EFFECTS OF STOCKING DENSITY WITHOUT OR WITH YEAST EXTRACT SUPPLEMENTATION ON THE GROWTH PERFORMANCE, DIGESTIVE ENZYMES, BLOOD METABOLITES, AND INTESTINAL MICROBIOTA OF GROWING JAPANESE QUAIL

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(Received 9/1/2022, accepted 16/3/2022)

SUMMARY

This study aimed at determining the role of dietary yeast extracts in improving the performance and health of quails reared under high stocking density. A total number of 340, 7-days old unsexed growing Japanese quail chicks with initial body weight of 30.42 g were used in this study. The quails were randomly distributed to six experimental groups and four replicates maintained per each group in a complete randomized design. The 1st group (40 quails) was stocked at a rate of 10 chicks/replicate (control, normal density (ND); 40 quails/m^2) and fed the basal diet without any supplementation; the 2nd, 3rd, 4th, 5th and 6th (60 quails/group; stocked at a rate of 15 chicks/replicate; high density (HD); 60 quails/m^2) and fed the basal diet supplemented with 0, 1, 2, 3 and 4 mg yeast extract (YE) /kg diet, respectively. Addition of YE at 1 mg/ kg diet to growing quail stocked at HD resulted in a significant (P<0.001) increase in LBW at 6 weeks of age and BWG through 3-5 and 1-5 stages when compared to all treatment groups. No significant differences were observed in feed intake among birds stocked at either ND or HD without dietary YE supplementation. Chicks reared in HD and received basal diet were recorded (P<0.001) the worst FCR values compared with the chicks reared in either ND and HD fed diet supplemented with YE at different levels. Dietary YE supplementation to HD groups resulted in significant enhances in digestive enzyme comparatively with groups kept at ND and HD fed diet without addition of YE. chicks reared under HD without dietary YE supplementation presented higher values (P<0.0001) of serum TC, TG, LDL and VLDL and lower values of serum HDL compared with the chicks reared under ND and HD with dietary YE supplementation at different levels. The values of complement 3 were significantly higher in ND group and HD treated with 1 mg YE/kg diet than the groups housed in HD and untreated and treated with 2, 3 and 4 mg YE/kg diet. Higher stocking density (HSD) fed diets treated with YE at different levels had highest *Enterococcus spp.* count and lowest total yeast and molds count, *E-coli*, *Salmonella SPP* and *Coliform.* It could be concluded that dietary addition of YE can positively mitigate the stress applied to quail raised under high stoking density by enhancing the antioxidant status, immunological parameters, intestinal pH, caecal microbial counts and as well as feed conversion and intake and growth performance.

***Keywords**: Yeast, stocking density, performance, blood, microbiota, quail.*

INTRODUCTION

Japanese quail is considered one of the important alternative resources of animal protein, because it have many advantages such as fast growth, early sexual maturity, short incubation period, small size and high egg production, and low housing costs (Padmakumar *et al.,* 2000). Also, quails are widely distributed in many countries of the world (Roshdy *et al.,* 2010 and El-Tarabany *et al.,* 2015).

Stocking density influences animals' welfare but lowering it without guaranteed optimal environmental conditions is of minor importance (Jones *et al.,* 2005 and Utnik-Banaś *et al.,* 2014). The best housing condition for rearing poultry are of great interest to researchers, and good production conditions are essential to promote poultry production and improve welfare and profit (Lewko and Gornowicz, 2011 and Mesa *et al.,* 2017). Housing conditions can also affect animal welfare and mortality of laying hens (Weimer *et al.,* 2019 and Schuck-Paim *et al.,* 2021).

Worldwide quail production has contributed to the poultry industry and will have a bright future due to low production costs and satisfy consumer demands (Yapici *et al.,* 2006). The target of the quail producers is to increase the stocking density to achieve further reductions in production costs, but an excessive crowding of chickens can decrease performance and welfare of chickens kept in cages (Tayeb, *et al.,* 2011 and Utnik-Banaś *et al.,* 2014). To this regard, no clear recommendations are available in the literature on optimal space allowance for quail rising. Stocking stress associated with increasing animal density can be relieved by using feed additives with strong antioxidant property (Attia *et al.,* 2021).

The role of feed supplements/additives in poultry industry is very important (Bolacali and Irak, 2017; Awais et al., 2021). The optimal applications of feed additives can improve feed utilization, productivity and public health. Probiotics such as yeasts (*Saccharomyces cerevisiae*; *SC*) are one of the popular feed additives and have been used as feed additives for improving animal health and performance (Ogbuewu *et al.,* 2018; Bilal et al., 2021). Probiotics are live microorganisms that enhance birds' health by competing with harmful bacteria, improve the intestinal balance of microbiota and absorption of nutrients (Al-Khalaifah, 2018). *Saccharomyces cerevisiae* is considered as one of the most yeast species that are supplemented to poultry diets during diet formulations (Duarte *et al.,* 2012). *Saccharomyces cerevisiae* contains high levels of vitamins, digestible proteins, and minerals including magnesium and zinc, also the wall of SC contains polysaccharides α-D-mannan, β-D-glucan and chitin (Elghandour *et al.,* 2019) which play a key role in improving microbial balance in poultry intestine (Alizadeh *et al.,* 2016). Therefore, this work was designed to study the effects of stocking density without or with yeast extracts supplementation on the growth performance, digestive enzymes, intestinal pH, blood biochemical parameters, antioxidant and immune measurements, and cecal microbial counts of growing Japanese quail.

MATERIALS AND METHODS

The present work was designed at Poultry Department, Faculty of Agriculture, Zagazig University, Egypt to study the impact of stocking density without or with yeast supplementation on the growth performance, digestive enzymes, intestinal pH, blood biochemical parameters, antioxidant and immune measurements, and cecal microbial counts of growing Japanese quail.

Experimental birds and management:

A total number of 340, 7-days old unsexed growing Japanese quail chicks with initial body weight of 30.42 g were used in this study. The quails were randomly distributed to 6 experimental groups and 4 replicates maintained per each group in a complete randomized design. The 1st group (40 quails) was stocked at a rate of 10 chicks/replicate (control, normal density (ND); 40 quails/m^2) and fed the basal diet without any supplementation; the 2nd, 3rd, 4th, 5th and 6th (60 quails/group); stocked at a rate of 15 chicks/replicate; high density (HD); 60 quails/m^2) and fed the basal diet supplemented with 0, 1, 2, 3 and 4 mg yeast extract(YE kg diet, respectively.

Birds were weighed, and randomly housed in cages. House temperature was kept at about 30°C through the 1st week, then gradually decreased by 2°C weekly until reached 24°C and kept until the end of the experimental period. In all the experimental groups, birds were subjected to 23 hours light at intensity of 3 watt/m^2 along the experimental period which extended to the age of 5 weeks, feed and water were available *ad libitum* throughout the experiment. All experimental birds were raised under similar environmental, hygienic and managerial conditions. The authors formulated the experimental diets to cover the requirements of quail during the fattening period (NRC, 1994, Table 1).

Measurements investigated:

Growth performance:

All performance parameters (live body weight (LBW) and body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR)) have been determined at 1, 3 and 5 weeks of age.

Biochemical characteristics:

Individual blood samples were collected into EDTA tubes from 4 birds within each treatment (on individual basis) at 5 weeks of age to determine the different biochemical characteristics. Each blood sample was centrifuged at 4000 rpm for 15 minutes to separate blood plasma.

The colorimetric determination of cholesterol was carried out using kit produced by Bio-system according to Allain *et al.* (1974). The principle of the method is that cholesterol forms a colored complex with acetic anhydride and concentrated sulfuric acid and the colored complex is measured photometerically. The colorimetric determination of triglycerides was carried out by specific diagnostic kit produced by Bio diagnostic according to Allain *et al.* (1974). After centrifugation, the high density lipoproteins (HDL), low density lipoproteins (LDL) and the very low density lipoprotein (VLDL) were determined by enzymatic methods (Friedewald *et al*., 1972 and Myers *et al.*, 1994).

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

Item	g/kg
Ingredient	
Yellow corn 8.5%	518.0
Soybean meal 44%	367.0
Corn gluten meal 62%	52.1
Soybean oil	29.0
Limestone	7.0
Di-calcium phosphate	16.5
NaCl	3.0
Premix[1]	3.0
L-Lysine	1.3
Dl-Methionine	1.1
Choline chloride	2.0
Total	1000.0
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
Methionine + Cysteine (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 B2 (riboflavin), 8.0 mg; Vitamin B6, 4.950 mg; Vitamin B12, 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)*

Immunity indices:

The levels of immunoglobulin G (IgG) and A (IgA) in the plasma were determined spectrophotometrically using commercial kit from Biodiagnostic Company (Giza, Egypt) according to (Akiba *et al*., 1982). The authors determined the level of complement (C_3) in quail plasma, using the ELISA Kit from MyBiosource.com.

Antioxidant parameters:

Plasma samples were subjected to the measurement of superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity and total antioxidant capacity (TAC) according Winterbourn *et al*. (1975) and Koracevic *et al.* (2001). Regarding lipid peroxide, malondialdehyde (MDA) in the plasma was determined according to Mihara and Uohiyama, (1978).

Digestive enzymes and cecal pH:

Digestive enzymes including the activity of amylase, lipase and protease was determined by the method of Somogyi (1960), Tietz and Fiereck (1966) and Lynn and Clevette-Radford (1984), respectively. Regarding pH, we used a digital pH meter to determine the pH value in the cecal content (Model 507; Crison Instruments S.A., Barcelona, Spain) and the pH value was recorded twice, and the mean of the recorded two values was kept for the statistical analysis.

Microbiological analyses:

10 g of cecal content of quail were separately transferred to a 250 ml Erlynmayar flask containing 90 ml of sterile peptone saline solution (0.85% NaCl and 0.1% peptone) and well mixed, then serial dilutions up to 10^7 were prepared. One tenth ml of each dilution was spread on the surface of plate count agar medium (P.C. agar, Oxide) then incubated at 30 ±2ºC for 48 hr., for enumeration the total bacterial count. For lactic acid bacteria (LAB) enumeration, MRS agar (De-Man, Rogosa and sharp) were used for counting *lactobacilli* after incubation at 30ºC for 24–48 hr. *Enterococcus* was enumerated on Kanamycin Aesculin Azide – agar medium, then incubated at 37ºC for 48 hr. Black colonies on Kanamycin Aesculin Azide-agar are typical colonies of *enterococci*. Plates were incubated at 37ºC for 24 hr. Pink colonies on MacConkey agar are typical colonies of *coliform*. *Salmonella* and *Shigella SPP* were counted using S.S. agar (Oxide CM 99). All plates were incubated at 37ºC for 24 hr. (Reda *et al.,* 2020a,b).

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. The significant differences between treatment means were separated by Duncan multiple range-test (Duncan 1955) ($P<0.05$).

RESULTS AND DISSCUTION

Growth performance:

Live body weight and body weight gain:

Results in Table (2) shows that increasing density from ND to HD without YE supplementation resulted in a significant ($P\leq0.001$) reduce in LBW and BWG through the interval periods studied. Addition of YE at 1 mg/ kg diet to growing quail stocked at HD resulted in significant ($P<0.001$) increase in LBW at 5 weeks of age and BWG through 3-5 and 1-5 stages when compared with all treatment groups.

The lack effect of stocking density on changing LBW and BWG is in consistent with those of previous studies (Cengiz *et al*., 2015) and El-Tarabany (2016), who showed that, reductions in the growth (LBW and BWG) in growing quails at high stocking densities. Recently, Gholami *et al.* (2020) observed that the final body weight in all treatments with a density of 10 chickens/m^2 was higher than other densities (15, 17 and 20 chickens) in the different climate conditions. In the same line, Al-Hamed (2020) showed a significant increase in LBW and BWG of broiler chicks within density 44 birds/m^2 as compared with other densities (52 and 60 birds/m^2). Likewise, Shewita *et al*. (2019) reported that broiler chicks kept under high stocking density showed a reduction of the final LBW than low stocking density. Moreover, Boontiam *et al.* (2019) showed that final body weight, and BWG were reduced in the growing quails at the density of 61.73 birds/cage (25 quails/m^2) compared with those at the density of 32.10 birds/cage (13 quails/m^2) and 41.98 birds/cage (17 quails/m^2).

Conversely, Vargas-Rodriguez *et al.* (2013) reported that weight gain was not significantly affected under HSD (16 birds/m^2) or low (10 birds/m^2). Also, Houshmand *et al.* (2012) showed that LBW and BWG of broiler chickens were not affected by different stocking density during the experiment. Similarly, Buijs *et al.* (2009) stated that the final LBW (39 days) of broiders was not different among chickens reared under different densities (6, 15, 23, 33, 35, 41, 47 and 56 kg/m^2). In the same line, stocking density in Japanese quail did not significantly effect of either LBW (Abdel-Hakim *et al*., 2005 and El-Sagheer *et al*., 2012) or BWG (Dhaliwal *et al*., 2008).

The significant decrease of LBW and BWG due to increase stocking density may be attributed to high stocking density limit the bird's movement to a confine area within the cage (Cengiz *et al.,* 2015). Also, higher ambient temperature that occurred due to less space per bird and overcrowding of the birds in the pen might cause stress on then. Askar and Assaf (2004) attributed the unfavorable effects of high stocking density on LBW and BWG of quail to the modification of the resting behavior due to the disturbances by the other quails.

Table (2): Live body weight and body weight gain of growing Japanese quail as affected by stocking density without or with yeast extract (YE) supplementation.

Item	Normal density	high density (HD)	HD + YE level (mg/kg diet)				SEM	*P* value
			1	2	3	4		
Live body weight (g)								
1 wk	30.50	30.32	30.39	30.39	30.46	30.45	0.141	0.9635
3 wk	105.82^{a}	97.82^{c}	103.29^{b}	104.33^{ab}	99.71^{c}	98.33^{c}	0.628	<.0001
5 wk	210.09^{b}	187.98^{e}	213.51^{a}	189.73^{de}	198.60^{c}	192.30^{d}	0.878	<.0001
Body weight gain (g / day)								
1-3 wk	5.38^{a}	4.82^{c}	5.21^{b}	5.28^{ab}	4.95^{c}	4.85^{c}	0.040	<.0001
3-5 wk	7.45^{b}	6.44^{e}	7.87^{a}	6.10^{f}	7.06^{c}	6.71^{d}	0.037	<.0001
1-5 wk	6.41^{b}	5.63^{e}	6.54^{a}	5.69^{de}	6.01^{c}	5.78^{d}	0.031	<.0001

Means within the same row with different common superscripts differ significantly (P<0.05).

The obtained improvements in the present study of LBW and BWG by dietary YE (1 mg/kg) supplementation to high stocking density agree with many investigators (Ashok *et al.,* 2016; Buba *et al.,* 2016 and Abd El wahab *et al.,* 2019) who medicated an improvements in BWG in broiler and quail chicks with yeast products supplementation of diet and during water.

In the other hand other investigations indicated that, yeast supplementation failed to obtain a significant increase in LBW and BWG of broiler chicks and turkey poults (AL-Mansour *et al.,* 2011 and Adebiyi *et al.,* 2012). Different authors showed that the improvement in LBW and BWG with yeast supplementation could be attributed to improve the absorption of nutrients by improving morphological structure of the gut (Pourabedin *et al*., 2014).

Feed utilization:

Concerning FI, statistical analysis indicated that, no significant differences were observed between birds stocked at either ND or HD without dietary YE supplementation (Table 3). However, the results revealed that, groups housed at HD and fed diet supplemented with YE at different levels, ~~were~~ consumed less fed when compared with groups housed in ND or HD and fed un-supplemented diet through interval periods. This reduction in FI may be attributed to the reduction in floor space, which increases the competition for positions at the feeder trough.

Table (3): Feed intake and feed conversion ratio of growing Japanese quail as affected by stocking density without or with yeast extract (YE) supplementation.

Item	Normal density	high density (HD)	HD + YE level (mg/kg diet)				SEM	*P* value
			1	2	3	4		
Feed intake (g / day)								
1-3 wk	14.38^{ab}	15.10^{a}	13.38^{c}	13.95^{bc}	12.58^{d}	13.55^{bc}	0.243	0.0003
3-5 wk	24.87^{a}	24.10^{a}	24.23^{a}	20.80^{c}	22.43^{b}	21.93^{bc}	0.429	0.0003
1-5 wk	19.62^{a}	19.60^{a}	18.80^{a}	17.38^{b}	17.50^{b}	17.74^{b}	0.255	0.0004
Feed conversion ratio (g feed/ g gain)								
1-3 wk	2.67^{bc}	3.13^{a}	2.57^{c}	2.64^{bc}	2.54^{c}	2.80^{b}	0.058	0.0003
3-5 wk	3.34^{b}	3.74^{a}	3.08^{c}	3.41^{b}	3.17^{bc}	3.27^{bc}	0.073	0.0009
1-5 wk	3.06^{b}	3.48^{a}	2.88^{b}	3.05^{b}	2.91^{b}	3.07^{b}	0.045	0.0001

Means within the same row with different common superscripts differ significantly (P<0.05).

Regarding to FCR, the results in Table (3) showed that, chicks reared in HD and received unsupplemented basal diet ~~were~~ recorded (P<0.001) the worst FCR values compared with the chicks reared in either ND and HD fed diet supplemented with YE at different levels. It could be noticed that, the addition of YE to HD diets achieved FCR values significantly equal to the ND and did not significantly different.

Concerning stocking density effect of FI and FCR, the obtained data are in a line with Al-Hamed (2020) who noted that, the best FCR was in 44 quail/m^2 and 52 quail/m^2 while it deteriorated through increasing the density to 60 quail/m^2. Also, Boontiam *et al*. (2019), found that, linear reductions in FI were detected as increasing stocking density from 17 to 23 quails/cage. Likewise, Cengiz *et al*. (2015) stated that, FI and FCR were significantly improved in broilers kept at low stocking density (10 birds/m^2) as compared to those at high stocking density (20 birds/m^2) during 0-21, 22-42 and 0-42 days of age. Attia *et al*. (2012) asserted that, quail housed at 24 birds/cage consumed less feed (P<0.01) compared with those housed at 12 birds/cage during 1-3, 3-6 and 1-6 weeks of age. Houshmand *et al*. (2012) reported that, FCR in broilers during the starter period (1-21 day) was similar between birds kept under two densities (5 and 8 birds/each cage). However, during the finisher period (22- 42 day), birds kept at a high density (8 birds/cage) recorded worst value of FCR compared with birds kept under a normal density (5 birds/cage). Moreover, the overall FCR (1 to 42 day) was better for birds at a normal stocking density. Seker *et al*. (2009) and Abdel-Azeem (2010) noted the increasing in stocking density of quail resulted in a linear reduction in feed consumption. Al-Homidan and Fahmy (2007) demonstrated that increasing stocking density of broiler chicks (Hybro) from 10 to15 bird/m^2 resulted in a reduction of FI. However, they showed that FCR was not significantly affected by increasing stocking density from 10 to 15 birds/m^2. On the other hand, Vargas- Rodriguez *et al*. (2013) found that FI and FCR of broiler chickens were not significantly affected with low (10 birds/m^2) or high stocking density (16 birds/m^2).

The effect of YE on FI and FCR in the current study, the obtained results confirmed the previous findings of several researches (Ahmed *et al*., 2015; Buba *et al*., 2016 and Shankar *et al*., 2017;) who indicated that, inclusion of *SC* yeast in broilers diet improved FCR. However, our results are in disagree with Markovic *et al*. (2009) and Gao *et al*. (2008) who indicated that yeast preparations failed to obtain a significantly effect on FI and FCR of broilers and quail chicks.

The inconsistency in the results of growth performance in response to stacking density may be attributed to many factors. High stocking density may limit the bird's movement to a confined area of the cage. The birds under low stocking density may have an access easier to drinkers and feeders than those kept under high stocking density. Thus, the FI may reduce in chicks housed in HD resulting in lowered BWG and poor FCR (Puron *et al*., 1995 and Feddes *et al*., 2002). Moreover, a reduction in the growth performance of broiler with high density may be attributed to disorders in the intestinal microbiota, which is associated with the digestion and absorption of nutrients (Biswas *et al*., 1999; Bedford and Apajalathi 2001; Banhazi, *et al*., 2008 and Guardia *et al*., 2011).

Digestive enzymes and intestinal pH:

In the current study, there were no significant differences in digestive enzymes (a- lipase and protease) value between ND and HD densities (Table 4). Dietary YE supplementation to HD groups resulted in significant enhances in digestive (amylase and protease) enzyme comparatively with groups kept at ND and HD fed diet without addition of YE. Use of *SC* in the diets plays positive roles in improving the public health and productivity of poultry, which promote the activity of digestive enzymes (Han *et al.,* 1999 and Yoon *et al.,* 2004).

Table (4): Digestive enzymes of growing Japanese quail as affected by stocking density without or with yeast extract (YE) supplementation.

Enzyme	Normal density	high density (HD)	HD + YE (mg/kg diet)				SEM	*P* value
			1	2	3	4		
Amylase	58.45^{cd}	42.25^{e}	116.00^{a}	54.60^{de}	74.45^{b}	69.25^{bc}	3.753	<.0001
Lipase	20.25^{ab}	17.99^{bc}	22.88^{a}	16.15^{c}	16.08^{c}	17.60^{bc}	0.949	0.0025
Protease	0.56^{b}	0.50^{b}	1.10^{a}	0.68^{b}	1.17^{a}	1.06^{a}	0.087	0.0007

Means within the same row with different common superscripts differ significantly (P<0.05).

Blood constituents:

Lipid profile:

Results in Table (5) showed that chicks reared under HD without dietary YE supplementation presented higher values (P<0.0001) of serum TC, TG, LDL and VLDL and also, lower values of serum HDL compared with the chicks reared under ND and HD with dietary YE supplementation at different levels. It could be observed that, addition of YE in the diets of chicks reared at HD alleviated the stress effect included by high SD through decreasing the level of serum TC, TG, LDL, and VLDL and increasing HDL compared with those reared in HD without YE supplementation and achieved lipid profile values equal significantly to ND values.

Table (5): Lipid profile of growing Japanese quail as affected by stocking density without or with yeast extract (YE) supplementation.

Items	Normal density	high density (HD)	HD + YE level (mg/kg diet)				SEM	*P* value
			1	2	3	4		
TC (mg/dL)	181.33^{c}	258.96^{a}	163.55^{d}	225.70^{b}	181.14^{c}	191.68^{c}	4.410	<.0001
TG (mg/dL)	243.05^{c}	367.10^{a}	240.60^{c}	292.25^{b}	184.31^{d}	200.25^{d}	11.224	<.0001
HDL (mg/dL)	48.57^{a}	31.93^{c}	37.93^{bc}	39.46^{b}	52.15^{a}	52.00^{a}	1.855	<.0001
LDL (mg/dL)	84.16^{cd}	153.62^{a}	77.51^{d}	127.79^{b}	92.13^{cd}	99.63^{c}	5.440	<.0001
VLDL (mg/dL)	48.61^{c}	73.42^{a}	48.12^{c}	58.45^{b}	36.86^{d}	40.05^{d}	2.245	<.0001

Means within the same row with different common superscripts differ significantly (P<0.05).
TC: total cholesterol; TG: triglycerides; HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein.

Our results ~~of effect~~ of stocking density agree**d** with Qaid *et al*. (2016) and Dozier *et al*. (2006) who declared that, serum cholesterol concentration in broiler chicks significantly increased at HSD. Contradicting with our results **those** obtained by Al-Hamed (2020), Shewita *et al.* (2019) and Houshmand *et al.* (2012), who showed that, there were no significant differences in TC, TG, LDL and VLDL due to stocking density in quail and broiler chicks .

The results in the current study of the impact of dietary YE supplementation on lipid profile are in the same line with the results obtained by Abd El-Wahab *et al*. (2019) who showed that quail fed diets enriched with yeast (0.5, 1.5, 2.5 and 3.5%) reduced (P<0.01 the concentrations of cholesterol and triglycerides in their serum comported to control. In addition, Shareef and AL-Dabbagh (2009) stated that, addition of yeast at 1.0, 1.5 and 2.0% reduced serum triglycerides, but only at highest level (2%) for cholesterol compared with other treatments. Likewise, Mohamed *et al.* (2015) demonstrated that, there was a linear decrease in blood cholesterol of broiler chickens fed graded yeast levels; but there was no significant difference in serum TG among all groups. Moreover, Koncea *et al.* (2009) reported that supplementation of yeast into the toms turkey diets did not affect serum triglycerides level in broilers. Yalcin *et al*. (2010) declared that, serum level of triglycerides in broiler chicks were unaffected significantly by dietary *S. Servisiae*. In addition, Pouraziz *et al*. (2013) demonstrated that, there is no significant impact of dietary *S. Serevisiae* on serum TG in broilers chicks.

Antioxidant and immunological indices:

Concerning immunity parameter, results in Table (6) showed insignificant differences among all trail groups in plasma IgA and lysozyme content. However, IgG level was higher (P<0.0001) in quail chick group housed in ND than those housed in HD and fed untreated diet with YE, while HD groups treated with YE at different levels had significantly (P<0.0001) higher IgG contents compared with groups housed in ND and untreated HD. A significant difference among experimental groups was observed in complement 3 (Table 6). It was noted that, the values of complement 3 were significantly higher in ND group and HD treated with 1 mg YE/kg diet than the groups housed in HD and untreated and treated with 2, 3 and 4 mg YE/kg diet.

Table (6): Antioxidant and immunological indices of growing Japanese quail as affected by stocking density without or with yeast extract (YE) supplementation.

Item	Normal density	high density (HD)	HD + YE level (mg/kg diet)				SEM	*P* value
			1	2	3	4		
Immunity								
IgG (mg/dl)	1.08^{cd}	0.86^{d}	1.33^{bc}	1.56^{ab}	1.82^{a}	1.75^{a}	0.083	<.0001
IgA (mg/dl)	0.72	0.56	0.79	0.90	0.79	0.81	0.072	0.0894
Complement 3	144.15^{a}	62.68^{b}	135.90^{a}	74.47^{b}	68.25^{b}	73.25^{b}	5.125	<.0001
Lysozyme	0.37	0.24	0.28	0.34	0.39	0.38	0.044	0.2317
Anti-oxidants								
SOD (U/mL)	0.37^{ab}	0.26^{bc}	0.29^{bc}	0.21^{c}	0.47^{a}	0.41^{ab}	0.043	0.0169
MDA (nmol/mL)	0.10^{d}	0.30^{a}	0.20^{bc}	0.25^{ab}	0.12^{cd}	0.14^{cd}	0.023	0.0008
TAC (ng/ml)	0.31	0.15	0.28	0.22	0.33	0.32	0.034	0.0556
GPX (ng/ml)	0.36^{a}	0.16^{c}	0.30^{ab}	0.24^{b}	0.33^{a}	0.32^{a}	0.020	0.0004

Means within the same row with different common superscripts differ significantly (P<0.05).
IgG: immunoglobulin G; IgA: immunoglobulin A; SOD: superoxide dismutase; MDA: malondialdehyde; TAC: total antioxidant capacity; GPX: glutathione peroxidase.

The results of stocking density are supported by 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, Heckert, *et al*. (2002) indicated that the HSD suppression of immunity in broilers. Houshmand *et al*. (2012) reported that broiler chicks housed at a normal stocking density had a higher antibody titer against New castle disease than those housed at a high stocking density (Heckert *et al*., 2002). Therefore, it could be concluded that the HSD in the present study resulted in poorer immunity.

Regarding ~~to~~ antioxidant parameters, the results indicated that, plasma, SOD content values were higher (P=0.0169) in the groups reared in ND and HD fed diet supplemented with either 3 or 4 mg/kg comparatively with other treatment groups. However, increasing stocking density from ND to HD without dietary YE supplementation resulted in insignificant effect between them. The GPX value were reduced (P=0.0004) in chicks kept at HD fed diet without YE supplementation when compared with chicks group kept in ND and other treatment groups (Table 6). The present results revealed that dietary supplementation with YE at different levels of HD groups resulted in enhance in GPX values to be as the same value obtained by the chicks kept in ND. No significant effect on averages of TAC values among all treatment groups.

The findings of stocking density effect, Cengiz *et al.* (2015) observed that, no significant difference between low stocking density and HSD of blood MDA, corticosterone and nitric oxide. These results are confirmed by Houshmand *et al*. (2012); Buijs *et al*. (2009) and Dozier *et al*. (2006) who reported no significant difference in corticosterone and nitric oxide in broiler chicks when reared under low stocking density and high stocking density.

Dietary yeast supplementation in Japanese quail increased the activities of TAC and SOD (Abd El-Wahab *et al*., 2019). A similar study reported that, the mechanism of oxidative defense could be improved by B-glucans and mannoligosaccharids, which are main component of *SC* (Ognik and Krauze, 2012). This action illustrates that use of yeast in poultry diets can protect the gut rather than just removing harmful microorganisms.

Microbiological analysis:

There were no significant differences in cecal microbial counts studied due to increasing stocking density in quail chicks from ND to HSD fed diets supplemented with YE. It could be noted that, HSD fed diets treated with YE at different levels had highest *Enterococcus SPP*. count and lowest total yeast and molds count, *E- Coli*, *Salmonella SPP* and *Coliform* (Table 7).

Table (7): Cecal microbial counts and Intestinal pH of growing Japanese quail as affected by stocking density without or with yeast extract (YC) supplementation.

Item	Normal density	high density (HD)	HD + YE level (mg/kg diet)				SEM	*P* value
			1	2	3	4		
Microbiological count (log CFU/g)								
Total bacterial count	6.65^{a}	6.53^{a}	6.08^{b}	5.77^{c}	5.59^{d}	5.66^{cd}	0.034	<.0001
Total yeasts and molds count	4.43^{a}	4.47^{a}	4.25^{b}	3.93^{c}	3.85^{c}	3.84^{c}	0.025	<.0001
E-Coli	5.33^{b}	5.51^{a}	4.97^{c}	4.54^{d}	4.34^{e}	4.38^{e}	0.038	<.0001
Salmonella spp	3.12^{b}	3.85^{a}	2.43^{c}	2.46^{c}	2.34^{d}	2.47^{c}	0.015	<.0001
Enterococcus spp.	5.57^{b}	5.69^{a}	4.77^{d}	5.13^{c}	5.20^{c}	4.73^{d}	0.020	<.0001
Coliform	6.30^{b}	6.56^{a}	6.22^{b}	5.38^{c}	5.35^{c}	5.36^{c}	0.025	<.0001
Intestinal Ph	6.44^{ab}	6.59^{a}	6.08^{d}	6.19^{cd}	6.12^{cd}	6.27^{bc}	0.048	0.0002

Means within the same row with different common superscripts differ significantly (P<0.05).

The results of stocking density supported by Cengiz *et al.* (2015) who found, no changes in total count of aerobs and *Salmonella* were experienced due to different stocking density. Similarly Guardia *et al*. (2011) observed, at 42 days all digestive microbiota did not change significantly between either low or high stocking density.

Contradicting results were obtained by Cengiz *et al.* (2015) who noted, there were fewer lactobacilli in broiler chicks at high stocking density as compared at low stocking density. Likewise, Tannock (1997) found that the counts of *Lactobacilli* in the intestinal content of broiler chicks are negatively affected by high stocking density.

Regarding YE findings, Abd El-Wahab *et al*. (2019) reported that, Japanese quail chicks fed diet supplemented with either 2.5 or 3% yeast had resulted in reduced the counts of *E. Coli* (P<0.05) and *C. Perfringens* CFU in excreta than those fed un-supplemented diet. However, Ghosh *et al*. (2012) reposted that dietary yeast supplementation had no significant impact in *E. Coli* CUF count in the intestinal digesta of broiler chicks.

Precise mode of yeast action to decrease intestinal gut microflora have not been understood, but years have increased the production of intestinal cytokines and IgA by macrophages (Gao *et al*., 2008). Furthermore, yeast contains prebiotics (fructo-oligosaccgarides and mannan-oligosaccharide), which have an important role in reducing harmful bacteria in poultry gut by removing them through the intestine without colonization (Iji *et al*., 2001). On the other hand, *Lactobacilli* can ferment probiotics such as fructo oligosacchlides, which can assist to decrease the growth rate of pathogens like *c. perfringens* (Hofacre *et al*., 2005). Suarez and Guevara (2018) pointed out that the yeasts have variable mechanisms including attachment and removal of pathogenic bacteria and improvements of the bird's immune response (Chichlowski *et al.*, 2007 and Ezema and Ugwu, 2014).

The intestinal pH was significantly (P=0.0002) influenced by treatments. It was mentioned that, the treatment with 0.1 mg YE/kg diet of groups stocked at HD resulted in significantly decreased intestinal pH comparable with either group kept at HD or untreated group kept at HD (Table 7).

CONCLUSION

From our results, it could be concluded that dietary addition of YE can positively mitigate the stress applied to quail raised under high stoking density by enhancing the antioxidant status, immunological parameters, intestinal pH, caecal microbial counts and as well as FCR, FI and growth performance.

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تأثير كثافة التربية بدون أو مع إضافة مستخلص الخميرة على أداء النمو ، الإنزيمات الهضمية ، نواتج التمثيل والميكروبات المعوية للسمان الياباني النامي

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هدفت هذه الدراسة إلى تحديد دور مستخلصات الخميرة الغذائية في تحسين أداء وصحة السمان المربي تحت كثافة تربية مرتفعة. تم استخدام 340 سمانة عمر 7 أيام غير مجنسة بمتوسط وزن جسم 30.42 جم. وزع السمان عشوائياً على ست مجموعات تجريبية بواقع أربع مكررات لكل مجموعة في تصميم عشوائي تام. تم وضع المجموعة الأولى (40 سمان) بمعدل 10 كتاكيت / مكرر (معاملة ضابطة ، كثافة طبيعية (ND)، 40سمان / م 2) وتم تغذيتها على العليقة الاساسية بدون أي اضافات. الثانية والثالثة والرابعة والخامسة والسادسة (60 سمان / مجموعة ؛ بمعدل 15 كتكوت / مكرر ؛ كثافة عالية (HD) ؛ 60 سمان / م 2) ويتغذى على العليقة الاساسية مع 0 ، 1 ، 2 ، 3 و 4 مجم مستخلص الخميرة / (YE)كجم علف ، على التوالي. أدت إضافة YE عند 1 مجم / كجم من العلف إلى السمان النامي المربي في HD إلى زيادة معنوية (P <0.001)في LBW في عمر 6 أسابيع و BWG خلال 3-5 و1-5 عند مقارنتها بجميع المعاملات. لم يلاحظ وجود فروق معنوية في الغذاء المأكول بين الطيور المرباة في ND أو HD بدون اضافة الخميرة. سجلت الكتاكيت المرباه تحت كثافة عالية والمغذاة على العليقة الاساسية (P <0.001)أسوأ قيم FCR مقارنة مع الكتاكيت التي تمت تربيتها تحت ND و HD مع اضافة الخميرة بمستويات مختلفة. أدت اضافة الخميرة لمجموعات HD إلى تحسينات كبيرة في الانزيمات الهضمية مقارنة بالمجموعات المرباه تحت ND و HD بدون إضافة YE. سجلت الكتاكيت التي تمت تربيتها تحت HD بدون اضافة YE قيمًا أعلى (P <0.0001) من TC و TG و LDL و VLDL وقيم أقل من HDL في الدم مقارنة مع الكتاكيت التي تمت تربيتها تحت ND و HD مع اضافة الخميرة بمستويات مختلفة. ارتفعت قيم المكمل 3 بشكل ملحوظ في المجموعة ND و HDالمعاملة بـ 1 مجم من / YE كجم عن المجموعات الموجودة في HD وغير المعاملة والمعاملة بـ 2 و 3 و 4 مجم من / YE كجم. سجلت الطيور المرباه تحت كثافة تربية عالية (HSD) والمغذاة علي YE بمستويات مختلفة أعلى مستوى من بكتيريا .Enterococcus spp وأقل عدد من الخميرة والعفن الكلي ، E- coli ، Salmonella SPP و Coliform. يمكن الاستنتاج أن اضافة الخميرة يمكن أن يخفف بشكل إيجابي من العبء الحادث من تربية السمان تحت كثافة تربية مرتفعة من خلال تعزيز حالة مضادات الأكسدة، والقياسات المناعية ، ودرجة الحموضة المعدة، وعدد الميكروبات الضارة، وكذلك تحويل العلف وتناوله وأداء النمو.