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Research Article

Effect of stocking density and supplementation of potato peel (*Solanum tuberosum* L.) powder and extract on the physiological and immunological performance of Japanese quail, *Coturnix coturnix japonica*

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Abstract
This study aimed to investigate the effects of stocking density and supplementation of potato peel (*Solanum tuberosum* L.) powder and extract on Japanese quail's physiological and immunological performance. A total of 810 quail chicks were randomly assigned to six dietary treatments and two stocking densities in a complete randomized design with a 6×2 factorial scheme. The quail were kept with a stocking density of 333cm^2/birds (15 birds/replicate), and 167cm^2/birds (30 birds/replicate). The first treatment served as a control diet without supplementation (T1). T2 and T3 were supplemented with potato peel extract (PPE) at 15, and 30ml/l in drinking water; T4, T5 with 15, and 30g/kg potato peel powder (PPP) in basal diet, and T6 with PPE at 15m/l+PPP at 15g/kg. A significant improvement in WBC (T2 and T6), total protein and globulin, and a significant decrease in relative weight of spleen in T3 and T6, bursa of fabricus and bursa index in all treatments, albumin in T2 and T3, glucose, and cholesterol were observed compared to control. Immune response, and value of antibodies titer against Newcastle and Gumboro disease were significantly better than control. In conclusion, the supplementation of PPE, PPP and their mixture showed the best physiological and immune response. Furthermore, the low density performed better in most studied traits.

Keywords: Quail, Potato peel powder and extract, Biochemical parameters, Immunity.

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Introduction

Stocking density, sensitivity to pathogens, metabolic complaints, breeding ability, and suitable slaughter age could affect overall economic performance in the poultry industry (Baeza et al. 2012; Berg & Yngvesson 2012; Kryeziu et al. 2018). Environmental stressors, including stocking density, humidity, and temperature, are crucial factors for the overall performance in this industry too (Gharaghani et al. 2015; Pompeu et al. 2018). Stocking density is one of the most common stressors in the poultry industry. The advantages of stocking density include enhancing productivity, using restricted space, and increasing income (Majid et al. 2020).

The potato peel has received excessive attention as an herbal antioxidant and is documented to have antioxidant effects 10 times greater than its flesh with approximately 50% of all of the polyphenolic contents found in potato tuber (Javed et al. 2019). There is interest in herbal antioxidants as useful components because they provide cell safety to oxidative damages restricting the danger of degenerative illnesses (Liang et al. 2014, 2015). There is a strong relationship between total phenol content in the extract and antioxidant properties; however, total polyphenol is decreased in potato peel during storage at -20ºC. Therefore, correct storage conditions in potato peels to keep their antioxidant properties are crucial (Al-Weshahy & Rao 2009; Al-Weshahy et al. 2013). Alansari (2020) pointed out that adding rosemary oil to potato starch edible films improves the oxidative stability of foodstuff. Since using by-products in food with dietary benefits is economically attractive (Pathak 2015). Therefore, this study aimed to investigate the effect of stocking density and supplementation of potato peel powder and its extract on some hemato-biochemical parameters and immune response of Japanese quails.

Materials and Methods

Animals, diets, and management: The experiment was conducted in a quail farm, the Department of Animal Production, College of Agriculture, the University of Basra, from 28 December 2020 to 7 February 2021. A total number of 810 Japanese quail (*Coturnix coturnix japonica*) chicks were used in this study. Six dietary treatments and two stocking densities were used in a completely randomized design with a 6×2 factorial arrangement of treatments. The quail chicks were kept in cages of 100×50cm^2 with a stocking density of 333 cm^2/birds (15 birds/replicate, 167cm^2/birds, and 30 birds/replicate). Chicks were fed six experimental diets from 7 to 42 days as a control diet without supplementation (T1). In the second and third treatments, the potato peel extract (PPE) was supplemented at 15 and 30ml/l in drinking water; in T4 and T5, 15, and 30g/kg potato peel powder (PPP) were added in the basal diet, and for treatment six, potato peel extract at 15ml/l+potato peel powder at 15g/kg were added, respectively. A basal diet was formulated according to NRC (1994) (Table 1). All chicks were kept under constant management conditions throughout the experiment period of 42 days.

Preparation of potato peel powder (PPP): Potato peels (*Solanum tuberosum* L.) were collected from some restaurants in Basra City. The collected potato peels were washed, sundried, crushed into a meal, and stored for later use.

Chemical composition of potato peels powder: Proximate composition of the potato peel powder was analyzed based on AOAC (2016) (Table 2).

Preparations of potato peel extract (PPE): To prepare the methanol extract of potato peel powder, 10g of the ground materials were used to extract by 100ml of methanol alcohol overnight in a shaker at room temperature, followed by filtration through Whatman No.1 filter paper. The residues were re-extracted under the same conditions. The combined filtrates were evaporated in a rotary evaporator below 40°C. The potato peel extracts obtained after evaporation of methanol were stored at -20°C for further use (Mohdaly et al. 2010).

Hematological and Biochemical traits: On day 42, six birds (three males + three females) were selected randomly from each group and slaughtered to collect blood samples in tubes containing anticoagulant (EDTA). The hematologicall analysis includes red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), and hemoglobin (Hb). For biochemical traits, the blood was collected in tubes without anticoagulant, allowed for an hour at room temperature for serum collection, then serum separated by centrifuge at a speed of 3000rpm for 15min and then stored at -20˚C for further analysis. Serum contents, including total protein, albumin, globulin, cholesterol, and glucose, were analyzed by a spectrophotometer using a commercial kite (Biolabo, SAS, France).

Immunological tests: Regarding immune response, six birds from each group were randomly chosen, weighed, slaughtered, eviscerated, and dressed. The relative weight for lymphoid organs, spleen and

Table 1. Ingredients and nutrient composition of quail basal diets.

Ingredients	Basal diet (%)
Maize	50.00
Wheat	8.75
Soybean meal (48%)	34.00
[1]protein concentrates (44%)	5.00
Dicalicum phosphate	0.50
Limestone	1.00
Mineral premix	0.30
Sodium chloride	0.30
DL-methionine	0.15
Total	100
Calculated composition[2]	
Metabolizable energy (Kcal /Kg)	2921
Crude protein	24.00
Crude fat	2.97
Crude fiber	2.54
Calcium (%)	0.84
Phosphorus available	0.36
Lysine	1.30
Methionine	0.48
Methionine + Cysteine	0.82

[1]Protein concentrate used from Al-Hayat Company, Jordanian origin, to provide the following per kg of diet: 44% protein, 2800 kcal/kg ME, 12% fat, 25% ash, 5% calcium, 2.9% phosphorus, 2.55% methionine+Cysteine, 2.8% lysine. The chemical composition [2]Was calculated according to of feedstuff contained in NRC (1994).

bursa of Fabricius were expressed as a percentage of live body weight. Blood samples were collected during slaughtering, allowed to clot, and then centrifuged immediately to separate serum and stored at -20˚c until the determination of antibody titer against Newcastle (ND) and Gumboro diseases (IBD) by the hem-agglutination inhibition test using ELISA test kit.

Statistical analysis: An experiment was assigned in a complete randomized design with a 6×2 factorial scheme (3 replications each). Data were analyzed based on variance analysis at a 5% significance level. Duncan's multiple ranges tests were used if the treatment indicated significant effects (Duncan's 1955).

Results and Discussion

The effect of stocking density and experimental treatments on the hematological parameters of quail are shown in Table 3. The results indicate that RBC and PCV did not affect, whereas a significant ($P≤0.05$) improvement was observed in WBC of T2 and T6. Birds fed diets supplemented with 15 PPP g/kg+15 PPE m/l (T6) showed significantly ($P≤0.05$) higher hemoglobin concentration and lower heterophils: lymphocytes (H/L) ratio. Regarding stocking density effects, the low density (D1) showed a higher ($P≤0.05$) WBC count and lower hetrophils: lymphocytes (H/L) ratio in comparison with the high density (D2). This positive effect of these parameters may be attributed to the vital function of potato peels. Phenolic compounds of potatoes are phenolic acids and flavonoids, including flavonols and anthocyanins (Deusser et al. 2012). The relationship between polyphenol intake and reduced incidence of some diseases has been noticed in several studies (Akyol et al., 2016); however, their positive effects could not be given only to their antioxidant properties. The health benefits of polyphenols are assigned to some "non-antioxidant" complex activities that could not be linked to free radical inhibition (Andre et al. 2014). Blood haematological parameters serve as indicators of the physiological state of birds (Mohammed et al. 2015). The antioxidant potential of potato peel extracts was examined as a defensive towards erythrocytes oxidative induced *in vitro*

Table 2. Chemical analysis of potato peel powder.

Compounds	Percentage%
Dry matter	88.60
Moisture	11.40
Crud protein	12.23
Crud fiber	2.31
Total Ash	1.82
Crud fat	0.22
Nitrogen free extract	83.42
Organic mater	98.18
*Metabolic energy kcal/kg	3431.74

*ME was calculated by the equation described by (Pauzenga 1985).

damage, observing morphological and structural variations in the cell membrane (Javed et al. 2019). Medicinal plants or their extract improve quails' productive and physiological traits (Abbas et al. 2017; AL-Salhie & AL-Waeli 2019). Our results disagree with Kpanja et al. (2020), who observed no effect of the test material on hematological parameters of broiler chicks when using graded levels of potato peels meal in their diet. Also, our findings disagree with Beloor et al. (2010), who found that the H:L ratio is independent of stocking density at 15, 20, and 25 birds/m2 in broiler chicks.

Table 4 indicates the effect of stocking density and experimental treatments on blood biochemical parameters of quail. The biochemical changes showed significant ($P\leq0.05$) improvements in total protein and globulin concentrations compared to control, while albumin (in T2, T3, and T6), glucose, and cholesterol levels were significant ($P\leq0.05$) decreased by the dietary treatments of broilers. The improvements in total protein and globulin in chicks fed potato peel powder (PPP) and its aqueous extract (PPE) may be due to potato peel antioxidant and antimicrobial properties, supporting birds' immune systems. In a previous study, potato peel extract was inspected for antimicrobial activity and found to be potentially effective against bacterial and fungal species. As compared to antibiotic (Streptocycline), potato peel extract effects significantly against *Clavibacter microgenesis* and *Pseudomonas aeruginosa* (Prasad & Pushpa 2007). Gebrechristos et al. (2020) reported that potato peel extract has antibacterial and antioxidant properties, and incorporated with potato starch, produces an active film that can be used for active food packaging.

Low stocking density (D1) significantly affected serum total protein and globulin levels. The result showed an interaction effect of stocking density and supplementary diets on the content of serum total protein, globulin, and cholesterol. A significant effect on the interaction of PPP and PPE and stocking density may be due to the potato peels content of Phenolic compounds, which have antioxidant activity and other features that could encourage health (Deusse et al. 2012). Also, chlorogenic acid, which is extensively found in potato samples, is caused an increase in insulin sensitivity, decreases gut glucose absorption, and prevents gluconeogenesis (Ong et al. 2013; Andre et al. 2014). In contrast, Raphael (2017) reported that serum albumin level was highest in broiler fed 100% sun-dried Irish potato peel meal.

Inclusion of PPE and PPP and their mixture in the diet decreased ($P\leq0.05$) total plasma cholesterol and glucose levels. The polyphenol and anthocyanin are rich in purple potato that play an important role in the safety against oxidative harm in rats fed a high-cholesterol diet (Ezekiel 2013). Althawab (2019) showed that the antioxidant ingredients in the peel of the purple sweet potato improved the lipid status in

Table 3. Effect of stocking density and experimental treatments on hematological parameters of quail at 42 days.

Parameters	Density	T1(control)	T2 (15 m/l PPE)	T3 (30 m/l PPE	T4 (15g/kg PPP)	T5 (30 g/kg PPP)	T6 (15 PPP + 15 PPE)	Mean	SEM	*P-value**
		Experimental diets								
RBC (10^6/ mm^3)	D1	2.69	3.52	3.46	3.75	3.32	3.40	3.35	0.11	T=0.37
	D2	3.62	3.60	3.24	3.46	3.46	3.58	3.49	0.07	D= 0.29
	Mean	3.15	3.56	3.35	3.61	3.39	3.49	3.42	0.06	T×D=0.12
WBC 10^3/mm^3))	D1	29.26	34.90	31.84	32.10	32.10	31.93	32.02^{A}	0.61	T = 0.01
	D2	27.66	32.16	29.83	30.60	30.04	32.58	30.48^{B}	0.57	D=0.05
	Mean	28.46^{b}	33.53^{a}	30.83^{ab}	31.35^{ab}	31.07^{ab}	32.25^{a}	31.25	0.43	T×D=0.84
Hb (g/100ml)	D1	14.10	16.02	16.14	16.01	15.80	15.50	15.59	0.28	T = 0.18
	D2	14.95	15.76	14.98	14.99	15.91	16.76	15.56	0.25	D= 0.9
	Mean	14.53^{c}	15.89^{b}	15.56^{b}	15.50^{b}	15.85^{b}	16.13^{a}	15.58	0.18	T×D=0.32
PCV%	D1	36.66	49.66	46.66	43.00	46.66	45.66	44.72	1.66	T = 0.39
	D2	52.66	43.33	50.00	44.00	42.66	54.33	47.83	1.47	D=0.12
	Mean	44.66	46.50	48.33	43.50	44.66	50.00	46.27	1.12	T×D=0.06
H/L	D1	0.29	0.19	0.16	0.19	0.19	0.16	0.19^{B}	0.010	T=0.001
	D2	0.34	0.21	.210	0.23	0.25	0.18	0.23^{A}	0.012	D=0.001
	Mean	0.31^{a}	0.2^{c}	0.18^{d}	0.21^{b}	0.22^{b}	0.17^{e}	0.21	0.008	T×D=0.01

^{abc} Means in the same row with no common superscript are different at $P \leq 0.05$, ^{ab}Means in the same column with no common superscript are different at $P \leq 0.05$. SEM: Standard error of the mean. PPE: Potato peel extract; PPP: Potato peel powder, *P-value**, T= Treat effect, D: Density effect, T×D = Interaction effect, D1: 16 chicks/replicate; D2: 30chicks/replicate.

the blood of mice fed a high-fat diet after giving them peel extract with fermented cow's milk at a level of 1 and 2%. Nasoetion et al. (2019) reported that the addition of purple sweet potato extract at a level of 25ml/ kg led to a reduction in cholesterol and low-density lipoproteins in the serum of broiler chickens raised in different densities. Furthermore, the control of lipid oxidation associated with stocking density entails the supplementary of normal antioxidant factors to block the production of free radicals (Xiao et al. 2021). Our results are in line with Arun et al. (2015) that reported that a freeze-dried powder of potato peel causes a remarkable decrease in blood glucose levels and efficiently reduces the diabetic change in rats. In contrast to our study, Park et al. (2018) reported that a high stocking density increased total cholesterol and triacylglycerol levels in the blood of broilers. Also, Majid et al. (2020) observed a mild and humid climate with 20 chick density treatments (high density) had the highest cholesterol levels, and Qaid et al. (2016) also reported that blood glucose increased due to stress caused by high stocking density.

The present results indicate a significant improvement in the relative weight of lymphoid organs (spleen and bursa of Fabricus), bursa index, antibody titers against Newcastle (ND), and Gumboro diseases (IBD) compared to control one (Table 5). A significant improvement was found in antibody titers against IBD and ND for low stocking density, while higher values were achieved at the higher density in the spleen. A significant interaction effect of stocking density and supplementary diets on serum antibody titers' content against IBD was also observed. It is widely known that high stocking density induces stress responses in broilers (Al-Benna et al. 2006). The antioxidants found in herbs and their extracts reduce stress on birds. Potato peel

Table 4. Effect of stocking density and experimental treatments on blood biochemical parameters of quail at 42 days.

Biochemical Parameters	Density	T1 (control)	T2 (15 m/l PPE)	T3 (30 m/l PPE	T4 (15g/kg PPP)	T5 (30 g/kg PPP)	T6 (15PPP + 15 PPE)	Mean	SEM	*P-value**
		Experimental diets								
Total Protein (g/dl)	D1	4.37	5.15	5.58	5.57	4.63	5.58	5.15^{A}	0.12	T=0.02
	D2	3.97	5.29	4.35	4.17	5.44	5.49	4.78^{B}	0.17	D= 0.01
	Mean	4.17^{c}	5.22^{ab}	4.96^{b}	4.87^{b}	5.03^{b}	5.53^{a}	4.96	0.11	T×D=0.001
Albumin (g/dl)	D1	2.49	1.84	1.89	2.68	2.16	2.12	2.20	0.10	T = 0.01
	D2	2.27	1.68	1.68	1.95	2.74	1.93	2.04	0.11	D=0.23
	Mean	2.38^{a}	1.76^{c}	1.79^{c}	2.31^{a}	2.45^{a}	2.02^{b}	2.12	0.07	T×D =0.14
Globulin (g/dl)	D1	1.88	3.30	3.68	2.89	2.46	3.46	2.95^{A}	0.16	T = 0.001
	D2	1.70	3.61	2.67	2.22	2.69	3.55	2.74^{B}	0.17	D=0.02
	Mean	1.79^{c}	3.46^{a}	3.17^{a}	2.55^{b}	2.58^{b}	3.51^{a}	2.84	0.11	T×D = 0.001
Cholesterol (mg/dl)	D1	153.49	128.96	115.79	118.90	119.06	122.80	126.50	3.25	T = 0.001
	D2	138.46	127.51	115.74	128.62	122.32	118.81	125.24	2.00	D=0.43
	Mean	145.97^{a}	128.23^{b}	115.77^{d}	123.76^{b}	120.69^{cd}	120.81^{cd}	125.87	1.88	T×D 0.001
Glucose (mg/dl)	D1	179.20	150.83	137.91	154.58	158.52	159.34	156.73	4.73	T = 0.001
	D2	178.40	148.28	138.78	177.51	167.31	166.57	162.81	4.05	D=0.23
	Mean	178.80^{a}	149.55^{bc}	138.35^{c}	166.06^{b}	162.92^{b}	162.96^{b}	159.77	3.11	T×D =0.69

abc: Means in the same row with no common superscript are different at $P \leq 0.05$, AB: Means in the same column with no common superscript are different at $P \leq 0.05$. SEM: Standard error of the mean, PPE: Potato peel extract; PPP: Potato peel powder, P-value*, T= Treat effect, D: Density effect, T×D = Interaction effect, D1: 15 chicks/replicate; D2: 30chicks/replicate.

Table 5. Effect of stocking density and experimental treatments on immunological parameters of quails at 42 days.

Immunological Parameters	Density	T1(control)	T2 (15 m/l PPE)	T3 (30 m/l PPE	T4 (15g/kg PPP)	T5 (30 g/kg PPP)	T6 (15PPP + 15 PPE)	Mean	SEM	*P-value**
		Experimental diets								
Spleen%	D1	0.08	0.05	0.03	0.07	0.0	0.02	0.05^{A}	0.010	T=0.001
	D2	0.09	0.05	0.05	0.08	0.05	0.04	0.06^{A}	0.012	D= 0.001
	Mean	0.08^{a}	0.05^{c}	0.04^{d}	0.07^{b}	0.05^{c}	0.03^{e}	0.05	0.008	T×D= 0.17
Bursa of fabric us %	D1	0.04	0.03	0.03	0.02	0.02	0.02	0.03	0.001	T = 0.001
	D2	0.04	0.02	0.02	0.03	0.02	0.02	0.02	0.002	D=0.07
	Mean	0.04^{a}	0.03^{b}	0.03^{b}	0.03^{b}	0.02^{b}	0.02^{b}	0.03	0.002	T × D =0.05
Bursa Index	D1	1.00	0.82	0.85	0.74	0.68	0.56	0.77	0.04	T = 0.001
	D2	1.00	0.64	0.65	0.74	0.6	0.72	0.72	0.03	D= 0.11
	Mean	1.00^{a}	0.73^{a}	0.75^{b}	0.74^{b}	0.64^{b}	0.64^{b}	0.75	0.03	T × D = 0.03
Antibody titers against ND	D1	3675.67	6468.33	5706.33	4486.67	4589.33	6550.33	5246.11^{A}	264.19	T = 0.001
	D2	3418.00	5751.67	5249.33	4178.00	4275.66	6505.33	4896.33^{B}	258.37	D=0.001
	Mean	3546.83^{e}	6110.00^{b}	5477.83^{c}	4332.33^{d}	4432.5^{d}	6527.83^{a}	5071.22	184.38	T × D = 0.33
Antibody titers against IBD	D1	2113.33	3328.00	3962.67	2551.67	3043.33	3843.00	3140.33^{A}	163.81	T = 0.001
	D2	2056.00	3271.33	2976.00	2309.00	2635.67	3245.33	2748.89^{B}	125.05	D=0.001
	Mean	2084.67^{d}	3299.67^{a}	3469.33^{a}	2430.33^{c}	2839.50^{b}	3544.17^{a}	2944.61	106.75	T × D = 0.01

abc: Means in the same row with no common superscript are different at $P \leq 0.05$, AB: Means in the same column with no common superscript are different at $P \leq 0.05$. SEM: Standard error of the mean, PPE: Potato peel extract; PPP: Potato peel powder, *P-value**, T= Treat effect, D: Density effect, T×D = Interaction effect, D1: 15 chicks/replicate; D2: 30 chicks/replicate, ND: Newcastle disease, IBD: Gumboro disease**.**

is rich in bioactive compounds, including antioxidants; the most important characteristic of peel is due to containing phenolic acids and flavonoids, including flavonols, and anthocyanins (Deusser et al. 2012). Potato peels seem to constitute a potential source of natural antioxidants used in the food industry (Carocho et al. 2018). The antioxidant effect of the extract resulted in low levels of peroxide value and carbonyl compounds (Sabeena et al. 2012). In this regard, Wang et al. (2016) revealed the antioxidant mechanism of immunomodulatory activity of sweet potatoes. Wang et al. (2016) found that water extract of sweet potato has the highest immunomodulatory activity by stimulated T- and B-cell proliferation and pro-inflammatory cytokine production, water extract restricted the production imbalance of the Th1-type, and Th2-type cytokine (Kim et al. 2015). Regarding density effect, Majid et al. (2020) noted that the density of 20chicks/m2 (high density) at 42 days of age had the lowest immune response, whereas, Houshmand et al. (2012) also showed the relative weight of the bursa and spleen were not affected by stocking density.

Conclusion

It is concluded that supplementation of PPE and PPP and their mixture achieved the best physiological and immune response in quails, with an interaction effect of stocking density and supplementary diets on the in hetrophils: lymphocytes (H/L) ratio, Hb%, the content of total protein, globulin, cholesterol and antibody titers against IBD of quails.

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