EFFECT OF ADDING POMEGRANATE PEELS TO GROWING JAPANESE QUAIL DIET ON PERFORMANCE, BLOOD AND IMMUNITY PARAMETRS

A.A. Abdel-Wahab[1] and A.S. Mosad[2]

[1]Poultry Production Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt.

[2]Ministry of Agriculture.

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SUMMARY

Pomegranate peel (PP) improved growth performance, nutrient digestibility and immunity moreover, reducing intestinal and fecal pathogenic microorganisms. So, the experimental work was designed to study the effects of PP powder as a natural feed additive on growing Japanese quails. Aggregate of 180 growing Japanese quail at ten days age were distributed into five groups, each group contain three replicates (12 birds each). The first group fed on basal control diet without any additives, the second group fed control diet plus sub-therapeutic dose of oxytetracyclin (1g/kg diet). While, third, four and five groups fed on basal control diet with 0.5 %, 1.0 % and 1.5 % PP powder respectively. The obtained results showed that: Pomegranate peel treatments significantly increased body weight (LBW_{38d}), body weight gain (BWG_{10-38}) and performance index (PI_{10-38}), while, feed intake (FI_{10-38}) was significantly lower and feed conversion (FC_{10-38}) was significantly improved in all treated groups especially with 1.0 % PP level compared with antibiotic and control groups. Females had higher LBW_{38d}, $BWG_{10-38,}$ PI_{10-38} and best FC_{10-38} than males. Except both very low density lipoprotein and triglycerides serum biochemical indices such plasma total cholesterol, low density lipoprotein and high density lipoprotein significantly decreased by PP addition. The best antioxidant parameters (except Glutathione peroxidase) and immune responses and intestinal microflora count, favoring the quail fed diet supplemented with PP which had the best growth performance, especially 1.0 % PP level. Quail fed diet containing 1.0% of PP had the lowest thiobarbaturic acid. Pomegranate peel (3% and 1%) supplementation desirably increased *Lactobacillus* count as compared with those fed diets appended with antibiotic and the control groups and decreased both *E- coli and Salmonella* counts compared to group of control. In conclusion, PP addition by 1.0% can improve productive and physiological parameters and also a good alternative to antibiotic for promoting quail growth.

Keywords**:** *Quail nutrition, pomegranate peel, antibiotics, growing, performance, plasma constituents, immune responses, microflora.*

INTRODUCTION

There are wastes production from fruits usually reinforced by some minerals, vitamins and other biological component and they a good provenance of fiber so. Compelling evidences vindicate and specify peel or extracts for much fruits as functional foods and nutraceuticals. Different sources for polyphenol (Sehm et al., 2011 and Benn et al., 2015) such as pomegranate and pomegranate peel (Bialonska et al., 2010) be obliged been found to progress growth of probiotic organisms in the hindgut, reduction different pathogens, causing higher production of fermentative metabolites which are useful for health. Also, several scientific studies have demonstrated the ability of these bioactive components as feed additives to improve growth performance, nutrient digestibility and immunity also, reducing intestinal and fecal pathogenic microorganisms and fecal noxious gas emissions (Yan et al., 2011, Abbas et al., 2017 and Ahmed and Yang, 2017).

Newly, attention has been increased highly in finding natural antioxidants for use in foods and medicine to substitute synthetic compounds which are being limited due to their carcinogenicity. Human body can be conserving from free radicals and arrest the progress of many chronic diseases as well as retard lipid oxidative rancidity in food by using natural antioxidants (Jang *et al.,* 2010). The preventative impact of

vegetables and fruits has been referred to the presence of antioxidants, especially antioxidant vitamins including α-tocopherol, β-carotene and ascorbic acid (Prior and Cao, 2000). Phenolic or polyphenols including flavonoid, commonly found in eaten plants, have received major interest due to their biological functions such as antitumor, anti-mutagenic and antioxidant activities (Othman *et al.,* 2007). Phenolic compounds act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators is mainly due to their antioxidant properties (Balasundram *et al.,* 2006). In many different cultures and countries for thousands of years pomegranate fruits have been widely used, pomegranate juice and peel contained a lot of essential polyphenols such as ellagic tannins, ellagic acid and gallic acid (Loren et al., 2005). Pomegranate peel considered as a polyphenol-rich source, where containing high levels of flavonoids and tannins, such as punicalin, pedunculagan, gallagic acid, ellagic acid, and its esters of glucose (Kaneria *et al.,* 2012 and El Din *et al.* 2014).

Pomegranate polyphenols contain flavonoids such as anthocyanins, flavonols and flavanols, compressed tannins like proanthocyanidins, ellagitannins and gallotannins that called hydrolysable tannins. The antioxidant effect of flavonoids acquired from pomegranate and its juice was punctate to be preferable or near to that of green tea and butylated hydroxyanisole. Pomegranate extract consists of admixture different phytochemicals, comprehensive the punicalagins, a kind of tannins adorable to pomegranates that have been shown to control free radical scavenging properties (Gil *et al.,* 2000 and Noda *et al.,* 2002). Schubert *et al.* (1999) displayed that the antioxidant activity was lower for empirical juices that were acquired from the arils only compared to mercantile juices that were obtained from whole pomegranates. Pomegranate is a strong anticancer factor that source the creation of apoptosis and cell cycle stopping in cancer cells, suppression of multiple signaling pathways in cancer cells and repression of tumorigenesis in animal models of various carcinomas (Mehta and Lansky, 2004; Jeune *et al.,* 2005 and Abu Hajleh and Al-Dujaili, 2016). Pomegranate peel powder is a byproduct of juice production industries containing a series of bioactive compounds such as minerals and fibers use for a wide range in dietary requests (Mirdehghan and Rahemi, 2007). The waste produce from fruit holds up relatively higher total phenolic concentration (1.261%) moreover to its properties as committing source for crude fibers (12.17%) and inorganic remains that health promotive countenance like prevention from the development of cardiovascular disorders, hypoglycemic, apoptotic, anti-inflammatory, anti-parasitic and as prebiotic (Abdel-Rahim *et al.,* 2013 and Anderson *et al.,* 2009). Also, Ellagitannin that isolated from pomegranate peel an active antioxidant compounds and anticancer activities responsible for protecting low density lipoprotein, cholesterol from oxidation in vivo a key step in the pathogenesis of atherosclerosis.

Bialonska *et al.* (2009) appeared that pomegranate polyphenols have positive effects on health-promoting bacteria (Bifidobacterium breve and B. infantis) besides inhibitory effects on pathogenic bacteria (clostridia and Staphyloccocus aureus). In addition, pomegranate polyphenols have resulted in modified metabolism for gut bacteria (Bialonska *et al.,* 2010). Also, the antibiotic activity for pomegranate returned to phenolic compounds particularly ellagic acid and punicalagin (Sarkhosh *et al.,* 2007). In recent decades, Japanese quail become an important experimental bird for scientific studies, because of short life period furthermore, greater resistance to many poultry diseases, and increasing consumption of meats and eggs, and represents an alternative to chicken production (Berto *et al.,* 2008; Cardozo *et al.,* 2010 and Jatoi *et al.,* 2013). So, the aims of this research was to define the effect of using three levels from pomegranate peel on performance, blood biochemical, blood antioxidant, immune responses and some microflora of intestinal in growing Japanese quails.

MATERIALS AND METHODS

The experimental work of the present study was carried out at the Poultry Research Station, Poultry Production Department, Faculty of Agriculture, Fayoum University to evaluate pomegranate peel powder as a feed additive in growing Japanese quails.

Experimental design:

Birds and diets:

A total number of 180 one day-old unsexed Japanese quail birds were used in this experiment and were initially fed a control diet (containing about 24% CP and 2900 Kcal ME/Kg) for ten days, according to

the requirement published by NRC (1994) At the end of 10^{th} day of age, the birds were wing-banded and randomly divided into five experimental groups each in three replicates (12 birds each).

Birds were individually weighed and placed in electrically heated battery till the end of experiment. The first group was fed basal control diet without any additives, the second fed control diet plus sub-therapeutic dose of oxytetracyclin, 1g/kg diet. While, third, four and five groups fed on basal control diet with 0.5 %, 1.0 % and 1.5 % from pomegranate peel powder, respectively. The basal diet composition is presented in Table (1). Chicks were exposed to continuous lighting, feed and watered *ad libitum*. In 31 day of age birds were vaccinated against Newcastle virus (Lasota) by projection at eye.

Table (1): Feed ingredients and chemical composition of basal experimental diet.

Item,%	Basal diet %
Maize, ground or yellow corn	56.30
Soybean meal (44 CP %)	31.00
Plant concentrate meal[1] (50 CP)	11.00
Vegetable oil	0.50
Calcium carbonate	0.20
Sodium chloride	0.30
Dicalcium phosphate[2]	0.40
Vitamin and mineral premix[3]	0.30
Calculated analysis	
Metabolizable energy (Kcal/kg)	2913
Crude protein (CP)	23.92
Crude fiber (CF)	3.52
Calcium	0.81
Available phosphorus (Av P)	0.47
Lysine	1.64
Methionine	0.50
Methionine+Cystine	0.90

[1]*Plant Concentrate meal%: CP 50, CF 1.3, Ca 5, Av P 3.1, lysine 6, methionine 2.1 and ME 2650 kcal/kg.*
[2]*Dicalcium phosphate Ca 28%, total P 19%*
[3]*Premix provided per kg of diet: vitamin A, 12000 IU; vitamin D3, 00 IU; vitamin E, 30 mg; vitamin K_3, 4 mg; vitamin B_1, 3 mg; vitamin B_2, 7 mg; vitamin B_6, 5 mg; vitamin B_{12}, 15 µg; niacin, 25 mg, Fe, 80 mg; folic acid, 1 mg; pantothenic acid, 10 mg; biotin, 45 mg; choline, 125,000 mg; Cu, 5 mg; Mn, 80 mg; Zn, 60 mg; Se, 150 µg.*

Growth Performance:

Live body weight of chicks (LBW) were individually weighed and feed consumption per cage were weekly recorded (FI), the uneaten feed discarded, body weight gain (BWG, g) as follows: $BWG_{10\ to\ 38} = LBW_{38} – LBW_{10}$, feed conversion ratio (FCR) and performance index (PI, $_{10\ to\ 38}$) based on North (1981) formula was calculated as follows: PI = LBW, kg / FCR x 100.

Blood biochemical, antioxidant and immunity:

Individual 20 blood samples were collected in dry clean centrifuge tubes at slaughter and serum was separated by centrifugation at 3000 rpm for 15 minutes and assigned for subsequent determination. Quantitative determinations were done for the following: total cholesterol (Chol), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and triglycerides (Trig). All blood biochemical parameters were calorimetrically determined using commercial diagnosing kits (produced by Spectrum Diagnostics Company, Egypt). The glutathione peroxidase (GPx, EC 1.11.1.9) was calorimetrically determined according to Paglia and Valentine (1967) and thiobarbaturic acid- reactive substances' (TBARS) were performed according to Yagi (1998) using commercial diagnosing kits produced by Cayman Chemical Company (USA). The method used for the assay of chicken Immunoglobulins Isotypes IgG, IgM, and IgA in Sandwich ELISA described by Erhard *et al*. (1992) the absorbance measured on an ELISA plate reader set at 450 nm.

Microbial analysis:

Immediately after slaughter, intestinal content was collected in sterile glass containers, digesta was evacuated and mixed. The sealed containers were kept in the laboratory at 4°C till enumeration of microbial population. Samples (1g of the mixed fresh mass) were taken into sterile test tubes, diluted 1:10 in sterile 0.1% peptone solution and homogenized for three min in a Stomacher homogenizer. Ten fold serial dilutions up to 10^{-7} of each sample were prepared in nine ml of 0.1% sterile peptone solution. Viable counts of *Salmonella ssp, E. coli* and *Lactobacilli ssp* were performed. One milliliter of the serial dilution was incubated into sterile Petri dishes and sealed with an appropriate medium. *Lactobacillus spp*. colony count was determined using MRS agar (Biokar Diagnostic, France) after incubation in an anaerobic chamber at 37° C for 72 h. *Salmonella* and *E. coli* colonies were counted on brilliant green agar plate and incubated at 37° C for 24 h). After cultivation in Petri dishes, the total colony count for *Lactobacilli, Salmonella* and *E. coli* was then calculated as the number of colonies by reciprocal of the dilution. The microbial counts were determined as colony forming units (cfu) per gram of sample.

Statistical analysis:

Using General Linear Models (GLM) procedure of SPSS (2013) studied traits were subjected to a two-way analysis of variance with treatment and sex as main effects as follows:

$$Y_{ijk}=\mu+ T_i + S_j+ e_{ijk}.$$

Where: Y_{ijk}: Observed value in the i^{th} treatment of the j^{th} sex of the k^{th} individual, μ: overall mean, T_i: treatment effect (i: 1 to 5), S_j: sex effect (j: 1 and 2) and e_{ijk}: random error term. When significant F values were obtained main effects means were compared by Duncan's new multiple range tests (Duncan, 1955).

RESULTS AND DISCUSSIONS

Data presented in Table 2 showed that quails fed diet supplemented with 1.0 % PP had the heaviest LBW_{38d}, BWG_{10-38}, better FC_{10-38} and higher PI_{10-38} ($P\leq .001$) and nearly less FI_{10-38} compared with other treatments studied (control and antibiotic groups). Significant sex effects were shown for LBW_{38}d, $BWG_{10-38,}$ FC_{10-38} and PI_{10-38} favoring females.

Table (2): Effects of treatment and sex on growth traits in Japanese quail (Main effects ± SE).

Item	LBW_{10d}	LBW_{38d}	BWG_{10-38}	FI_{10-38}	FC_{10-38}	PI_{10-38}
Treatment effect:						
Control diet (C)	60.20±1.18	208.31±2.80^{c}	148.11±2.45^{c}	636.07±0.81^{a}	4.38±0.06^{a}	4.92±0.17^{d}
C+oxytetracyclin1g/kg diet	59.92±1.19	213.08±2.81^{c}	153.17±2.46^{c}	631.03±0.81^{b}	4.17±0.06^{b}	5.23±0.17^{d}
C+0.5 % PP	60.15±1.21	233.68±2.86^{b}	173.54±2.51^{b}	597.44±0.83^{c}	3.46±0.06^{c}	6.82±0.17^{c}
C+1.0 % PP	60.52±1.20	253.44±2.83^{a}	192.91±2.48^{a}	563.66±0.82^{d}	2.94±0.06^{e}	8.73±0.17^{a}
C+1.5 % PP	59.96±1.36	234.05±3.22^{b}	174.08±2.83^{b}	547.30±0.93^{e}	3.16±0.07^{d}	7.47±0.19^{b}
P	0.9971	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Sex effect:						
Females	62.47±0.69^{a}	238.69±1.64^{a}	176.22±1.44^{a}	595.00±0.47	3.47±0.04^{b}	7.26±0.10^{a}
Males	57.83±0.86^{b}	218.33±2.02^{b}	160.51±1.77^{b}	595.19±0.58	3.77±0.05^{a}	6.01±0.12^{b}
P	<0.0001	<0.0001	<0.0001	0.7988	<0.0001	<0.0001

SE: standard error, BWG: body weight gain FI: feed intake, PI: performance index, FC: feed conversion,
$^{a-e}$: Means within the same column with different superscript, PP: pomegranate peels.

These results are in accordance with those reported by Azoz and Basyony (2012) when pomegranate dried waste was included in diets of mature does at levels of 0.5, 1.0 and 1.5%. In the same trend Mady *et al.* (2016) showed that rabbits fed diet of 1.5% and 3% pomegranate peel powder (ppp) recorded the highest values of daily weight gain as compared to those fed the control diet (27.16 and 27.02 vs.21.71 gm) and FC

was improved (1.10 and 1.09 vs.1.27) and increasing significantly final body weight, BWG and PI, appreciable improvement of FC. Similarly, Yassein *et al.* (2015) observed that quails fed on diet supplemented with 10 and 15 g PPP/kg diet had significant (P≤0.01) increased BWG compared to butylated hydroxy toluene supplemented diet and the control group. While, there was a significant (P≤0.05) decrease in FI and FC in all treated groups. Tannins are astringent, bitter plant polyphenols, which react with salivary muco-protein or directly with taste receptors in the mouth, reducing palatability. Ahmed and Yang (2017) stated that inclusion of PGB in the broiler diet reduced the FI and improved FC values, the reduction in FI can be explained by reduced diet palatability due to the presence of a considerable amount of tannin in the experimental PGB (hydrolysable tannin 14.26 mg/g on a DM basis). Also, Ahmadipour *et al.* (2018) confirmed that in broiler. The highly rendering for pomegranate may be coming from ingredients of pomegranate that show health promoting influence out of the alteration of physiological and biochemical pathways. Recently evidences submitted that pomegranates fruits, peels and seeds clarify therapeutics modulations in health directing via inhibition of free radical effect and modulation of enzymes activity linked with diseases development and progression (Rahmani *et al.,* 2017). In contrast, Abbas *et al.* (2017) showed that final body weight was similar among quails fed on treated diets by PPP, whereas, the highest FI was listed in quail that fed on diets with 7.5% PPP; however, for 2.5 and 5.0% was insignificant affect FI as compared to control, and this agree with (Rajani *et al*., 2011 and Saki *et al*., 2014). This effect on BWG or even cause weight reduction could be attributed to several factors as pomegranate peel contains considerable amounts of polyphenols together with the high fiber content which reduced FI and the restricted calorie intake (Mahmoud *et al.,* 2011); contains polyphenols may suppress growth of the adipose tissue through their anti-angiogenic activity and by modulating adipocyte metabolism or reduce fat digestion and absorption, nutrient digestibility (CP and NFE) and nutritive value of TDN, DE and DCP (Fayed *et al.,* 2012).

Results in Table (3) showed that insignificant effect influenced all serum biochemical indices studied (*P*>0.05). All serum biochemical indices significantly affected by sex, except total VLDL and Trig. Females had higher total Chol, HDL and LDL (*P*≤0.05) concentration than males.

Table (3): Serum biochemical indices at slaughter as affected by treatment and sex (Main effects ± SE).

Item	Total Chol, mgdl	HDL mgdl	LDL mgdl	VLDL mgdl	Trig mgdl
Treatment effect					
Control diet (C)	211.75±14.16	31.25±2.92	145.75±9.34	34.75±9.01	149.50±44.21
C+oxytetracyclin1g/kg diet	254.75±14.16	35.50±2.92	175.25±9.34	44.00±9.01	220.00±44.21
C+0.5 % PP	202.75±14.16	28.50±2.92	142.75±9.34	31.50±9.01	157.50±44.21
C+1.0 % PP	188.25±14.16	32.50±2.92	130.75±9.34	25.00±9.01	125.00±44.21
C+1.5 % PP	191.25±14.16	29.50±2.92	139.25±9.34	22.50±9.01	112.50±44.21
P	0.0571	0.53422.92	0.0797	0.4512	0.4725
Sex effect					
Females	230.42±8.17[a]	37.33±1.68[a]	159.58±5.39[a]	33.50±5.20	160.00±25.52
Males	185.67±8.17[b]	26.50±1.68[b]	133.58±5.39[b]	25.58±5.20	127.33±25.52
P	0.0022	0.0007	0.0052	0.3028	0.3833

Chol: cholesterol, HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low density lipoprotein, Trig: triglycerides, [a,-b:] Means within the same column with different superscript. SE: standard error, PP: Pomegranate peels.

Babu and Srinivasan (1997) reported that Chol and LDL were decreased significantly as compared with control by PP treatments, which may be due to mediated motivation of hepatic cholesterol-7- hydroxylase activity. While, PP significantly (P<0.01) increased HDL and the ratio of HDL/LDL in the blood of doe rabbits feeding on diets contains PP, thus play important and positive role in the treatment of lipid metabolic unrest and obesity. Aviram and Rosenblat (2013) found that PP has good protection for high-density lipoprotein from oxidation compared to other antioxidants. Additionally, PP has the ability to stimulate HDL-associated paraoxonase 1, which depredated harmful oxidized lipids in lipoproteins (Fuhrman *et al.,* 2005 and Rosenblat *et al.,* 2006). Dietary PP modulate fat metabolism (Medjakovic and Jungbauer, 2013). In fact, blood Chlo levels were decreased by PP consumption (Aviram *et al.,* 2008). Many action mechanisms might explain these effects. Among them one can that include metabolic effects such as: (1) suppression of

energy intake and inhibition of pancreatic lipase activity, leading to decreased absorption of fat (Lei *et al.*, 2007), (2) changing the interplay between the metabolic hormones leptin and insulin (decrease of both) and adiponectin (increase) (McFarlin *et al.*, 2009). Al-Moraie *et al.* (2013) who showed that oral treatment for hypercholesterolemic rats by pomegranate juice were significantly increased HDL levels and antioxidant enzymes as compared with the control positive group. Also, the same results were found by (Yassein *et al.*, 2015 and Abbas *et al.*, 2017) in quails. Results founded by (Sharifiyan *et al.*, 2016) showed no significant differences are observed in total Chlo, Trig, LDL, HDL and VLDL for treatment groups in comparison with hypercholesterolemic control.

All Antioxidant parameters and immune responses studied were significantly affected (Table 4) by treatment effect (except GPx). Quail fed the diet supplemented with 1.0 % PP had the highest Ig_G, higher Ig_A and Ig_M but the lower thiobarbaturic acid (TBAR) followed by those fed the diet supplemented with 1.5% PP, 0.5 % PP and control. On the contrary, antioxidant parameters and immune responses tested insignificantly affected by sex (Table 4).

Table (4): Antioxidant parameters and Immune response as affected by different dietary treatments and sex (Main effects ± SE).

Item	Antioxidant parameters		Immune response		
Treatment effect					
	GPXnmolminmgprotien	TBARµgg	IgGmgdl	IgA mgdll	IgMmgdll
Control diet (C)	1864.50±117.03	1.53±0.10^{a}	947.63±35.26^{cd}	95.12±4.25^{ab}	181.70±7.96^{ab}
C+oxytetracyclin1g/kg diet	1683.75±117.03	1.50±0.10^{a}	902.08±35.26^{d}	86.07±4.25^{b}	164.72±7.96^{b}
C+0.5 % PP	2068.50±117.03	1.25±0.10^{ab}	978.33±35.26^{bc}	91.19±4.25^{ab}	174.33±7.96^{ab}
C+1.0 % PP	2009.00±117.03	1.15±0.10^{b}	1067.96±35.26^{a}	100.15±4.25^{ab}	191.14±7.96^{ab}
C+1.5 % PP	1912.75±117.03	1.16±0.10^{b}	1053.38±35.26^{ab}	98.70±4.25^{ab}	188.40±7.96^{ab}
P	0.1616	0.0069	0.0100	0.1019	0.1019
Sex effect					
Females	1859.42±67.57	1.29±0.06	1000.42±20.36	94.90±2.45	181.29±4.60
Males	1879.00±67.57	1.22±0.06	1018.85±20.36	96.91±2.45	185.05±4.60
P	0.8411	0.4071	0.5342	0.5731	0.5731

GPX: glutathione peroxidase, TBAR: thiobarbaturic acid, IgG, IgA, IgM Immunoglobulins G, A, M
$^{a...d:}$ Means within the same column with different superscript, SE: Standard error, PP: pomegranate peels.

The results obtained are consistent with Ahmed and Yang (2017) who indicated that dietary supplementation with Punica granatum L. by-product (PGB) led to a significant increase in serum IgA and IgG concentration relative to the control. Also, Sharifiyan *et al.* (2016) indicated that PP extract significantly increases serum antioxidant capacity in the extract recipient group in comparison with hypercholesterolemic control (P<0.05). Wang *et al.* (2000) reported that dietary supplementation with polyunsaturated fatty acid (PUFA), especially n-3 fatty acid, increased the growth of immune organs. Pomegranate seeds are a rich source of PUFA, particularly α-linolenic acid, linoleic acid and conjugated α- linolenic acid (CLA) (Fadavi *et al.*, 2006 and Melo *et al.*, 2014). Dietary CLA is also reportedly a potent enhancer of IgG production (Kohno *et al.*, 2004 and Yamasaki *et al.*, 2004). Yamasaki *et al.* (2006) reported significantly enhanced IgG and IgM production in spleen lymphocytes of rats fed diet supplemented with pomegranate seed oils. In addition, ellagitannin (Ramstead *et al.*, 2013) and a polysaccharide (PSP001) (Joseph *et al.*, 2012) isolated from pomegranate peel was also found to have immunomodulatory activity via stimulation of the growth of normal lymphocytes. Also, Yassein *et al.* (2015) found that the TBARS values was significantly decreased in birds fed 15 g PP/kg compared with that received butylated hydroxy Toluene (BHT) supplemented diet and the control group and these results are consistent with our findings. Many antioxidant compounds that found in PP including, a precursor of ellagic acid and (a) ellagitannins have ability to lower malondialdehyde levels, Which, have antioxidative properties (Mass *et al.*, 1991). Also, Zeweil *et al.* (2013) reported that lipid peroxide (malondialdehyde) levels decreased significantly to reach around 54% of the heat stressed bucks and value of PP were 1.5, 3.0 and 4.5% of PP dietary used.

Dietary treatments represented useful and harmful intestinal bacteria (Table 5). Both 1.0 and 3.0 % PP supplementation desirably increased *Lactobacillus* count and decreased both *E- coli and Salmonella* counts as compared with control group.

Table (5): Useful and harmful intestinal bacteria in growing quails as affected by different dietary treatments and sex (Main effects ± SE).

Item	*Lactobacillus* log 10 cfug	*E coli* log 10 cfug	*Salmonela* log 10 cfug
Treatment effect:			
Control diet (C)	5.21±0.24^{b}	7.29±0.25^{a}	7.04±0.30^{a}
C+oxytetracyclin1g/kg diet	4.63±0.24^{b}	4.80±0.25^{b}	5.23±0.30^{c}
C+0.5 % PP	5.96±0.24^{a}	6.46±0.25^{b}	6.27±0.30^{ab}
C+1.0 % PP	6.39±0.24^{a}	6.69±0.25^{ab}	6.21±0.30^{abc}
C+1.5 % PP	6.04±0.24^{a}	6.41±0.25^{b}	5.93±0.30^{bc}
P	0.0010	0.0003	0.0256
Sex effect:			
Females	5.99±0.14	6.30±0.14	5.80±0.17^{b}
Males	5.58±0.14	6.24±0.14	6.38±0.17^{a}
P	0.0600	0.7644	0.0353

E coli: Escherichia coli cfug: logarithm of colony forming unit per gram of digesta

$^{a...c:}$ Means within the same column with different superscript, PP: Pomegranate peels.

The lowest number of *E- coli* and *Salmonella* counts were shown for the group fed the diet supplemented with antibiotic whereas the control group had the highest harmful intestinal bacteria. Insignificant differences due to sex effect were obtained for useful and harmful intestinal bacteria studied except for *Salmonella* counts whereas males had higher than females. This results agree with Mady *et al.* (2016) who found that inclusion of pp whether 1.5 % or 3% in the diet leads to marked improvement of caecal eco-system through decreasing significantly numbers of pathogenic bacteria and justifying each of values, ammonia concentration and total volatile fatty acids profile in the caecum toward healthy circumstances. The antimicrobial action may be due tannins that found in PP the potency of tannin compounds to precipitate proteins, thus causing transpiration of cell membrane of the microorganism (Endo *et al.*, 2010), and aiding cell lysis which ultimately leads to cell death. Also, may be due to the bactericidal and bacteriostatic effects of PP which confirmed by Viuda *et al.* (2010) due to its contents of phenolic, anthocyanine, gallic acid and hydrolysable tannins, (mainly punicalin, pedunculagin and punicalgin) by which the total bacterial count, *E-coli* and *Clostridium spp* in caecum content were reduced, then improved the intestinal microbial balance and in turn directed the fermentation pattern and its end products toward favorable circumstances. On the other hand, the essential oils contents of PP evidenced by Brenes *et al.* (2010) may be responsible for enhancing the production of digestive secretions, stimulating blood circulation, excreting antioxidant properties and reducing the level of pathogenic bacteria. Ahmed and Yang (2017) found that feeding on diets with PGB resulted a linear lowering in *Salmonella spp* and *Escherichia coli*. Also, PGB in diets reduced the pH in ileal digesta. Contrariwise, the concentration of *Bacillus bacteria* in cecal digesta were increased linearly in response to both levels of dietary PGB, while *Salmonella and E. coli* concentrations decreased when birds feeding on diets were supplemented by 1% PGB, as did cecal pH. At 21 day the concentration of S. cerevisiae was increased by 1% PGB only, but at 35 day, fecal Bacillus concentration increased in both PGB levels. Fecal *E. coli* at 21 and 35 day was reduced by increasing levels of PGB, whereas *Salmonella* only at 21 day. Regarding the average of 48 h, dietary PGB effectively reduced the emissions of ammonia and methanethiol from broiler excreta. In conclusion, the results suggest that, dietary PGB improved immunity and the intestinal microbial ecosystem of broilers along with reduced odorous gas emissions from excreta. In contrast, Yassein *et al.* (2015) concluded that there were no significant effects of PP and BHT on micro bacterial count in the small intestine.

CONCLUSION

Considering the results of the current study it could be concluded that supplementation of PP, particularly 1.0 % level can improve productive, physiological parameters, antioxidant parameters, immune responses, intestinal microflora count and also a good alternative to antibiotic for promoting quail growth.

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تأثير إضافة قشر الرمان إلى علائق السمان الياباني على الأداء الإنتاجي وبعض صفات الدم والمناعة خلال مرحلة النمو

عبد الوهاب عبد الله عبد الوهاب[1] و أحمد سلامه مسعد[2]

[1] قسم انتاج الدواجن - كليه الزراعه - جامعة الفيوم - الفيوم- مصر.

[2] وزارة الزراعة- مصر.

إضافة قشر الرمان فى علائق السمان اليابانى تعمل على تحسين أداء النمو، وكفاءة هضم الغذاء ورفع المناعة مع الحد من الكائنات الحية الدقيقة الضارة المسببة للأمراض المعوية .لذلك ، تم تصميم هذه التجربة لدراسة تأثير إضافة مسحوق قشر الرمان كأحد الإضافات الطبيعية لعلائق السمان الياباني النامي. فى هذه الدراسة تم استخدام 180 كتكوت سمان يابانى نامي عمر 10 أيام موزعة على خمس مجموعات، كل مجموعة تحتوي على ثلاث مكررات بواقع 12 طائر لكل مكرر. حيث غذيت المجموعة الأولى على عليقة الكنترول بدون أي إضافات، بينما المجموعة الثانية غذيت على عليقة الكنترول مضافا إليها المضاد الحيوي الأوكسي تتراسيكلين (oxytetracyclin) بمعدل 1جم/ كجم عليقة. في حين أن كل من المجاميع الثالثه، الرابعة والخامسة غذيت على عليقة الكنترول مضافا إليها مسحوق قشر الرمان بنسبة 0.5 ٪ ، 1.0 ٪ و 1.5 ٪ على التوالي، وقد أظهرت النتائج التي تم الحصول عليها مايلي: أدت إضافة مسحوق قشر الرمان إلى علائق السمان الياباني النامى إلى زيادة معنوية فى الوزن الحى (LBW38d)، معدل الزيادة في وزن الجسم (BWG10-38d) ومؤشر الأداء(PI 10-38d) ، في حين حدث إنخفاض فى معدل إستهلاك الغذاء (FI 10-38d) مع تحسن فى كفاءة تحويل الغذاء المأكول (FC 10-38d) في جميع المجموعات المضاف إليها مسحوق قشر الرمان وخاصة مع مستوى 1.0٪ (المجموعة الرابعة) مقارنة مع مجموعة المضاد الحيوي والكنترول. إضافة إلى ذلك كان هناك تأثير للجنس حيث كانت الإناث أعلى في LBW38d، BWG10-38d ، PI 10-38d وأفضل FC 10-38d من الذكور. بالنسبة لمقاييس الدم فإنه فيما عدا كل من Very low density lipoprotein و Triglycerides فإن بقية المؤشرات البيوكيميائية في الدم مثل Total Cholesterol و Low density lipoprotein و High density lipoprotein انخفضت بشكل كبير بإضافة مسحوق قشر الرمان. وبالنسبة لمقاييس مضادات الأكسدة، باستثناءGlutathione peroxidase والاستجابات المناعية والميكروفلورا المعوية، فإن أفضل مضادات أكسدة للطيور التى غذيت على علائق مضافا إليها مسحوق قشر الرمان وخاصة عند استخدامه بمستوى 1.0 % فى علائق السمان الياباني النامي، ووجود قشر الرمان مضافا للعلائق أدى إلى خفض وتقليل حمض ثيوباربانوريك. إضافة قشر الرمان بمستويات (1 ٪ و3 ٪) أدى إلى زيادة أعداد بكتيريا حامض اللاكتيك (*Lactobacillus*) بالمقارنة مع مجموعات المضاد الحيوي والكنترول مع خفض في تعداد كل من *E coli* و *Salmonella* مقارنة بمجموعة الكنترول. في الختام، يمكن إضافة مسحوق قشر الرمان بنسبة 1.0 ٪ لتحسين الأداء الإنتاجي والفسيولوجي وأيضا أستخدامه كبديل جيد للمضادات الحيوية لتحفيز وتنشيط نمو السمان.