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The effect of using different levels of pomegranate (*Punica granatum*) peel powder on productive and physiological performance of Japanese quail (*Coturnix coturnix japonica*)

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Abstract

The experiment was conducted to evaluate the efficacy of pomegranate peel powder (PPP)(as an unconventional feed stuff) to partially replace yellow maize on productive performance, egg quality, some blood characteristics and jejuna morphology in Japanese quail (*Coturnix coturnix japonica*) diets. A total of 120 ten weeks of age Japanese quail were randomly and equally assigned to four treatments (n=30). Each treatment was replicated three times (containing 10 birds each). Birds were fed on experimental diets that were formulated to contain the same components as the control diet substituting PPP instead of yellow maize at levels 2.5 or 5.0 and 7.5 % in other three experimental diets respectively.

Results indicated that final body weight was similar among quails fed on replaced diets, whereas, the highest feed intake was recorded in quail that received 7.5 % PPP; however, for 2.5 and 5.0% feed intake was not affected as compared to control. Substitution with PPP improved feed conversion ratio, egg production, egg numbers, egg weight and egg mass. Serum cholesterol, triglyceride, glucose concentration and GPT (5% PPP) were decreased and total protein was increased, whereas the GOT/ GPT ratio was not affected. Quails on experimental diets hadd the highest relative weight in liver and heart, as well as villus height, crypt depth compared to control. Ratio of villi length /villi depth in diets substituted with 5.0 and 7.5% PPP had the highest values as compared to other treatments. Females had greater liver weight, villus length and crypt depth than males. It can be concluded that the substitution of pomegranate peel powder at a level 7.5 %, instead of yellow maize, can improve productive and physiological parameters and jejuna morphology in quails.

Keywords: *biochemical parameter, jejuna morphology, yellow maize*

Introduction

Pomegranate (*Punica granatum* L.) peel is a nutritive, antioxidant rich by-product, easily available after production of pomegranate juice and ready to eat arils (Sayed 2014). A large quantity of pomegranate peel waste is produced, and its disposal is an environmental problem (Kanatt et al 2010). Pomegranate peels which constitute up to 40% of the whole fruit remain as a by-product after pomegranate juice production (Panichayupakaranant et al 2010). Pomegranate peel is a good source of tannin's, flavonoids, phenolic and other polyphony's compounds **(**Li et al 2006), alkaloids, aromatic compounds and enzymes (Dhinesh and Ramasamy 2016). Pomegranate peel was considered to have high content of polyphony compounds, which possess antioxidant properties that can play an important role against cancer cells (Lansky and Newman 2007, Cam and Hisil 2009, Dahham et al 2010).

Many studies have been focused on pomegranate as having antimicrobial activity against a wide range of microorganisms including bacteria (Gram positive, Gram negative), fungi, yeasts and viruses (Naz et al 2007). Several investigators reported that pomegranate peel has antioxidant and antibacterial properties (Reddy et al 2007, Opara et al 2009 , Al-Zoreky 2009) . Pomegranate peel extract (PPE) showed good antimicrobial activity against *Staphylococcus aureus* and *Bacillus cereus* having minimum inhibitory concentration of 0.01%, while *Pseudonyms* could be inhibited at a higher concentration of 0.1% in chicken meat products (Kanatt et al 2010), as well as it has high inhibitory effect against both Gram positive and Gram negative bacterial species (Devatkal et al 2013, Hamady et al 2015).

Moreover, pomegranate peel didn't get much attention as an unusual feed in quails ration. Several investigators reported that using Pomegranate (peel extract or seed pulp) in chicken diets improved egg production, productive, dressed weight percentages and extended meat shelf-life ((Kanatt et al 2010, Saki et al 2014, Hamady et al 2015).

Therefore, we focused our interest in the objective of this study on the pomegranate peel powder to investigate its effect as partial replacement of yellow maize on the productive performance, some physiological parameters and jejuna morphology of Japanese quail.

Material and methods

Animal Husbandry and Treatments

The experiment was carried out in Quail's Field, College of Agriculture, University of Basra from 15/ 3/ 2016 to 9/ 5/ 2016. A total of 120 ten weeks of age Japanese quails (*Coturnix coturnix japonica*) were weighed. Initial body weight was compared at the beginning of the study. The birds were randomly assigned to four treatments, with three replicates (cages) of ten quails each. All diets were formulated to meet the nutrient requirements of the Japanese quails. A basal control diet was formulated to contain 20.03% CP and 2904 kcal/kg metabolized energy (ME). In the other three experimental diets yellow maize was partially replaced by

pomegranate peel powder (PPP) at levels of 2.5 or 5.0 and 7.5%. During the experiment (8 weeks), feed and water were supplied *ad libitum.* The composition of the basal diet is presented in Table1. Chemical analysis of PPP was carried out according to AOAC (2000) (Table 2) All birds reared in cages (replicates) of (45×70×75 cm), on a 17 h lighting was provided per day.

Table 1. Ingredients and composition of experimental diets

Ingredient, %	Pomegranate peel powder levels, %			
	0	**2.5**	**5**	**7.5**
Yellow Maize	56	53.5	51.0	48.5
Wheat	4.0	2.5	2.5	2.5
Soybean meal, 44%	28	28	28	28
pomegranate peel powder	0.0	2.5	5.0	7.5
Protein concentrate, 43%	5.0	6.0	6.0	6.3
Oil plant	1.0	1.5	1.7	1.8
Limestone	4.4	4.4	4.4	4.4
Dicalcium Phosphate	1.0	1.0	0.8	0.5
Vitamin / mineral premix	0.3	0.3	0.3	0.25
Common Salt	0.3	0.3	0.3	0.25
Total	100	100	100	100
Chemical analysis, %				
[#] ME, Kcal /Kg diet	2904	2905	2908	2901
Crude protein	20.0	20.2	20.0	20.0
Ether extract	3.93	3.92	3.86	3.82
Crude fiber	3.49	3.71	3.95	4.21
Calorie : protein ratio	145	144	145	145
Calcium	2.31	2.33	2.30	2.30
Phosphorus available	0.46	0.48	0.42	0.40
Methionine	0.38	0.41	0.43	0.46
Lysine	1.06	1.09	1.08	1.09
Methionine + Cystine	0.83	0.88	0.91	0.96

[#] *ME, Kcal /Kg diet = Metabolized energy*

Table 2. Gross chemical composition,% on dry weight basis of pomegranate peel powder

Component, %	Pomegranate peel powder
Moisture	11.9
Crude protein	3.97
Crude fat	2.34
Ash content	4.19
Crude fiber	12.1
Available carbohydrate	77.4
Organic matter	95.8
Metabolized Energy, Kcal/Kg[#]	2825

[#] *ME was calculated according to Lodhi et al (1976)*

Data collection

Body weight (BW) was recorded at the beginning and at the end of the study. Egg production was recorded daily and feed consumption, cumulative egg number, egg mass and egg weight were recorded at two weekly intervals. Feed conversion ratio (FCR) was calculated by determining the amount of feed consumed per one kg of egg.

Blood samples and physiological parameters

Four quails per treatment (two males, two females) were randomly selected at the end of the experiment to determine blood serum parameters. Blood samples were collected separately in non-heparin's tubes for biochemical assays, and centrifuged (3000 rpm, 15 min, 25^{o}C) to obtain serum. Serum samples were stored at –20ºC until analyzed for total protein by a colorimetric method using commercial kits (Bio lab AS, France). Blood serum cholesterol, triglycerides and glucose concentrations were determined according to the methods of Tietz (1999) using commercial kits (Biolabo AS, France). The activities of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were determined spectrophotometncally by measuring the oxidation rate of NADH (nicotinamide adenine dinucleotide, reduced) in a thermostated cuivette at 340 nm after incubation of samples with L-aspartate and L-alanin, respectively (Bergmeyer and Bernt 1974 a and b).

Intestinal histology

At the end of the feeding two birds from each replicate were sacrificed after starving them for 12 h to limit intestinal throughput. To ensure clean histology samples, the intestinal samples were removed with sterile surgical tools in a cool environment. Livers and hearts were removed and weighed. The whole length of the small intestine was removed, washed and weighed. A length of 3 cm jejunum from two birds per replicate was cut midway between the end of the duodenum where bile duct and pancreatic ducts meet and the embryonic sac junction, washed with sodium chloride (1%) to remove intestinal contents, then weighed and fixated with 10% neutral buffered formalin over 24 hr. Tissue sections of the preserved small intestine samples were processed for 24 h in a tissue processor with ethanol as dehydrant and samples embedded in paraffin wax. Five µm sections were made from the tissue and were stained with haematoxylin-eosin (Gracia et al 2009). Samples were viewed under a light microscope. Morphometric variables were measured under a light microscope and the height and width of the villi were measured using an eyepiece micrometer. Villus height was obtained from the top of the villus to the crypt–villus junction, while crypt depth was defined as the depth of the invagination between villi (Hu and Guo 2007).

Statistical analysis

The performance data from experiment were executed to a one-way ANOVA, whereas the data (heart and liver weight, villus height, crypt depth and villus height to crypt depth ratio) were subjected to a two-way ANOVA (feeding × gender) by using SPSS program software (2012). Means separated using the Least Significant Difference at p <0.05.

Results and discussion

Productive performance

There were no differences among treatments in body weight (Table 3). Birds fed 7.5% of PPP consumed more feed than on other dietary treatments, while there were no differences in feed intake between 2.5 and 5.0 % PPP and control diets. Data in Table 3 show that there was improvement on feed conversion ratio, egg mass and egg weight in PPP diets compared with the control. Hen day egg production and cumulative egg number were highest in both 5% and 7.5% PPP diets compared with groups fed on 2.5 % PPP diet and the control (Figures 1 and 2).

Table 3. Effect of diets containing different levels of pomegranate peels powder on quail performance at 10-18 weeks of age

Quail performances	Dietary pomegranate peels powder, %				SEM	*p*
	0.0	2.5	5.0	7.5		
Initial body weight, g	182	182	183	182	0.61	0.833
Final body weight, g	223	229	228	227	1.74	0.643
Feed intake, g	960^{b}	943^{b}	952^{b}	1013^{a}	9.48	0.012
Egg mass, g/bird/8wks	347^{d}	357^{c}	374^{b}	390^{a}	5.04	< 0.001
Feed conversion ratio, g/g	2.77^{c}	2.64^{b}	2.55^{a}	2.60^{ab}	0.03	0.002
Hen day egg production, %	71.8^{c}	72.2^{c}	75.3^{b}	78.1^{a}	0.78	< 0.001
Egg weight, g	11.5^{c}	11.7^{b}	11.8^{ab}	11.9^{a}	0.05	< 0.001
Number of eggs, egg/bird/8wks	30.2^{c}	30.3^{c}	31.6^{b}	32.8^{a}	0.33	< 0.001

$^{abc\ d}$ *Means without common superscript are different at p < 0.05 ,SEM: Standard error of the mean*

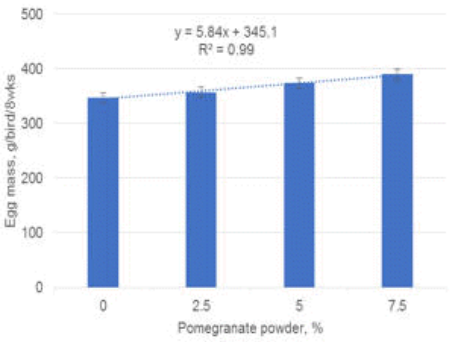

Figure 1. Effect different levels of pomegranate peels powder on egg mass (g/bird/8 wks) of quail at 10-18 weeks of age

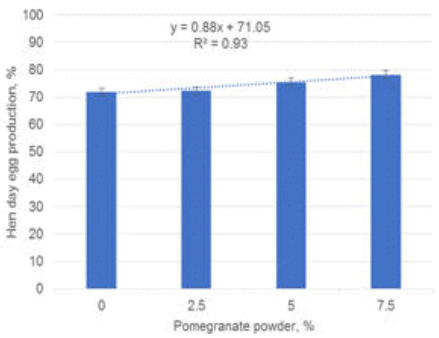

Figure 2. Effect different levels of pomegranate peels powder on hen egg production (%) of quail at 10-18 weeks of age

Plasma parameters

Serum cholesterol, glucose and triglyceride declined in all dietary groups in comparison with the control (Figure 3). Quails that received diets with 2.5 % PPP had the lowest GPT activity, whereas GOT and GOT/ GPT ratio was not affected as PPP was increased, while serum total protein was superior in dietary treatments compared with the control.

Table 4. Effect of diets containing different levels of pomegranate peel powder on some blood characteristics of quail at 18 weeks of age

Parameters	Dietary pomegranate peels powder, %				SEM	*p*
	0.0	2.5	5.0	7.5		
Total Protein, g/dL	5.1^{b}	5.7^{a}	5.7^{a}	5.6^{a}	0.08	0.043
Glucose, mg/dL	276^{a}	264^{b}	262^{b}	261^{c}	2.46	0.033
Cholesterol, mg/dL	296.7^{a}	275.7^{bc}	258.0^{cd}	246.7^{d}	6.28	0.002
Triglycerides, mg/dL	354.7^{a}	324.7^{bc}	333.7^{b}	311.0^{c}	5.37	0.004
GOT#	221	220	224	218	1.81	0.811
GPT##	18.3^{ab}	17.4^{c}	18.7^{a}	17.8^{bc}	0.17	0.003
GOT/ GPT ratio	12.1	12.6	12.0	12.3	0.14	0.354

#GOT: Glutamic Oxaloacetic Transaminases enzyme, ##GPT: Glutamic Pyruvic Transaminases enzyme

$^{abc\ d}$ *Means without common superscript are different at p < 0.05 , SEM: Standard error of the mean,*

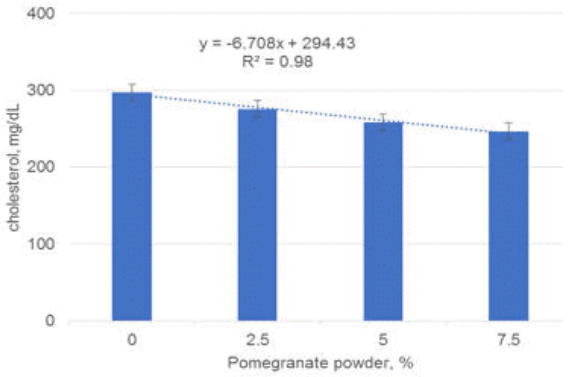

Figure 3. Effect different levels of pomegranate peels powder on serum cholesterol (mg/dL) of quail at 18 weeks of age

Organ weight and histology measurement

On diets with pomegranate peel at 5% level, heart weight was higher as compared with control, whereas, but there were no differences in heart relative weight between birds fed 2.5 and 7.5% of PPP and control. Liver weights were higher in PPP replaced diets as compared to control. Females were superior to males in these traits. Jejunum villus height, crypt depth and ratio of the villus height to the crypt depth (V/C) were increased with increasing PPP in the diets (Table 5; Figures 4-6) The results also revealed that females were superior compared to males in villus height and crypt depth, while the V/C)ratio was not affected between sexes. The results did not indicate any evidence of treatment × gender interaction for all traits examined (Table 5).

Table 5. Morphometric measurements of the jejunum, heart and liver relative weight of quail fed pomegranate peels powder at 18 weeks of age

Parameter	Sex	Dietary pomegranate peels powder, %				Mean	SEM	*p*
		0.0	2.5	5.0	7.5			
Heart#	M	0.87	0.91	0.94	0.92	0.91	0.012	0.222
	F	0.89	0.92	0.96	0.93	0.93	0.012	0.222
	mean	0.88^{b}	0.92^{ab}	0.95^{a}	0.93^{ab}	0.92	0.008	0.048
Liver#	M	2.21	2.27	2.26	2.31	2.26_{B}	0.014	< 0.001
	F	2.34	2.42	2.41	2.41	2.40^{A}	0.014	< 0.001
	mean	2.27^{b}	2.35^{a}	2.34^{a}	2.36^{a}	2.33	0.017	0.003
Villus length, µm	M	290	343	470	493	400^{B}	2.672	0.015
	F	307	377	513	517	428^{A}	2.791	0.015
	mean	298^{c}	360^{b}	492^{a}	505^{a}	414	1.913	< 0.001
Crypt depth, µm	M	34.7	39.3	48.7	50.3	43.3^{B}	0.201	0.007
	F	36.7	42.3	52.3	51.7	45.8^{A}	0.205	0.007
	mean	35.7^{c}	40.8^{b}	50.5^{a}	51.0^{a}	44.5	0.143	< 0.001
Villus/ crypt ratio	M	8.39	8.74	9.64	9.88	9.17	0.219	0.672
	F	8.27	8.89	9.80	10.0	9.25	0.239	0.672
	mean	8.33^{b}	8.81^{b}	9.72^{a}	9.94^{a}	9.21	0.159	< 0.001

 # as percent to body weight

^{abc} Means in the same row with no common superscript are different at $p < 0.05$, ^{AB} Means in the same column with no common superscript are different at p <0.05. SEM : Standard error of the mean,

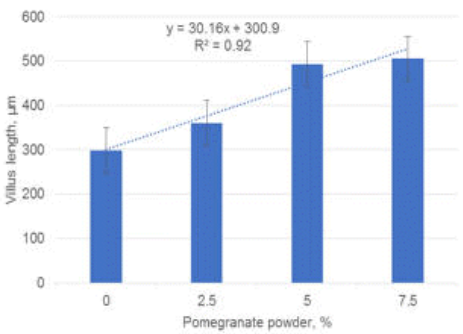

Figure 4. Effect different levels of pomegranate peel powder on villus length (µm) of quail at 18 weeks of age

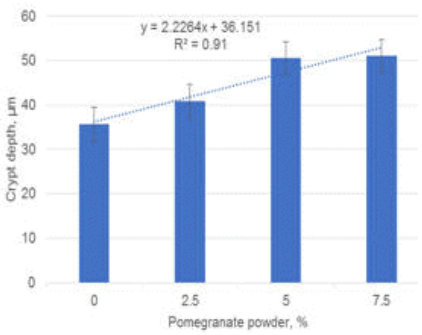

Figure 5. Effect different level of pomegranate peels powder on crypt depth (µm) of quail at 18 weeks of age

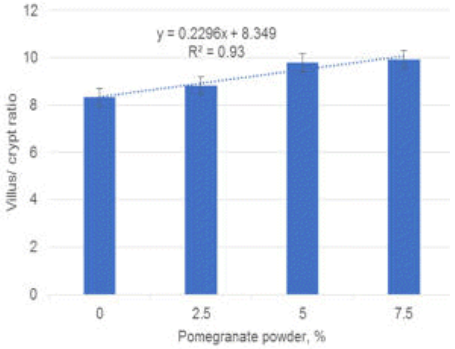

Figure 6. Effect different level of pomegranate peels powder on villus/crypt ratio of quail at 18 weeks of age

Discussion

Similar results to ours were reported by Rajani et al (2011) and Bostami et al (2015) on body weight gain, for pomegranate peels powder (PPP) inclusion in broiler diets. Differently from the present study, Yassein et al (2015), recorded decrease in FI and FCR in Japanese quails fed diet supplemented with 10 and 15 g PPP/kg diet. Improvements in feed intake, feed conversion ratio and egg production traits in birds fed pomegranate peel powder may be related to the palatability and digestion stimulating effects of these additives. Positive effects that plant feed additives exert on gastro-intestinal enzymatic activity and enhanced nutrient absorption and digestibility were reported by Banerjee et al (2013). The presence of active substances in PPP that improve digestion and metabolism have been attributed to antioxidant and antibacterial properties. These help to decrease combat pathogens in the intestine and as a consequence more nutrients are available in the intestinal lumen for absorption to convert to body mass (Jamroz et al 2006, Reddy et al 2007, Opara et al 2009, Al-Zoreky 2009). Health benefits of pomegranate peel are thought to be due to its biological activities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Prakash and Prakash 2011), as well as tannins (Hassanpour et al 2011) , could be the main reasons for its bioactive functions.

The findings related to serum cholesterol, glucose and triglyceride levels, obtained in this study agree with Yassein et al (2015) who found a decrease in lipid profile and glucose in Japanese quails fed with PPP. In contrar to our results, Saki et al (2014) reported an increase in serum cholesterol levels, when using up to 15% of PPP in diets of laying hen. Depending on the presence of phenolic compounds (punicalagin, punicalin, gallic acid and particularly elegiac acid), PPP may affect serum lipid concentrations and other blood indices (Rajabian et al 2007). Moreover, some investigations showed that pomegranate peels contain high ratios of antioxidants material in addition to phenolic materials and metals (Ibrahium 2010, Ullah et al 2012, Taher-Maddah et al 2012). Furthermore, polyphony's of pomegranate may accelerate and promote cholesterol metabolism by reversing cholesterol transport via HDL (Aviram et al 2002, Esmaillzadeh et al 2004).

In respect to sex effects on liver weight, our results are in line with those of Selim et al (2006), who reported higher weight of liver in females than in males. As for the improvement in jejunum measurements recorded in the present study, Svihus (2014) indicated that the jejunum is largely responsible for digestion and absorption of all the major nutrients, thus, increased villi height may be a consequence of greater need for digestive capacity. Accordingly, the change in villus lengths of jejunum may influence health and may increase nutrient uptake, which is positively reflected in egg production. In intestinal parameters, females showed the highest values in micro metric criteria in all ages compared to males (Rahmat et al 2016).

Conclusions

- Data from the present study indicate that up to 7.5 % level of pomegranate peel powder could be utilized effectively by Japanese quails for egg production with improvements in productivity, and in some physiological parameters and jejuna morphology in both males and females.

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