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Artigo

The effect of using pomegranate peel powder and protexin probiotic on carcass traits, blood parameters, jejunal tissue morphology and intestinal microbial population of Japanese quails

El efecto del uso de polvo de cáscara de granada y probiótico de protexina en las características de la canal, los parámetros sanguíneos, la morfología del tejido yeyunal y la población microbiana intestinal de las codornices japonesas

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Abstract: To evaluate the effect of using pomegranate peel powder and protexin probiotic on carcass traits, blood parameters, jejunal tissue morphology and intestinal microbial population of Japanese quails, two hundred and forty-one days-old Japanese quails were divided as an experimental randomized design plan into the eight treatments with 3 replicates and 10 quails each. The treatments were basal diet basis corn and soybean meal with no any supplementation kept as control, and three 0.2, 0.4 and 0.6 perctengae of pomegranate peels powder and two levels (0 and 0.1) percentage of diet of protexin probiotic respectively. At the age of 35 days, two quail pieces (male and female) were randomly selected from each replication and blood sampling were done to determine blood parameters. Mentioned quails were slaughtered to evaluate the carcass traits also and to determine the histomorphology of the intestinal tissue samples near Meckel's diverticulum from ileum were chosen and to determine the microbial population of the small intestine, fresh sample of the digestive contents of the ileum was taken. The results showed that the percentage of live weight and carcass weight increased significantly with the consumption of pomegranate peel powder and protexin ($p \leq 0.05$). The live and carcass weight and relative organ weight of gizzard, heart and intestine increased, but the relative weight of the liver showed a significant decrease ($p \leq 0.05$). Data also showed that the cholesterol and triglyceride levels in blood serum of quails were significantly reduced under the influence of pomegranate peel powder and protexin consumption ($p \leq 0.05$). Intestinal evaluation of ileum showed that by consuming pomegranate peel powder and protexin, the population of Lactobacillus increased and the

population of Escherichia coli decreased none significantly .Additionally the histomorphology evaluation of the intestinal jejunum tissue showed that the width, length, height of the intestinal villi and the mucosal muscle increased with the increase in the consumption of pomegranate peel powder and protexin probiotic, but no statistically significant difference was observed between the treatments. In general, the results showed beneficial effects of increasing levels of pomegranate peel powder up to 0.6 percentage level and protexin probiotic on carcass traits, blood parameters, jejunal tissue morphology and intestinal microbial population in quails.

Key words: Pomegranate peel, Protexin probiotic, Blood parameters, Jejunal tissue morphology, Intestinal microbial population, Japanese quails.

Resumen: Para evaluar el efecto del uso de polvo de cáscara de granada y probiótico de protexina sobre las características de la canal, los parámetros sanguíneos, la morfología del tejido yeyunal y la población microbiana intestinal de las codornices japonesas, se dividieron doscientos cuarenta y un días de codornices japonesas en un diseño experimental aleatorizado. planifique los ocho tratamientos con 3 repeticiones y 10 codornices cada uno. Los tratamientos fueron dieta basal a base de harina de maíz y soya sin suplementación mantenida como control, y tres porcentajes de cáscara de granada al 0.2, 0.4 y 0.6 por ciento y dos niveles (0 y 0.1) porcentaje de dieta de probiótico protexina respectivamente. A la edad de 35 días, se seleccionaron aleatoriamente dos piezas de codorniz (macho y hembra) de cada repetición y se tomaron muestras de sangre para determinar los parámetros sanguíneos. Las codornices mencionadas fueron sacrificadas para evaluar las características de la canal y también para determinar la histomorfología de las muestras de tejido intestinal cerca del divertículo de Meckel del íleon y para determinar la población microbiana del intestino delgado, se tomó una muestra fresca del contenido digestivo del íleon. Los resultados mostraron que el porcentaje de peso vivo y peso en canal aumentó significativamente con el consumo de polvo de cáscara de granada y protexina ($p \leq 0.05$). El peso vivo y de la canal y el peso relativo de los órganos de la molleja, el corazón y el intestino aumentaron, pero el peso relativo del hígado mostró una disminución significativa ($p \leq 0.05$). Los datos también mostraron que los niveles de colesterol y triglicéridos en el suero sanguíneo de las codornices se redujeron significativamente bajo la influencia del polvo de cáscara de granada y el consumo de protexina ($p \leq 0.05$). La evaluación intestinal del íleon mostró que al consumir polvo

de cáscara de granada y protexina, la población de Lactobacillus aumentó y la población de Escherichia coli no disminuyó significativamente. Además, la evaluación histomorfológica del tejido del yeyuno intestinal mostró que el ancho, largo y alto de las vellosidades intestinales y el músculo de la mucosa aumentó con el aumento del consumo de polvo de cáscara de granada y probiótico protexina, pero no se observó diferencia estadísticamente significativa entre los tratamientos. En general, los resultados mostraron efectos beneficiosos del aumento de los niveles de polvo de cáscara de granada hasta un nivel de 0,6 por ciento y probiótico de protexina sobre las características de la canal, los parámetros sanguíneos, la morfología del tejido yeyunal y la población microbiana intestinal en las codornices.

Palabras clave: cáscara de granada, probiótico Protexin, parámetros sanguíneos, morfología del tejido yeyunal, población microbiana intestinal, codornices japonesas.

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Introduction

The use of antibiotic as growth promoters in livestock has been banned due to concern about their residues in animal tissue and induction of bacterial resistance (VALI, 2009). It have evaluated alternative growth promoter such as probiotics and prebiotics (Fuller.R ,1989), which benefits the health of digestive tract (KHEIRI et al.,2015). The importance of feed additives especially of plant origin has greatly increased in recent years and a number of studies have reported that the beneficial effect of herbals or their active components on digestion process and better performance in birds (Hashemi and DAVOODI, 2011 and FAGHANI et al., 2014).

Pomegranate peel is among the spectrum of natural feed additives that has received increased attention over the years as prophylactics and growth enhancers in broiler nutrition (DHINESH and

RAMASAMY, 2016). It obtained from pomegranate fruit, often lefts over from production of the juice (YASOUBI et al., 2007). It possesses potential health benefits due to a rich content of antioxidant polyphenols (SHARIFIAN et al. 2019). The main compounds in pomegranate peels are phenolic compounds, such as punicalagin, gallic acid, fatty acids, catechin, quercetin, rutin, flavonols, flavones, flavanones, and anthocyanidins and have a great antioxidant capacity (DERAKHSHAN et al., 2018). Further, pomegranate peels can improve immune properties, activate enzymatic antioxidants, and control free radicals during stress conditions due to antioxidant, antimicrobial, anti-inflammatory, and anticancer properties and helps to reduce pathogens in the intestine; thus, additional nutrients are accessible in the intestinal cavity to be absorbed to convert into body mass (OPARA et al.,2009).

Ahmadi pour et al (2018) showed that no significant effect was observed among dietary treatments in terms of body weight gain and feed intake was linearly reduced by pomegranate peel inclusion and there was a significant difference between the control and pomegranate peel ($p \leq 0.05$). Sarica and Urkmez (2016) reported that the improved feed conversion ratio when broiler diets supplemented with ethanoic extract of pomegranate peel. They also showed that reduced accumulation of fat in abdominal cavity in birds fed pomegranate peel further confirms this observation.

Esmaillzadeh et al. (2006) also noted that pomegranate extract decreased total cholesterol and LDL-cholesterol by modulating the activity of HMG-CoA reductase. Sharifian et al (2019) showed that dietary pomegranate peel addition did not affect the relative weights of carcass, breast, thigh, abdominal fat, liver, pancreas, gallbladder, heart, gizzard, bursa of Fabricius, and spleen. Saleh et al. (2017) reported that dietary pomegranate peel reduced the feed efficiency, improved the nutritional value of meat and enhanced humoral immune response, decreased plasma triglycerides concentration. It seems that the use of pomegranate peel for reducing antibiotic effects is crucial in production and quality of birds (RAHIMIAN et al., 2018 and REZAIAN, 2006).

The few researches have been carried out regarding the use of pomegranate peel powder in poultry nutrition yet; hence, the aim of this study was conducted to determine the effect of using pomegranate peel powder and protexin probiotic on carcass traits, blood parameters, tissue histomorphology and

intestinal microbial population of Japanese quails.

Material and methods

Pomegranate peels were prepared from local fruit juice factory in Yasuj city. Pomegranate peels were clean, free of impurities, foreign substances and dust, and were dried. They were with a size of one mm were ground using a special mill and, after the milling process was completed; they were transferred to the field to complete the diet processing process in quail's farm. Two hundred and forty-one days-old Japanese quails were divided as an experimental randomized design plan into the eight treatments with 3 replicates and 10 quails each.

The treatments were basal diet basis corn and soybean meal with no supplementation kept as control, and three levels (0.2, 0.4 and 0.6 percentage) of pomegranate peels powder and two levels of protexin probiotic (0 and 0.1 percentage) supplemented diet respectively. At the end of the experimental period (35 days old), two quails from each replicate of each dietary treatment were randomly selected, weighed individually then slaughtered and allowed to bleed. The blood samples were gathered into clean dry test tubes and allowed to clot and serum was separated, collected, frozen and later analyzed. Hot carcasses weight was recorded and the dressing percentage was determined by expressing hot carcass weight to the live weight. The blood samples from each bird were collected and stored at refrigerator at $+4^{\circ C}$ for 24 ^{h} and then they were subjected to biochemical determine for their serum lipids such as cholesterol, triglycerides, HDL and LDL toward method described by (Ellefson and Graway.1967) and (FRINGS and DUNN. 1970).

To determine the microbial population about 7-8 cm from the length of the ileum was sampled and one gr of ileum content (digesta) was used to make 10-fold dilution using buffered peptone water and then 0.1 mL of the appropriate ileum dilution was spread on Lactobacillus MRS1 Agar-Hi Media Laboratories to detect lactic acid bacteria and Violet Red Bile Agar to detect Escherichia.Coli form. The cultures of Lactobacillus and Escherichia. Coli bacteria were made an aerobically form. The sample plates were incubated at $37.5^{\circ C}$ for 48 ^{h}. After counting the number of colonies in each plate, the number so obtained was multiplied by inverse of the dilution and the result was stated as the number of colony forming unit (cfu) in one gr of the sample as described by

(DOWNES and ITO.2001).

Additionally the jejunal tissue morphology examination was performed by light microscopy, and the measurement was done using public domain image analysis software (Image J, National Institute of Mental Health, Bethesda, MD, USA).

Statically model

The statically model was Yijk = μ+ ά i+ ßj+ eijk

Whereas: Yijk = average effect observed, μ = total average, άi= effect of treatments,ßj= effect of replications,eijk = effect of errors.

Statically analysis

Obtained data were analyzed by ANOVA procedure for completely randomized experimental design with eight treatments and three replicates using SAS (Version 6.12; SAS Institute, Cary, USA) and the means were compared by (Duncan,1995) test. In addition, a p value less than 0.05 were considered as statistically significant (P≤0.05).

Result and Discussion

Data from this study showed that the live body weight, carcass weight and heart efficiency percentage of Japanese quails in diets receiving different levels of pomegranate peel powder, protexin probiotic and different levels of pomegranate peel powder combined with protexin probiotic showed a significant increase (p<0.05). Table 1.

Table 1- The main and reciprocal effects of pomegranate peel powder with protexin on live weight, carcass weight and relative efficiency of carcass components and internal organs of Japanese quail.

Treatments	Live body weight (g)	Carcass weight (g)	Liver (%)	Gizzard (%)	Heart (%)
Pomegranate peel (%)					
0	220.14bc	186.12bc	2.99	2.32	1.18c
0.2	225.24b	194.34b	2.48	2.51	1.24c
0.4	226.89ab	193.21ab	2.26	2.74	1.46b
0.6	241.76a	200.76a	2.11	2.89	1.58a
Protexin probiotic (%)					
0	229.01b	197.53b	2.86	2.65	1.01b
0.1	237.14a	201.34a	2.75	2.41	1.49a
Pomegranate × Protexin					
Control	223.14bc	190.56bc	2.76	2.25	1.16c
0.1 Protexin	225.87bc	192.10bc	2.58	2.26	1.22c
0.2 Pomegranate with no Protexin	231.36b	198.71b	2.36	2.43	1.31c
0.4 Pomegranate with no Protexin	238.78ab	200.02ab	2.38	2.55	1.50bc
0.6 Pomegranate with no Protexin	204.60ab	202.20ab	2.45	2.30	1.68ab
0.2 Pomegranate with 0.1Protexin	244.76a	206.62a	2.28	2.44	1.71ab
0.4 Pomegranate with 0.1Protexin	247.39a	209.76a	2.31	2.69	1.96b
0.6 Pomegranate with 0.1Protexin	250.85a	211.74a	2.14	2.65	2.18a
SEM	21.42	20.69	0.630	1.78	0.013
P- value	0.0163	0.014	0.29	0.296	0.0001

*Means within row with no common on letter are significantly different (p≤ 0.05).

The blood serum cholesterol and triglyceride levels had significant decrease in quails under study by consuming pomegranate peel powder without and with protexin probiotic (P<0.05). The results of this study also showed that the highest reduction in cholesterol and triglycerides belonged to the groups were feed of 0.6 percentage of pomegranate peel powder combined with 0.1% protexin probiotic. Table 2.

Table 2-The main and reciprocal effects of pomegranate peel powder and protexin probiotic on some serum blood parameters in Japanese quail.

Treatments	Total Protein (g.dl)	Glucose (mg.dl)	Triglyceride (mg.dl)	Cholesterol (mg.dl)
Pomegranate peel (%)				
0	3.10	169.22	211.82a	211.21a
0.2	3.22	170.45	206.52b	207.46b
0.4	3.43	172.34	202.22c	202.24b
0.6	3.55	174.45	197.65d	191.17c
Protexin probiotic (%)				
0	3.46	170.17	200b	195.38b
0.1	3.60	172.36	204.29a	204.46a
Pomegranate × Protexin				
Control	3.22	170.10	216.16b	208.43a
0.1 Protexin	3.25	171.25	203.45b	207.21a
0.2 Pomegranate with no Protexin	3.44	169.45	200.16dc	206.52a
0.4 Pomegranate with no Protexin	3.61	168.54	197.36d	202.74ab
0.6 Pomegranate with no Protexin	3.44	170.00	207.36a	208.34ab
0.2 Pomegranate with 0.1Protexin	3.48	169.31	202.25ab	205.65a
0.4 Pomegranate with 0.1Protexin	3.51	170.25	200.29ab	205.33a
0.6 Pomegranate with 0.1Protexin	3.63	171.62	198.65c	206.55a
SEM	0.36	16.24	20.16	34.28
P- value	0.541	0.106	0.071	0.098

*Means within row with no common on letter are significantly different (p≤ 0.05).

The results of effects of pomegranate peel powder and protexin probiotic on intestinal morphology in Japanese quail are shown in Table 3. With the consumption of higher levels of pomegranate peel powder and the use of protexin probiotic, the length, width, height and mucosal muscle increase, but no significant differences were observed in this regard (p≤0.05).

The mutual effects of the combined consumption of pomegranate peel powder and protexin probiotic increased the width, length, and height of the intestinal villi and the mucous muscle of the intestinal tissue of Japanese quails compared to the control group, although no significant difference was observed in this regard.

Table3-The main and reciprocal effects of pomegranate peel powder and protexin probiotic on Intestinal morphology in Japanese quail.

Treatments	Villus length (Micron)	Villus width (Micron)	Villus height (Micron)	Muscularis (Micron)
Pomegranate peel (%)				
0	42.42	7.00	4.36	1.01
0.2	48.37	7.13	5.15	1.18
0.4	50.23	7.68	5.36	1.19
0.6	54.31	8.32	6.17	1.25
Protexin probiotic (%)				
0	48.29	7.16	5.02	1.00
0.1	52.11	7.68	5.55	1.19
Pomegranate × Protexin				
Control	45.45	7.14	4.10	1.00
0.1 Protexin	47.48	7.38	4.67	1.11
0.2 Pomegranate with no Protexin	49.14	8.19	5.11	1.08
0.4 Pomegranate with no Protexin	50.38	8.38	5.26	1.26
0.6 Pomegranate with no Protexin	52.51	8.65	5.45	1.45
0.2 Pomegranate with 0.1Protexin	54.16	9.11	5.16	1.66
0.4 Pomegranate with 0.1Protexin	56.69	9.39	6.98	1.86
0.6 Pomegranate with 0.1Protexin	58.34	10.11	7.20	1.89
SEM	0.814	0.746	0.751	0.748
P- value	0.126	0.324	0.326	0.331

*Means within row with no common on letter are significantly different ($p \leq 0.05$).

The result of intestinal microbial population determination showed that the of Escherichia coli pathogenic bacteria colonies decreased and the population of beneficial Lactobacillus bacteria increased by pomegranate peel powder and protexin probiotic supplementation. The maximum increase of lactobacillus population and the minimum decrease of Escherichia coli in the experimental groups were shown with higher levels of pomegranate peel powder and probiotic protexin, although no significant difference was observed between the treatments. Table 4.

Sunder et al (2013) showed that the quails on experimentais diets had the highest relative weight in liver and heart, as well as villus height, crypt depth compared to control. Ratio of villi length to villi depth in diets substituted with 5.0 and 7.5 percentage of pomegranate peel powder had the highest values as compared to other treatments.

Eid et al (2021) noted that the addition of pomegranate peel powder led to an increase in the level of glucose, cholesterol and triglycerides in the blood plasma of birds at the expense of the control treatment. Results related to levels of

cholesterol and triglycerides in the blood, obtained in the current study, were consistent with other studies which found a decrease in the level of fats and glucose as a result of feeding birds pomegranate peels, which may be due to the high percentage of fiber which is associated with bile salts in the intestine, reduces intestinal transit time, and increases bile secretion (ABBAS et al.,2017, SHARIFIAN et al., 2019).

Table 4-The main and reciprocal effects of pomegranate peel powder and pprotexin probiotic on intestinal microbial population count in Japanese quail.

Treatments	Escherichia coli (CFU)	Lactobacillus (CFU)
Pomegranate peel (%)		
0	6.2	4.5
0.2	6.0	4.8
0.4	5.8	4.9
0.6	5.5	4.9
Protexin probiotic (%)		
0	6.1	4.7
0.1	6.0	4.3
Pomegranate × Protexin		
Control	5.9	4.6
0.1 Protexin	5.5	4.9
0.2 Pomegranate with no Protexin	5.4	5.1
0.4 Pomegranate with no Protexin	5.4	5.4
0.6 Pomegranate with no Protexin	5.3	5.5
0.2 Pomegranate with 0.1Protexin	5.1	5.7
0.4 Pomegranate with 0.1Protexin	5.0	5.6
0.6 Pomegranate with 0.1Protexin	4.8	5.9
SEM	0.122	0.291
P- value	0.235	0.367

*Means within row with no common on letter are significantly different ($p \leq 0.05$).

The result of the current study showed that the live body weight, carcass weight and heart efficiency percentage of Japanese quails in diets receiving different levels of pomegranate peel powder, protexin probiotic. Abbas et al (2017) also showed that pomegranate peel powder has the ability to increase the size of the heart, with resultant increase in oxygen and nutrient supply as blood, which was reflected in the growth of the birds. Saki et al (2019) found a decrease in the level of fats and glucose because of feeding birds pomegranate peels, which may be due to the high percentage of fiber. The lower cholesterol in the blood and triglycerides by PPP could possibly be due to phenol compounds such as pontiacagen and pontiacin, in particular and it may stimulate

pomegranate polyphenols and promote cholesterol metabolism by modifying HDL transport (ESMAEILLZADEH et al.,2004).

The beneficial effects of herbal plants and probiotics or their active substances may include the stimulation of appetite and increase feed intake (Gunal et al.,2006), the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial, and antioxidant activities (Kheiri et al., 2015). Ghazaleh et al. (2013) reported that the tannins in pomegranate peels treat stomach acidity that it is one of the main causes of which is an increase in the secretion of hydrochloric acid from the stomach, or due to a disruption of the acid neutralization process inside the stomach due to a lack of some enzyme.

Javed et al (2006) suggested that there is a possibility of gathering these to antimicrobial herbs made a remarkable decrease in the intestine microbial pathogen colonies and this prevented from lyses of amino acids and they may use in formation of protein issues and increased the breast percentage. Ahamdipour et al (2018) showed that the existence of harmful microbes in digestive system causes an increase protein and amino acids lysis of nutrients, di-amination activity of proteins and amino acids and rapid decomposition of these molecules due to secretary substances from bacteria like urease and dietary pomegranate peels can improve the digestion and metabolism of fatty acids and have antibacterial and anti-atherosclerotic activity.

Conclusion

Based on the results mentioned above we could demonstrate that the beneficial facts of pomegranate peel powder and protexin probiotic on carcass traits, blood parameters, tissue histomorphology and intestinal microbial population in experimental Japanese quails. This improvement may be due to the biological functions of pomegranate peel improve growth or that may be due to its role as stimulant, carminative, enhanced digestibility and antimicrobial and antioxidant properties. The lack of widespread data quails feed with supplemental pomegranate peel powders the diets is available. Therefore, further studies are needed for more explanation

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