J. of Animal and Poultry Production, Mansoura Univ., Vol . 13 (2):33 - 41, 2022

Journal of Animal and Poultry Production

Journal homepage & Available online at: www.jappmu.journals.ekb.eg

Effect of Dietary Licorice Supplementation on Performance of Growing Japanese Quail Reared in High Stocking Density

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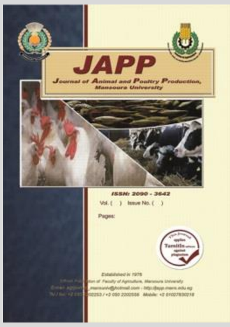

ABSTRACT

This study was conducted to evaluate the influence of dietary levels of licorice on quail performance stocked under high stocking density. A total number 255 one week old unsexed quail were randomly distributed into six groups. Chicks 1 were fed on a basal diet without licorice and reared at normal stoking density (10 birds/ replicate). The chicks of groups 2,3,4,5 and 6 distributed to be in high stocking density (15 birds/ replicate) and fed on basal diet supplemented with licorice at levels of 0.0, 0.3, 0.6, 0.9 and 1.2 g/kg diet, respectively. Addition of 0.9g licorice/kg diet to quail reared in HD increased LBW and BWG at wk 3 and 6. The quail chicks housed at HD without or with dietary supplementation at 0.3 g/kg presented higher values ($p<0.05$) of plasma TC, TG, LDL and lower plasma HDL and VLDL values. IgA and IgM levels were higher ($p<0.01$) in the quail chicks kept under HD treated with 0.9 g licorice /kg diet. Plasma SOD was higher ($p<0.05$) in the experimental group housed under HD treated with 0.6 g licorice/kg diet comparatively with other experimental groups. There were no significant differences in digestive enzymes, total yeasts, molds count and enterococcus SPP. In conclusion, increasing stocking density of growing Japanese quail from 10 to 15 negatively affect their performance. But, supplementing diets with licorice could reduce these negative effects and improve the performance at high stocking density.

***Keywords**:* licorice, high stoking density, performance, health, quail.

INTRODUCTION

In recent years, poultry meat production has witnessed development to face the increasing demand in the market. Hence, the production for poultry turned to quail intensive breeding (Aboul- Ela 2005). So, high stocking density (HD) is considered one of stressful factor which have deleterious impact on quail performance, and health as it reduction bird access to water and feed (Thaxton *et al.*, 2006; Pandurang *et al.*, 2011; Mahrose *et al.*, 2019; Bilal *et al.*, 2021).

Many studies have reported the impacts of HD on broiler production have been reported (Vargas, 2013), while others observed decrease in the performance due to stocking density (Whietehead and Keller, 2003, Capo and Davila, 2002; Zhang *et al.*, 2013). Some studies showed that, inclusion of natural or synthetic antioxidants in poultry diet could enhance feed consumption and alleviate deleterious impacts of oxidative stress (Wang *et al.*, 2008; Alagawany *et al.*, 2022; Alagawany and Abd El-Hack 2021).

Glycyrrhiza glabra (licorice) is a plant belonging to the Fabaceae (Shebl *et al.*, 2012; Alagawany *et al.*, 2019a).

According to phytochemical analysis, licorice contains flavonoids, glycyrrhetinic acid, glycyrrhizin, asparagine, isoflavonoids, and chalcones (Tiwari *et al.*, 2018; Fiore *et al.*, 2005). Licorice considers a herbal product, antioxidative, antimicrobial, anti-inflammatory, radical scavenging activities, antiatheroclerotic, antinephritic, oestrogen rich beverage antiviral, and pharmaceutical hematic pills (Fukai *et al.*, 2003, Shalaby *et al.*, 2004).

Several pharmacological impacts and health benefits have been reported for licorice active principles in animals (Nakgawa *et al.*, 2004; Aoki *et al.*, 2007; Rackova *et al.*, 2007, Suchitra and Shakunthala 2014, Moradi *et al.*, 2014; Parvaiz *et al.*, 2014; Reda *et al.*, 2021a; Dosoky *et al.*, 2021). Therefore, the present study was designed to assess the effect of licorice on growing Japanese quail performance when reared at high stocking density.

MATERIALS AND METHODS

A total number of 255 one week old unsexed Japanese quail chicks were used in this experiment. The chicks were randomly distributed into six groups with three replicates of each. Group 1 was reared in normal stocking density (10 birds/ replicate) and fed on the basal diet without licorice (*Glycyrrhiza glabra*) supplementation and served as control. Chicks in other groups (2, 3, 4, 5 and 6) were reared in high stocking density (15 birds replicate) and fed in basal diet supplemented with licorice at 0, 0.3, 0.6, 0.9 and 1.2 g/kg diet, respectively.

Licorice root powder was purchased from the commercial market, Cairo, Egypt. The basal diet prepared form soybean meal corn diet and was formulated to meet the nutrient requirements of quail chicks from 1 to 5 weeks of age.

The composition and calculated analysis were presented in Table 1.

Performance and carcass parameters were determined according to Reda *et al.* (2021a).

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DOI: 10.21608/jappmu.2022.121540.1030

Table 1. Composition and calculated analysis of the experimental basal diet.

Items	g/kg
Ingredient	
Yellow Corn, 8.5%	518.0
Soybean meal, 44%	367.0
Gluten meal, 62%	52.1
Soybean oil,	29.0
Limestone	7.0
Di-calcium phosphate	16.5
Salt	3.0
Premix[1]	3.0
L-Lysine	1.3
Dl-Methionine	1.1
Choline chloride	2.0
Calculated composition[2]	
Metabolizable energy (MJ/kg)	12.53
Crude protein (g/kg)	240.0
Calcium (g/kg)	8.0
Nonphytate phosphorus (g/kg)	4.5
Lysine (g/kg)	13.0
Total sulfur amino acids (M+CY; g/kg)	9.2

1 Provides per kg of diet: Vitamin A, 12,000 I.U; Vitamin D3, 5000 I.U; Vitamin E, 130.0 mg; Vitamin K3, 3.605 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B_2 (riboflavin), 8.0 mg; Vitamin B6, 4.950 mg; Vitamin B_{12}, 17.0 mg; Niacin, 60.0 mg; D-Biotin, 200.0 mg; Calcium D-pantothenate, 18.333 mg; Folic acid, 2.083 mg; manganese, 100.0 mg; iron, 80.0 mg; zinc, 80.0 mg; copper, 8.0 mg; iodine, 2.0 mg; cobalt, 500.0 mg; and selenium, 150.0 mg.

2 Calculated according to NRC (1994).

Biochemical characteristics

Individual blood samples were obtained into EDTA tubes from 4 birds within each treatment (on individual basis) at 5 weeks of age to determine the distinct biochemical features. After centrifugation, low density lipoproteins (LDL), very low-density lipoprotein, high-density lipoproteins (HDL), triglyceride, and total cholesterol were determined by enzymatic methods. Regarding immunity indices, immunoglobulin G (IgG) and A (IgA) levels in the plasma were determined. The authors determined the level of complement (C3) in quail plasma, using the ELISA Kit from MyBiosource.com. Concerning antioxidant parameters, superoxide dismutase (SOD), glutathione peroxidase (GPX) activity and total antioxidant capacity (TAC) were determined according to Koracevic *et al.* (2001). Malondialdehyde-MDA was determined according to Uohiyama and Mihara, (1978).

Digestive enzymes and microbiological analyses

Digestive enzymes were determined (Tietz and Fiereck, 1966 and Lynn and Clevette-Radford, 1984). Regarding microbiological analyses, 10 g of cecal content of quail were separately transferred to a 250 ml Erlynmayar flask to determine the count of total yeasts, and molds count, *E. coli***,** *Salmonella* spp**.**, *Enterococcus* spp**.**, and *Coliform according to* Reda *et al*. (2020a,b and 2021b).

Statistical analysis

The differences among treatments were statistically analyzed by one-way ANOVA using the SAS General Linear Models Procedure (SAS, 2002) by adopting the following model:

$$X_{ij} = \mu + T_i + e_{ij}$$

Where: **X_{ij} = An observation, μ = Overall mean, T_i = Effect of treatments (i = 1, 2, …… and 6). e_{ij} = The experimental random error. Tukey's Multiple Range-test (P<0.05) was used to study the significant differences between treatment means.**

RESULTS AND DISCUSSION

Growth performance

Results in Table 2 revealed that, using HD without or with dietary licorice supplementation at 0.3 g/kg resulted significant decrease (p<0.05) in LBW at wk 3 and 5 and BWG through all the experimental periods studied (1-3, 3-5 and 1-5 weeks of age. However, the addition of 0.9 g licorice/kg diet to quail chicks reared in HD resulted significantly (p<0.05) increase in LBW and BWG at wk 3 and 5. It is worth to note that the supplementation of licorice to HD diets at levels 0.6 or 1.2 g/kg diet achieved LBW and BWG equal to the ND and did not significantly different.

The lake effect of HD on changing LBW and BWG were consistent with El-Tarabany *et al.*, (2016); Congiz *et al.*, (2015) who observed that, reduced in the LBW and BWG in growing quails at high stocking density. Recently, Gholami *et al.*, (2020) found that final BW was higher in all experimental treatments reared at 10 chicks/m^2 than other density treatments reared at 15, 17 and 20 chicks/m^2 in the different climates.

In the same line, Anwer and Hamed (2020) found a significant increase in LBW and BWG in broiler chicks stocked at either 52 birds/m^2. Likewise, Shewita *et al.*, (2019) stated that, broiler chicks stocked in HD showed a reduction of their final BW when compared with reared at LD. Moreover, Rashidi *et al.*, (2018) stated that BWG of broiler chicks was negatively affected (p < 0.05) due to increasing density in the growing period.

In addition, Boontiam *et al.*, (2018) reported that, final LBW and BWG of growing quails were reduced in the treatment stocked at 25 quail/m^2compared with those reared at either 13 or 7 quail/m^2. In contrast to our findings, Vargas Roderiguez *et al.*, (2013) demonstrated that, BWG did not differ significantly between high (16 birds/m^2) and low (10 birds/m^2) density. Similarly, Houshmand *et al.*, (2012) showed that BW and BWG of broilers were not affected by HD.

In the same line, stocking density in Japanese quails did not affected significantly of either BW (Abdel-Hakim *et al.*, 2005; El-Sagher *et al.*, 2012) or BWG (Dhaliwal *et al.*, 2008). In our study, The improvement of LBW at 3 and 6 weeks of age and BWG through 1-3 and 3-5 periods in growing Japanese quail due to addition of 0.9 g licorice/kg diet consistent with Sedghi *et al.*, (2010) and Salary *et al.*, (2014) who observed significantly increase of BW and BWG of broilers when supplementing their water (0.2 and 0.4%) or diet (0.05, 0.1 and 0.2%), respectively with extract of licorice.

In the same line Rashidi *et al.*, (2018) found that, at high density, BWG was increased by licorice extract supplementation in their diet. Also Lashin *et al.*, (2017) stated that, BW was improved by increasing licorice levels. In contrast, of our finding, Kalantar *et al.*, (2017) found BWG (up to 42 days) of broiler chicks was reduced due to dietary licorice supplementation. Other studies showed no significant impacts of licorice on BWG and BW (Naser *et al.*, 2017; Khamisabadi *et al.*, 2015; Moradi *et al.*, 2014) when added to their diets and drinking water.

Different anthers observed that, the improvement in BW and BWG by addition licorice in either diet or drinking water may be attributed to chemical composition in licorice (Kageyama *et al.*, (1994). It could be noted that, in the current study the improvements in LBW and BWG in growing Japanese quail were associated with its role in promoting immunity system (Table 2).

Table 2. Live body weight and body weight gain of quail as affected by HD without or with licorice supplementation.

Items	ND	HD	HD + Licorice (g/kg diet)				SEM	*P* value
			0.3	0.6	0.9	1.2		
Body weight (g)								
1 wk	30.01	30.00	29.90	29.85	29.86	30.02	0.306	0.9972
3 wk	105.40^{b}	98.50^{cd}	97.58^{d}	106.27^{b}	112.31^{a}	103.00^{bc}	1.606	0.0005
5 wk	210.53^{b}	188.89^{c}	193.40^{c}	213.77^{ab}	218.11^{a}	212.13^{ab}	1.927	<.0001
Body weight gain (g / day)								
1-3 wk	5.39^{b}	4.87^{cd}	4.84^{d}	5.46^{b}	5.89^{a}	5.21^{bc}	0.106	0.0002
3-5 wk	7.51^{a}	6.46^{b}	6.85^{b}	7.68^{a}	7.55^{a}	7.79^{a}	0.108	<.0001
1-5 wk	6.45^{b}	5.66^{c}	5.84^{c}	6.57^{ab}	6.72^{a}	6.50^{ab}	0.069	<.0001

Means within the same row with different common superscripts differ significantly (P<0.05).
ND: Normal stoking density HD: high stoking density

Feed intake and feed conversion ratio

Concerning feed intake (FI), the results in Table 3 did not show any significant effects in FI among all treatments throughout all ages due to dietary licorice supplementation and stocking density. Results of stocking density effect of FI in the present study were disagree with Boontiam *et al.*, (2018) who found that linear reduction in FI were detected as increasing stocking density from 17 to 23 quails/cage. Similarly, AL-Homsdan and Rebortson (2017) stated that increasing stocking density of broiler chicks from 10 to 15 birds/m^2 resulted in a reduction of FI. Moreover, Attia *et al.* (2012) found that quails housed at 24 birds/cage consumed less feed (p < 0.01) comparatively with those housed at 12 birds/cage through all periods studied (1-3, 3-6 and 1-6 weeks of age). However, our results of feed intake due to stocking density agree with Vargas-Rodriguez *et al.* (2013) who revealed that, FI of broilers were not significantly affected between chicks kept under low (10 birds/m^2) or high (16 birds/m^2) stocking density.

Table 3. Feed intake and feed conversion ratio of quail as affected by HD without or with licorice supplementation.

Items	ND	HD	HD + Licorice (g/kg diet)				SEM	*P* value
			0.3	0.6	0.9	1.2		
Feed intake (g / day)								
1-3 wk	14.29	15.18	14.48	15.56	15.84	14.59	0.403	0.1600
3-5 wk	24.73	24.48	24.95	25.17	25.08	26.01	0.503	0.4378
1-5 wk	19.51	19.83	19.71	20.37	20.46	20.30	0.423	0.5629
Feed conversion ratio (g feed/ g gain)								
1-3 wk	2.65	3.12	3.00	2.86	2.69	2.80	0.106	0.0900
3-5 wk	3.29^{b}	3.79^{a}	3.65^{a}	3.28^{b}	3.32^{b}	3.34^{b}	0.087	0.0061
1-5 wk	3.02^{b}	3.51^{a}	3.38^{a}	3.10^{b}	3.04^{b}	3.12^{b}	0.069	0.0048

Means within the same row with different common superscripts differ significantly (P<0.05).
ND: Normal stoking density HD: high stoking density

The insignificant effect of FI due to licorice supplementation to high density diet in the present study were in line the findings of Khamiabadi *et al.*, (2015), Naser *et al.*, (2017), Rashidi *et al.*, (2018) and Beski *et al.*, (2019) who indicated that using licorice as a dietary supplementation or via drinking water had no significant effect on the feed intake.

With regard to feed conversion ratio (FCR), the results in Table 3 indicated that, increasing stocking density from ND to HD without or with addition 0.3 g licorice/kg diet had the worst FCR values compared with chicks stocked in ND and HD treated with 0.6, 0.9 and 1.2 g licorice/kg diet. It could be noticed that, the addition of 0.6, 0.9 and 1.2 g/kg of licorice to chicks stocked HD diets achieved FCR values equal to the chicks stocked at ND value and did not significantly different.

It is worth to note that chicks reared in HD and received diet containing 0.9 g/kg licorice recorded the best FCR value compared with other treatment groups. Concerning the stocking density effect of FCR, the obtained data in the present study were similar to those reported by Al-Hamed (2020) and GenGiz *et al.*, (2015), who indicated that FCR values were significantly improved in broiler and quail chicks reared under normal density compared to those reared at high density. However, the investigations failed to obtain a significant effect in FCR of broiler chicks between chicks stocked at ND and HD density (Houshmand *et al.*, 2012, Vargas-Rodriguez, 2013 and Homidan and Robortson, 2017).

The effect of licorice on FCR values in the present study were in the with findings of (Al-Darag, 2013; Kalantar *et al.*, 2017; Rashidi *et al.*, 2018; Alagawany *et al.*, 2019) who indicated that delivering the licorice via feed or drinking water significantly improved the FCR value of broiler chicks. However, in broiler chicks, other researches indicated that, licorice supplementation via feed or drinking water had no effects on FCR.

Carcass traits

Relative weight of all carcass traits studied was not significantly affected by either stocking density or dietary licorice supplementation of groups stocked at HD (Table 4). However, intestinal PH was significantly by affected by treatments. It was observed that dietary licorice supplementation at 0.6 and 0.9 g licorice/kg diet of groups stocked at HD had significantly (p) reduced intestinal PH compared with other treatments (Table 4).

Several studies observed that, carcass percentages and carcass component ratio were not significantly affected by HD (Rashidi *et al.*, 2018; Cengiz *et al.*, 2015; Vargas-Rodriguez, 2013; Attia *et al.*, 2012).

In the current study, inclusion of licorice in diets of chicks reared in high density did not affect carcass traits. In addition, these chickens were insignificant when compared with the ND group. These findings are in agreement with

Rashidi *et al.* (2018) who pointed out that carcass % were not influenced by licorice extract in HD experimental diets. Also, Pooryousef and Hosseini (2012) stated that carcass % was not significantly affected by dietary probiotic and licorice root extract supplementation in Japanese quail. Furthermore, Sedghi *et al.*, (2010) added licorice extract on the diet of broiler and did not find any significant effect on relative weight of carcass. Likewise, Beski *et al.*, (2019) stated that, there was no significant effect of aqueous licorice extract administrated to the drinking water on carcass cuts, visceral organs and intestinal histomorphology. In contrast, Daraji *et al.*, (2013) clarified that, broiler chicks receiving 450 mg licorice extract in their drinking water have increased dressing percentage.

Table 4. Carcass traits of quail as affected by HD or with licorice supplementation.

Items	ND	HD	HD + Licorice (g/kg diet)				SEM	*P* value
			0.3	0.6	0.9	1.2		
Carcass, %	73.40	73.09	75.32	75.54	74.17	72.81	2.352	0.9384
Liver, %	2.54	2.21	2.12	2.25	2.81	2.92	0.238	0.1888
Gizzard, %	2.02	2.15	2.40	2.17	2.23	2.29	0.131	0.5433
Heart, %	0.89	0.83	0.97	1.01	0.97	0.94	0.111	0.8891
Giblets, %	5.45	5.19	5.49	5.44	6.02	6.14	0.262	0.2125
Dressing, %	78.85	78.28	80.81	80.97	80.19	78.95	2.314	0.9394
Intestinal pH	6.45[ab]	6.61[a]	6.39[ab]	6.12[c]	6.07[c]	6.25[bc]	0.075	0.0025

Means within the same row with different common superscripts differ significantly (P<0.05).
ND: Normal stoking density HD: high stoking density

Digestive enzymes

In view of the results in Table 5, it seems that, there were no significant differences in the digestive enzymes (amylase, lipase and protease) values between quail reared under normal density and reared under high density without licorice supplementation in their diet. It could be observed that, dietary licorice supplementation at different levels to quail stocked at high density groups resulted enhances significantly (p) in fore mentioned digestive enzyme. Similar results were obtained by Sabry (2021).

Table 5. Digestive enzymes of quail as affected by HD without or with licorice supplementation.

Items	ND	HD	HD + Licorice (g/kg diet)				SEM	*P* value
			0.3	0.6	0.9	1.2		
Amylase	57.90	42.93	76.00	60.20	64.55	61.90	6.358	0.0902
Lipase	20.53[b]	17.87[b]	26.93[a]	27.80[a]	25.08[a]	26.42[a]	1.086	0.0002
Protease	0.54[b]	0.52[b]	0.97[a]	1.21[a]	0.99[a]	1.03[a]	0.123	0.0190

Means within the same row with different common superscripts differ significantly (P<0.05).
ND: Normal stoking density HD: high stoking density

Blood biochemical

In the present study, results in Table 6 showed that total protein (TP) and albumin (ALB) values of growing Japanese quails did not differ between low and high density. However, quails supplemented with licorice at 0.6 and 0.9 g/kg had the highest TP, ALB and GLOB values compared with birds supplemented the licorice at 0.0 and 0.3 g/kg diet. In addition, these birds had higher TP, ALP and GLOB values when compared with ND. It is worth noting that, chicks group reared under HD without licorice supplementation have increased (p) ALG % compared with ND and HD with dietary licorice supplementation at different levels.

Table 6. Liver and kidney functions of quail as affected by HD without or with licorice supplementation.

Items	ND	HD	HD + Licorice (g/kg diet)				SEM	*P* value
			0.3	0.6	0.9	1.2		
TP (g/dL)	2.65[bc]	2.27[c]	2.82[bc]	3.54[a]	3.66[a]	3.24[ab]	0.190	0.0016
ALB (g/dL)	1.38[c]	1.37[c]	1.47[bc]	1.82[a]	1.79[ab]	1.51[abc]	0.099	0.025
GLOB (g/dL)	1.27[b]	0.90[c]	1.36[b]	1.72[a]	1.87[a]	1.73[a]	0.091	<.0001
A/G (%)	1.09[b]	1.54[a]	1.08[b]	1.06[b]	0.96[bc]	0.87[c]	0.032	<.0001
ALT (IU/L)	7.66	15.12	12.43	13.97	14.03	12.80	1.680	0.1155
AST (IU/L)	140.90[b]	200.50[a]	202.50[a]	195.00[a]	145.10[b]	153.20[b]	7.178	<.0001
LDH (IU/L)	183.35[ab]	193.15[ab]	209.20[a]	203.60[a]	135.71[c]	162.90[bc]	9.855	0.0022
Creatinine (mg/dL)	0.54	0.62	0.51	0.41	0.50	0.52	0.091	0.7292
Urea (mg/dL)	4.70	5.44	5.05	5.14	5.27	4.95	0.517	0.9403

Means within the same row with different common superscripts differ significantly (P<0.05).
ND: Normal stoking density HD: high stoking density TP: Total protein
ALB: Albumin GLOB: globulin ALT and AST: liver function enzymes

Similar to present results, Rezaei *et al.*, (2014) demonstrated that, the inclusion of licorice at 0.5% in broiler chick's diet increased globulin concentration. Rashidi *et al.*, (2018) showed that stocking density had significant effect on blood parameters of broiler chicks.

In contrast, El-Tarabany *et al.*, (2015) found that quail layers housed at low stocking density (143 cm^2/birds) had a significantly higher total protein and albumin concentration, in comparison with layers housed at high stocking density (200 cm^2/bird). Likewise, they found the highest ALB/GLB ratio has been recorded in low stocking density group while the lowest value was in high stocking density group

Liver and kidney functions

In view of the data presented in Table 6, it seems that the chicks housed at high density without or with dietary licorice supplementation at 0.3 and 0.6 g licorice/kg had significantly (p) higher AST and ALT concentrations compared with chicks housed at ND and HD with dietary licorice supplementation at 0.9 and 1.2 g licorice/kg. The present finding indicated that, increasing stocking density in growing Japanese quail form 10 to 15 in cage impairs their AST and ALT supplementation HD diet by licorice at 0.9 and 1.2 g/kg a meliorated these negative effect.

However, there were no significant differences observed among all experimental groups due to increasing density without or with dietary licorice supplementation. The increased plasma AST and ALT activity in birds housed under high stocking density indicated that, high stocking density might cause oxidative lesions which are in accordance with the results obtained by Simsek *et al.,* (2009). Similar to present results, Shewita *et al.,* (2019) declared that, high stocking density altered the activity of liver function enzymes (AST and ALT). In contrast, Anwar and Al-Hamad, (2020) and Bontiam *et al.,* (2018) demonstrated that, AST and uric acid concentrations did not significantly affect when broiler chicks stocked in high stocking density.

Lipid profile

In the current study, it was suggested that, density condition is one of the physiological stressor and altered the concentrations of blood indices. Table 7 shows that, the quail chicks housed at HD without or with dietary supplementation at 0.3 g/kg presented higher values ($p<0.05$) of plasma TC, TG, LDL and lower plasma HDL and vLDL values in compared with chicks housed at normal density and HD with addition licorice more 0.3 g/kg diet (0.6, 0.9 and 1.2 g/kg). It is worth noting that, addition of licorice more than 0.3 g/kg diet, the stress effect caused by high SD through decreasing the level of plasma TC, TG, LDL and increasing HDL and VLDL compared with those reared in HD without licorice supplementation in their diets and achieved lipid profile values significantly equal to ND values. Our results agree with Dozier *et al.,* (2006) and Qaid *et al.,* (2016) who demonstrated that, plasma cholesterol concentration was significantly higher at high stocking density in broiler chicks. However, others reported that serum concentrations of TC, TG, LDL and VLDL did not affect significantly in broiler and quail chicks when reared in high stocking density conditions (Abdel-Azeem, 2010; Houshmand *et al.,* 2012; Shewita *et al.,* 2019 and Anwar and Hamed, 2020).

Table 7. Lipid profile of quail as affected by HD without or with licorice supplementation.

Items	ND	HD	HD + Licorice (g/kg diet)				SEM	*P* value
			0.3	0.6	0.9	1.2		
TC (mg/dL)	181.16^{b}	261.53^{a}	248.57^{a}	171.21^{b}	185.32^{b}	178.87^{b}	8.034	<.0001
TG (mg/dL)	244.65^{b}	371.30^{a}	350.30^{a}	250.01^{b}	195.33^{c}	257.00^{b}	10.593	<.0001
HDL (mg/dL)	49.17^{b}	32.40^{c}	45.81^{b}	34.88^{c}	61.80^{a}	54.60^{ab}	3.098	0.0002
LDL (mg/dL)	83.07^{c}	154.88^{a}	132.70^{b}	86.33^{c}	84.45^{c}	72.87^{c}	5.506	<.0001
VLDL (mg/dL)	48.93^{b}	74.26^{a}	70.06^{a}	50.00^{b}	39.07^{c}	51.40^{b}	2.118	<.0001

Means within the same row with different common superscripts differ significantly (P<0.05).
ND: Normal stoking density HD: high stoking density TC: total cholesterol TG: triglyceride
HDL: high-density lipoproteins LDL: low density lipoproteins VLDL: very low-density lipoprotein

Consistent with the current results, Naser *et al.,* (2017) reported, addition of licorice to broilers chicks diet resulted increase in serum HDL level and HDL/LDL ratio because of its high levels of ascorbic acid and flavonoids. Furthermore, the use of 0.4% licorice extract in the drinking water of broiler chicks increased the HDL levels but reduced ALT level (Salary *et al.,* 2014). However, Rashidi *et al.,* (2018) and Pooryousef and Hosseini (2012) found that blood total triglycerides, glucose and total cholesterol of broiler chicks and Japanese quail were not affected by licorice root extract supplementation.

The hypocholesterolemic effects of licorice extract may be due to high secretion of cholesterol, neutral sterols, bile acids, and enhancement in the level of bile acid in the liver. In addition, to the bioactive molecules of licorice-saponin are able to inhibit the formation of lipid peroxides, reduce the level of LDL-associated carotenoids, and improvement the conversion rate of cholesterol to bile acids. Also, the presence of phytosteroids and saponins in licorice extract could be essential for lowering cholesterol in animals fed licorice extract diets (Alagawany *et al*., 2019).

Immunity and antioxidant parameters

Concerning immunity parameters, Table 8 shows that HD and use of licorice supplementation in HD had no significant effect on lysozyme content. However, IgA and IgM levels were higher ($p<0.01$) in the quail chicks kept under high density treated with 0.9 g licorice/kg diet. It was observed that IgA and IgM levels were not significantly affected by HD without dietary licorice supplementation. However, although not significant the IgA and IgM levels were numerically higher in the group kept under ND when compared with chicks kept under HD. This was consistent with Gholami *et al.,* (2020) who found that, broiler chicks kept in the density of 20 chicks/m^2 had the lowest immune response than those kept at 10, 17 and 20 chicks/m^2 at 42 days of age.

Likewise, Houshmand *et al.,* (2012) suppression of immunity in broiler chicks consistent with the present results, Sagadeeswaran and Selvasubramanian, (2014) revealed that broiler chicks received 0.1% licorice extract powder showed an improvement in the immunity. In contrast with our findings, Moradi *et al.,* (2014), Myandoab and Mansoub, (2012) demonstrated that, using licorice extract as a dietary supplementation or via drinking water had no significant effects on the immunological parameters of Japanese quails.

The improvements in immunity due to licorice supplementation may be attributed to the addition of licorice in the rations increased the weight of immune organs, like the bursa or spleen, thereby promoting immune response and the situation of health and livability (Kalantar *et al.,* 2017). With regard to antioxidant parameters, in view of the results in Table 8, it could be observed that, plasma SOD values were higher ($p<0.05$) in the experimental group housed under high stocking density treated with 0.6 g licorice/kg diet comparatively with other experimental groups. Plasma MDA value was ($p<0.05$) higher and plasma

TAG value was lower due to increasing stocking density without addition of licorice.

Inclusion of licorice at 1.2 g/kg diet had significantly ($p<0.05$) higher MDA and TAC values compared with other experimental groups. Other addition licorice levels to HD groups enhanced MAD and TAC to be as the same value obtained by chicks kept in ND and equal significantly. In contrast to our findings, other investigations found no significant differences between high and low stocking density of blood MDA, corticosterone and nitric oxide (Dozier *et al.,* 2006; Houshmand *et al.,* 2012; Cengiz *et al.,* 2015).

Table 8. Immunity and oxidative, state of quail as affected by HD without or with licorice supplementation.

Items	ND	HD	HD + Licorice (g/kg diet)				SEM	*P* value
			0.3	0.6	0.9	1.2		
Immunity								
IgG (mg/dl)	1.08^{bc}	0.89^{c}	1.42^{b}	0.94^{c}	1.63^{a}	1.50^{ab}	0.129	0.0067
IgM (mg/dl)	0.76^{b}	0.62^{b}	0.62^{b}	0.86^{b}	1.36^{a}	0.76^{b}	0.091	0.0020
Lysozyme	0.39	0.25	0.32	0.39	0.27	0.22	0.054	0.2348
Anti-oxidants								
SOD (U/mL)	0.38^{bc}	0.24^{c}	0.31^{c}	0.59^{a}	0.49^{ab}	0.37^{bc}	0.047	0.0045
MDA (nmol/mL)	0.12^{c}	0.29^{ab}	0.09^{c}	0.18^{bc}	0.31^{a}	0.40^{a}	0.035	0.0005
TAC (ng/ml)	0.33^{b}	0.17^{c}	0.31^{bc}	0.27^{bc}	0.42^{ab}	0.49^{a}	0.046	0.0056
GPX (ng/ml)	0.37	0.24	0.31	0.41	0.43	0.29	0.077	0.5082

Means within the same row with different common superscripts differ significantly ($P<0.05$).
ND: Normal stoking density **HD: high stoking density** **IgG and M: immunoglobulin G and M**
SOD: Super oxide dismutase **MDA: Malondialdehyde** **TAC: total antioxidant capacity**
GPX: glutathione peroxidase

Cecal microbial counts

Results in Table 9 revealed that there were no significant differences in total yeasts, molds count and enterococcus SPP. between chicks kept under HD and ND. However, HD received diet supplemented with licorice at different levels had lowest ($p<0.05$) cecum microbial counts studied compared with quail housed in ND and HD received un-supplemented diet.

Our results agree with Gurdia *et al.,* (2011) who observed that, all digestive microbiota did not significantly change between low and high stocking density at 42 days.

Likewise, Cengiz *et al.,* (2015) demonstrated that, no alteration in total aerobes and salmonella count were experienced by different stocking density. This may be attributed to inability of high stocking density to reduce the intestinal Lactobacilli count. On the contrary, Cengiz *et al.,* (2015) noted that, there were fewer Lactobacilli in broiler chicks at high stocking density as compared to low stocking density. Also, Tannock, (1997) stated that, the intestinal count of lactobacilli is negatively affected by high stocking density of broiler chicks.

Table 9. Bacteriology of quail as affected by HD without or with licorice supplementation.

Items	ND	HD	HD + Licorice (g/kg diet)				SEM	*P* value
			0.3	0.6	0.9	1.2		
Total yeasts and molds count	4.44^{a}	4.46^{a}	3.04^{d}	3.86^{b}	3.35^{c}	3.43^{c}	0.055	<.0001
E. coli	5.27^{b}	5.50^{a}	4.79^{c}	3.37^{e}	4.62^{cd}	4.46^{d}	0.069	<.0001
Salmonella spp.	3.16^{b}	3.91^{a}	2.39^{c}	2.33^{cd}	2.17^{d}	1.83^{e}	0.054	<.0001
Enterococcus spp.	5.59^{ab}	5.78^{a}	5.48^{abc}	5.16^{c}	5.39^{bc}	5.30^{bc}	0.095	0.0131
Coliform	6.26^{b}	6.49^{a}	5.09^{d}	5.22^{d}	5.28^{d}	5.53^{c}	0.058	<.0001

Means within the same row with different common superscripts differ significantly ($P<0.05$).
ND: Normal stoking density **HD: high stoking density**

CONCLUSION

Increasing stocking density of growing Japanese quail from 10 to 15 negatively affect their performance. Supplementing diets with licorice could reduce these negative effects and improve the performance at high stocking density.

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تأثير إضافة العرقسوس على اداء النمو للسمان اليابانى النامى المرباه تحت كثافة التربية العالية

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أجريت هذه الدراسة بهدف لتقييم تأثير المستويات الغذائية من العرقسوس على أداء السمان الياباني عند التربية تحت كثافة عالية. تم توزيع عدد ٢٥٥ سمان غير مجنس عمر أسبوع بشكل عشوائي على ست مجموعات غذائية. غذيت كتاكيت المجموعة الاولي على العليقة الأساسية بدون عرقسوس وتمت تربيتها بكثافة طبيعية (١٠ طيور/مكرر) مجموعة الكنترول. وتم توزيع كتاكيت المجموعات ٢ ، ٣ ، ٤ ، ٥ و ٦ بكثافة تربية عالية (١٥ طائر/مكرر) وتتغذى على علف قاعدي مدعم بالعرقسوس بمستويات ٠,٠ ، ٠,٣ ، ٠,٦ ، ٠,٩ و ١,٢ جم/كجم علف، على التوالى. أدت إضافة ٠,٩ جم من العرقسوس/كجم إلى عليقة السمان المربي تحت الكثافة العالية إلى زيادة الوزن الحي في عمر ٣ و ٦ أسابيع و الزيادة الوزنية خلال ١-٣ و ١-٥ أسابيع من العمر. سجلت أحسن قيمة للمعمل التحويل الغذائى للدجاج المربى تحت الكثافة العالية والمغذى على علائق محتوية على ٠,٩ جم من العرقسوس/كجم مقارنة مع باقى المجاميع التجريبية. سجلت كتاكيت السمان المرباه تحت الكثافة العالية بدون أو مع العرقسوس عند ٠,٣ جم/كجم قيم أعلى (p <0.05) من قيم TC و TG و LDL في البلازما و HDL و VLDL في البلازما مقارنة باقي المجموعات. كانت مستويات IgA و IgM أعلى (p <0.01) في كتاكيت السمان المرباه تحت الكثافة العالية والمعاملة بـ ٠,٩ جم من العرقسوس/كجم علف مقارنة باقي المجموعات. كان SOD البلازمي أعلى (p <0.05) في المجموعة التجريبية المرباه تحت الكثافة العالية والمعاملة بـ ٠,٦ جم من العرقسوس/كجم علف مقارنة بالمجموعات التجريبية الأخرى. لا توجد فروق ذات دلالة إحصائية في إنزيمات الجهاز الهضمي والعد البكتيرى. الكتاكيت المرباة تحت الكثافة العالية مع التغذية على علائق مضاف اليها العرقسوس جم/ كجم عليقة أظهرت أعلى قيمة معنوية لإنزيمات الليبيز والبروتيز وأقل قيمة معنوية فى العدد الكلي للبكتيريا، الخمائر الكلية ، عدد العفن والمكورات المعوية SPP. نستخلص من النتائج ان زيادة كثافة التربية للسمان الياباني من ١٠ إلى ١٥ تؤثر سلبًا على أدائها. ولكن استخدام العرقسوس في العلائق ممكن أن يخفف من هذه الآثار السلبية ويحسن الأداء عند كثافة التربية المرتفعة.