xiaogang, Jiang; Bo Zhong, Muhammad Shahzad; Fujun and Zhangand, Lu Shemin (2010). NO in both bronchoalveolar lavage and serum is associated with severity of antigen-induced pulmonary inflammation in rats .Journal Of Asthma, 47: 135-14.

Zhao, G.R.; Xiang, Z.J.; Ye, T.X.; Yaun, J.Y. and Guo, X.Z. (2006). Antioxidant activities of Salvia miltiorrhiza and Panaxnotoginseng. Food Chem. 99: 767-74.