P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article

The Impact of High Stocking Density and *Saccharomyces cerevisiae boulardii* on Productive Performance, Intestinal Microbiota and Gut Integrity of Broiler Chickens

Awaad MHH[1*], MA Elmenawey[2], MA Afify[1], SA Zouelfekar[1], FF Mohamed[1], M Elhariry[1] A Samir[1] and V Demy[3]

[1]Faculty of Veterinary Medicine, Cairo University; [2]Faculty of Agriculture, Cairo University; [3]Lallemand SAS, Blanca, France
***Corresponding author:** awaad3@gmail.com

Article History: Received: July 20, 2019 Revised: August 17, 2019 Accepted: September 13, 2019

ABSTRACT

Day-old male Arbor Acres plus broiler chickens (n=1120) were used to study the effect of dietary supplementation of the Probiotic *S.* c. boulardii on production performance, intestinal microbiota and gut integrity under HSD challenge. Duration of the trial extended from one day of age up to slaughter (42 days). The birds were allotted into 4 groups (1-4). Those of groups 1(NC) and 3 (T1) consisted of 240 birds. While those of groups 2 (PC) and 4(T2) consisted of 320 birds each. All groups ran contemporaneously. The birds of each group assigned into 10 replicates. Those of groups NC and T1 consisted of 10 birds/replicate/m^2 floor area; while those of groups PC and T2 consisted of 13 birds/replicate/m^2 floor area. Chickens of groups T1 and T2 were dietary supplemented with S.c. bululardii; while PC and NC groups were kept as controls. All experimented birds were vaccinated against different diseases according to the vaccination programs usually adopted in Egyptian chicken broiler farms. The used dietary supplement in challenged and unchallenged HSD improved productive performance variables including BW, BWG, FCR. It improved V/C ratio, fecal and cecal LAB counts and GIT integrity. It also Reduced macroscopic and microscopic lesion scores post vVND virus challenge.

Key words: High Stocking Density Challenge, Productive performance, S.c. boulardii, Intestinal microbiota, Gut integrity.

INTRODUCTION

Broilers are housed at different densities, relying on nearby rules, production device, and target marked body weight. Stocking density is defined as the numbers of birds or weight of birds being produced in a unit of house area. Presently, broiler production has accelerated and high stocking density was used to increase production and decrease constant charges (Gopinger *et al.,* 2015). The final aim of broiler producers is maximizing the profitability by increasing the body weight of chicken in a unit of house floor area and preventing production losses due to high stoking density (Rashidi *et al.,* 2019).

On the other hand, stocking density is considered as a crucial stress aspect in broiler production (Qaid *et al.,* 2016). It's far properly documented that, in broilers, excessive stocking densities adversely influences productive performance, livability, and immunity (Zhang *et al.,* 2013). Such stress factor consequences in serious economic losses (Kabir, 2009c). It has caused the need for researching new products used as growth enhancers under stress conditions and promising results that can replace antibiotics. Enzymes, acids, prebiotics and probiotics, herbs or etheric oils are some examples of classes of products that are used as growth enhancers' alternatives to antibiotics (Huyghebaert *et al.,* 2011). Probiotics are used to beneficially have an effect on the host animal, promotion intestinal microbiota balance. Supplementation with probiotics and/or prebiotics can enhance the productive performance of broiler chickens (Bozkurt *et al.,* 2014). They enhance feed consumption and digestion, and stimulate the immune system (Perumalla *et al.,* 2011). They improve sensory characteristics of dressed broiler meat (Pelicano, 2003) and promote microbiological meat

Cite This Article as: Awaad MHH, MA Elmenawey, MA Afify, SA Zouelfekar, FF Mohamed, M Elhariry, A Samir and V Demy, 2019. The impact of high stocking density and *saccharomyces cerevisiae boulardii* on productive performance, intestinal microbiota and gut integrity of broiler chickens. Inter J Vet Sci, 8(4): 362-370. www.ijvets.com

quality of broilers (Kabir *et al.*, 2005b). They are one of the possible approaches employed to avoid physiological stress in broilers (Cengiz *et al.,* 2015). It is expected that dietary supplementation of the probiotics can reduce the bad outcomes of HSD stress.

The probiotic arrangements are being increasingly more used in broiler diets to increase growth rate, enhance food utilization and to protect against intestinal infections (Xavier *et al.,* 2011). Chicken GIT provides a way with the aid of which the body derives nutrients, furnishes protective mechanisms to shield the host and serves as an environment for other live organisms (microbiota). Functions of probiotics inside the digestive system aren't known exactly, but a few optional mechanisms are; supplying nutrient, assisting in digestion of foods and inhibition of harmful bacteria in GIT. Ghadban (2002) stated that probiotics are mono-or mixed culture of living microorganisms, which result in useful effect to the host by enhancing the residences of the indigenous microflora. The useful impact of probiotics is based on their capability to alter gut microflora which necessitates that the microorganisms attain the intestine in a viable structure. The mode of action of probiotics includes; competitive exclusion (Berchieri *et al.,* 2006) and microbial antagonism (Mountzouris *et al.,* 2006).

We hypothesized that the use of Probiotics as supplements, would alleviate the adverse effects of HSD in broilers. Therefore, the aim of this study is to investigate the impact of dietary supplementation with a probiotic of viable cells of the yeast. S. c. *boulardii*, on productive performance, gut integrity and gut microbiota of naturally disturbed microflora balance of broiler chickens under HSD stress.

MATERIALS AND METHODS

The probiotic: *S. c. boulardii* 10 ME Titan [a microbial feed additive (E1703) highly concentrated in viable cells of yeast *Saccharomyces cerevisiae* strain CNCM I 1079] was used in this trial in an incorporation rate corresponding to 2.10^9 cfu/kg of complete pelleted feed for the first phase (starter) (100 g/Ton Levucell SB 10/T) and 1.10^9 cfu/kg feed for grower and finisher feed (50 g/T) lot No.87A3622P659.

Experimental design: One-day-old male Arbor Acres plus broiler chickens (n=1120) were used to study the effect of dietary supplementation of the Probiotic *S. c. boulardii* on productive performance, intestinal microbiota and gut integrity under HSD challenge. Duration of the trial extended from one day of age up to slaughter (42 days). The birds were allotted into 4 groups (1-4). Those of groups 1 (NC) and 3 (T1) consisted of 240 birds. While those of groups 2 (PC) and 4 (T2) consisted of 320 birds each. All groups ran contemporaneously. The birds of each group were assigned into 10 replicates. Those of groups NC and T1 consisted of 10 birds/replicate/m^2 floor area, while those of groups PC and T2 consisted of 13 birds/replicate/m^2 floor area. Chickens of groups T1 and T2 dietary supplemented with *S.* c. *bulularii*, while groups PC and NC were kept without supplementation as controls. All experimented birds were vaccinated against different diseases according to the vaccination programs usually adopted in Egyptian chicken broiler farms. All chickens were floor reared in separate pens and kept in environmentally controlled rooms. The experimental design is illustrated in Table 1.

Diets: Chickens were fed *ad libitum* a pellet commercial starter diet (23% crude protein and 3000 k cal ME/kg diet) during the first 2 weeks of age, commercial grower diet (22% crude protein and 3150 kcal ME/kg diet) from 2-4 weeks of age and then commercial finisher diet from 4-6 weeks (19% crude protein and 3200 kcal ME/kg diet). The diets compositions are indicated in Table 2. Birds had free access to water. No other microbial additive or AGP were added. Semduramicin was included to ration at a concentration of 25 ppm as a coccidiostat.

Measured parameters

Productive performance: Chicken performance response variables were determined according to Brady (1968), Sainsbury (1984) and North (1984). For BW, all birds were weighted individually at 1st day and weekly for the entire period of the experiment (6 weeks). FI was measured on the same days of birds weighting. FCR (g feed/g live BW), and carcass characteristics (dressing wt.%, front part wt.%, hind part wt.%, breast meat wt.%, thigh drumstick meat wt.%, carcass meat wt.%, heart wt.%, gizzard wt.%, liver wt.%, spleen wt.% bursa of fabricus wt.% thymus glands wt.% and intestinal length and diameter) were measured on 20 birds for each group at the end of the trial. The mortality rates have been recorded daily for each replicate. To keep the bird density, per replicate, constant, dead birds were replaced on the same day of death.

Microbiological assay: Random fresh droppings as well as specimens of caeci of 3 sacrificed chickens of the 4 investigated groups were collected at 14th day, 28th day, 35th day and 42nd day of age for enumeration of total *LAB* and *E. coli.* MRS agar (MERCK, 1.10660) was used for *LAB* counts incubated at 30°C for 3 days. *E. coli* was grown on VRB agar (MERCK, 1.01406) aerobically at 37°C for 24-48 hours. The bacterial colonies were enumerated, and the average number of live bacteria was calculated based on per gram of original intestinal contents. All quantitative data were converted into logarithmic colony forming units (cfu/g).

Table 1: Experimental design

Group No.	Chicken groups	No. of birds/ m2 floor area	Increased Density Challenge	*Probiotic* supplementation (1.10^9 cfu/kg feed) (100 g/Ton)
1	NC (Negative control: untreated unchallenged by HSD)	10	-	-
2	PC (Positive control: untreated challenged by HSD)	13	+	-
3	TUch (Treated unchallenged by HSD)	10	-	+
4	Uch (Untreated challenged by HSD)	13	+	+

Table 2: Composition of the broilers 3-phase diets (g/kg as fed) and their calculated chemical composition (on as fed basis)

Ingredients	Starter	Grower	Finisher
Yellow corn	524.5	544.2	628.5
Soybean meal 44%	332.4	299.1	221.1
Corn gluten meal 60%	70	70	66.5
Oil	30	43.8	40
Di-calcium phosphate	18	18	18
Lime stone	13	13	13
D.L. Methionine	2.2	2.1	2.3
Lysine hydrochloride	2.9	2.8	3.6
Sodium chloride	4	4	4
Premix*	3	3	3
Calculated analysis:			
Crude protein %	23.0	22.0	19.0
Metabolizable energy (kcal/kg)	3000	3150 3200	3200

*Each gram of mineral mixture contained: vitamin A (trans-retinyl acetate), 9,000 IU; vitamin D3 (cholecalciferol), 2,600 IU; vitamin E (dl-α-tocopheryl acetate), 16 mg; vitamin B1, 1.6 mg; vitamin B2, 6.5 mg; vitamin B6, 2.2 mg; vitamin B12 (cyanocobalamin), 0.015 mg; vitamin K3, 2.5mg; choline (choline chloride), 300 mg; nicotinic acid, 30 mg; pantothenic acid (d-calcium pantothenate), 10 mg; folic acid, 0.6 mg; d-biotin, 0.07 mg; manganese (MnO), 70 mg; zinc (ZnO), 60 mg; iron (FeSO4 H2O), 40 mg; copper (CuSO4 5H2O), 7 mg; iodine [Ca(IO3)2], 0.7 mg; selenium (Na2SeO3), 0.3 mg.

Response to vaccination: For overall judgment on immune modulation; challenge with vVND virus was adopted on 15 birds from each group at 42 days of age. These birds were under close observation for further 21 days for clinical signs and mortality. Macroscopic and microscopic (histopathologic) lesion scoring were determined at 7 and 14 days PI on 5 sacrificed chickens/ group.

Gut integrity assay (Histomorphometric assay): For histomorphometric assay; 5 birds from each group were sacrificed at the end of the trial, dissected and 1 cm-thick samples were taken from jejunum and ileum [the intestinal segmentation according to Samanya and Yamauchi (2002) as jejunum from the bile duct to Meckel's diverticulum and ileum from the Meckel's diverticulum to ileo-cecalcolonic junction]. Routine histological laboratory methods were adopted and villus histomorphometry for recording the histological indices were measured using digital photography and light microscopy. The photos were taken and morphometric analyses were performed. The villus height was measured from the apical to the basal region. Crypts were measured from the basis until the region of transition between the crypt and the villus. Five measurements per section were made for each parameter and averaged into one value.

Statistical analysis

One way analysis of variance for the data was done using the SAS General Linear Model Procedure (SAS Institute, 2000). Mean values were compared using Duncan's Multiple Rang Test, (Duncan, 1955) when significant differences existed. The significance level was set at 5%.

RESULTSAND DISCUSSION

The results of productive performance including, body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) and mortality rate are given in Table 3 (a, b and c). BW, BWG and FI were significantly affected adversely by increasing density in the growing period ($P<0.05$). Whereas, FCR and mortality rate were not significantly affected by stocking density. Similar results have been described by other investigators (Abudabos *et al.*, 2013; Qaid *et al.*, 2016). Our results were consistent with those reported by Zhang *et al.* (2013) who observed that final body weight and average daily gain were negatively affected by increasing the stocking density, but FCR was not affected.

Influence of *S.c. boulardii* supplementation on HSD challenge revealed, a significant increase in TUCh and Uch vs. PC group at the end of the trial ($P<0.05$). In the high stocking condition, BW and BWG were significantly

Table 3a: Effects of high stocking density and *Saccharomyces cerevisiae boulardii* supplementation on BWt, BWt gain and feed intake

	Body Weight (g)						
Chicken Groups	Age						
	1 day	7 days	14 days	21 days	28 days	35 days	42 days
NC	38.9±0.18	152±1.4^{a}	384 ±2.8^{a}	826±7.9^{a}	1372±12.3^{a}	1828±17.1^{a}	2478±21.5^{a}
PC	39.1±0.15	147±0.9^{b}	365±2.2^{b}	806±5.6^{b}	1327±8.2^{bc}	1782±14.3^{b}	2410±17.2^{b}
TUCh	38.9±0.18	152±1.1^{a}	381±2.8^{a}	831±6.6^{a}	1341±11.1^{b}	1833±15.6^{a}	2487±17.5^{a}
UCh	38.9±0.15	147.6±1.1^{b}	367.2±2.5^{b}	795.3±6.0^{b}	1301.2±9.9^{c}	1779.1±13.0^{b}	2454.6±14.6^{a}
Probability	0.8535	0.0001	0.0001	0.0002	0.0001	0.0107	0.0078
	Body weight gain (g)						
	0-7 days	8-14 days	15-21 days	22-28 days	29-35 days	36-42 days	1-42 days
NC	113±1.13^{a*}	231±2.7^{a}	442±8.0	546±14.1	451±19.8	649±24.6	2439^{a}
PC	108±0.92^{b}	218±2.3^{b}	440±6.0	522±9.9	454±16.4	629±22.8	2371^{b}
TUCh	113±1.1^{a}	229±2.9^{a}	449±6.9	510±11.9	489±18.5	653±22.9	2448^{a}
UCh	108±1.06^{b}	219±2.7^{b}	428±6.6	505±12.0	474±16.7	677±20.3	2415^{ab}
Probability	0.0001	0.0003	0.1641	0.0941	0.4291	0.4641	0.0077
	Feed intake (g/bird/day)						
	0-7 days	8-14 days	15-21 days	22-28 days	29-35 days	36-42 days	1-42 days
NC	30±0.45^{a*}	57±0.7^{a}	107±2.0	139±3.4^{a}	170±5.5	165±5.4	4692±100.1^{a}
PC	25±0.5^{b}	47±2.0^{b}	107±1.8	134±2.9^{ab}	157±5.3	164±4.5	4453±57.4^{b}
TUCh	30±0.6^{a}	56±1.1^{a}	105±1.4	136±2.5^{ab}	172±5.1	152±1.8	4576±63.0^{ab}
UCh	26±0.3^{a}	48±0.9^{b}	104±1.7	128±1.9^{b}	160±5.7	161±4.7	4395±71.6^{b}
Probability	0.0001	0.0001	0.5801	0.360	0.1598	0.1475	0.0376

*Means followed by different superscripts, between treatments, within trait within age are significantly different ($P\leq0.05$).

Table 3b: Effects of high stocking density and *Saccharomyces cerevisiae boulardii* supplementation on FCR.

	FCR (g feed/g live BW)					
	1-7 days	1-14 days	1-21 days	1-28 days	1-35 days	1-42 days
NC	1.4±0.0[a]*	1.6±0.0[a]	1.6±0.0[a]	1.7±0.0	1.9±0.0	1.8±0.0
PC	1.2±0.0[b]	1.3±0.0[b]	1.5±0.0[b]	1.6±0.0	1.8±0.0	1.8±0.0
TUCh	1.4±0.0[a]	1.6±0.04[a]	1.6±0.0[ab]	1.7±0.0	1.9±0.0	1.8±0.0
UCh	1.2±0.0[b]	1.4±0.0[b]	1.5±0.0[b]	1.6±0.0	1.8±0.0	1.7±0.0
Probability	0.0001	0.0001	0.0083	0.1453	0.1422	0.2822

*Means followed by different superscripts, between treatments, within trait within age are significantly different (P≤0.05).

Table 3c: Effects of high stocking density and *Saccharomyces cerevisiae boulardii* supplementation on mortality rate

	Mortality rate (%)						
	1-7 days	8-14 days	15-21 days	22-28 days	29-35 days	36-42 days	1-42 days
NC	0.00±0.0	0.42±0.4	2.50±0.9	2.92±1.4	1.67±0.9	1.67±0.7	9.18±2.6
PC	0.00±0.0	0.31±0.3	2.19±1.1	2.82±0.8	1.56±0.7	3.44±1.3	10.32±2.4
TUCh	0.00±0.0	0.00±0.0	1.67±0.7	3.75±1.2	1.67±0.9	0.83±0.6	7.92±2.0
UCh	0.00±0.0	0.62±0.4	2.50±0.8	2.19±0.8	0.93±0.7	2.19±0.7	8.43±2.3
Probability	-	0.6129	0.8922	0.7566	0.9046	0.1885	0.8958

*Means followed by different superscripts, between treatments, within trait within age are significantly different (P≤0.05).

Table 4a: Effects of high stocking density and *Saccharomyces cerevisiae boulardii* supplementation on Carcass quality

Organ Treatment	Dressing Wt. %	Front parts Wt. %	Hind parts Wt. %	Breast Meat Wt. %	Thigh + Drumstick meat Wt. %	Carcass meat Wt. %	Heart Wt. %
NC	69±0.5	39±0.4	30±0.3	16±0.6	13±0.4	30±0.9	0.67±0.0
PC	69±0.4	39±0.4	30±0.4	16±0.3	13±0.3	30±0.5	0.67±0.0
TUCh	69±0.5	39±0.5	29±0.4	16±0.4	13±0.2	30±0.5	0.76±0.7
UCh	69±0.7	39±0.5	30±0.5	16±0.4	13±0.3	30±0.6	0.78±0.1
Probability	0.8332	0.6242	0.8339	0.9400	0.6020	0.8847	0.2110

*Means, between treatment, within organ followed by different superscripts, differ significantly (P ≤ 0.05)

Table 4b: Effects of high stocking density and *Saccharomyces cerevisiae boulardii* supplementation on Carcass quality

Organ Treatment	Gizzard Wt. %	Liver Wt. %	Giblets Wt. %	Spleen Wt. %	BF Wt. %	Thymus Wt. %	Intest. Length cm	Intest. width cm
NC	1.8±0.1	2.6±0.1	5.1±0.1	0.2±0.0	0.2±0.0	0.5±0.0	199.5±1.8	1.00±0.0
PC	1.9±0.1	2.6±0.1	5.2±0.2	0.2±0.1	0.2±0.1	0.5±0.03	200.8±2.1	1.01±0.0
TUCh	1.9±0.1	2.7±0.1	5.4±0.2	0.2±0.1	0.2±0.1	0.5±0.0	198.1±2.3	1.01±0.0
UCh	2.0±0.2	2.8±0.1	5.6±0.2	0.2±0.0	0.2±0.0	0.5±0.0	196.7±2.3	0.99±0.0
Probability	0.4940	0.4528	0.2084	0.3543	0.1373	0.2050	0.5574	0.9208

*Means, between treatment, within organ followed by different superscripts, differ significantly (P≤0.05)

Table 5: Macroscopic lesion scoring of *S.C. boulardii* supplemented and non-supplemented broilers challenged or unchallenged by stoking density post vVND infection.

Chicken Groups	Days post vVND challenge	
	7 days	14 days
NC	5.8±1.59[ab]	6.8±1.71
PC	8.2±1.36[a]	9.2±0.58
TUCh	1.8±0.37[b]	6.0±0.32
UCh	5.0±1.92[ab]	8.0±1.14
Probability	0.0436	0.2122

*Means followed by different superscripts, within age, are significantly different (P≤0.05).

higher during the whole period due to adding the probiotic *S. c. boulardii*. On the other hand, there was 44 g numerical decrease in BWG in UCH group vs. PC group and only 9 g increase in BWG in TCh vs. NC group. Final FCR (0-6 weeks), revealed 48 points less in T1 group vs. NC group, while T2 group showed 58 points less vs. PC group. HSD challenge resulted in 1.14% higher in mortality rate in PC vs. NC group. Supplementation of *S. c. boulardii* to group TCh reduced mortality by 1.26% vs. NC group and reduced mortality by 1.89% in UCh vs. PC group. However all these differences were not statistically significant.

The slaughter yield (carcass quality) was not significantly influenced by the stocking density or dietary supplementation of *S. c. boulardii (*Table 4 a and b). Our results are in agreement with those reported by Cengiz *et al.* (2015) and Rashidi *et al.* (2019) who observed that the high stoking density or dietary probiotics supplementation did not significantly affect the carcass (carcass, breast, thighs, abdominal fat, liver and gizzard) parameters. Also, Kumprechtová *et al*. (2000) on studying the effect of *S. cerevisiae* on broiler chicken performance reported that the slaughter yield was not statistically significantly influenced by applications of the probiotic. Shareef and Al-Dabbagh (2009) studied the effect of *S. cerevisiae* on performance of broiler chickens and reported that there was no significant difference in the relative organ weights in all treated groups. Yadav, (2017) investigated the influence of dietary probiotic *S. c. cer*evisiae, supplementation on the performance, slaughter and carcass properties of broilers and concluded that it did not significantly affect the live performance and slaughter variables.

The negative effects of HSD on broiler chicken productive performance has been generally attributed to reduction of access to feed and water (Thaxton *et al.,* 2006). Additionally; air drift at bird level is decreased, hindering the dissipation of body heat (Pandurang *et al.,* 2011). Moreover; HSD can increase the ambient temperature around the bird (Feddes *et al.,* 2002) consequently; induces oxidative stress by generating reactive oxygen substance

(Lan *et al.*, 2004). Sohail *et al.* (2011) reported that oxidative stress can be stimulated by disturbing situations such as high ambient temperature and high stoking density, which result in an oxidative disruption in lipids, nucleic acids and proteins. Moreover, more bird density leads to more litter moisture, air ammonia and microbial counts in the house (Jayalakshmi *et al.*, 2009) and consequently body weight gain could be restricted.

Alleviation of HSD negative effects on productive performance by probiotics supplementation might be due to their mode of action in maintaining normal intestinal microbiota by competitive exclusion and antagonism. They also improve the metabolism by rising digestive enzymatic activity and reduce bacterial enzymatic activity and ammonia production and increase FI and digestion (Perumalla *et al.*, 2011).

Table 6: Microscopic intestinal lesion scoring of *S.C. boulardii* supplemented and non-supplemented broilers challenged or unchallenged by stocking density post vVND infection.

Chicken	Duodenum				
Groups	Villous fusions	Dilated Blood capillaries	Haemorhage	Epithelial defect	Total lesion score
NC	3.2±0.25^{a*}	1.5±0.28^{b}	0.5±0.28	3.7±0.25^{a}	9
PC	3.8±0.16^{a}	3.5±0.34^{a}	1.4±0.40	4.0±0.00^{a}	12.73
TUCh	1.7±0.85^{b}	1.3±0.21^{b}	0.3±0.20	2.5±0.042^{b}	5.91
UCh	2.8±0.40^{ab}	2.1±0.20^{b}	0.7±0.35	3.8±0.14^{a}	9.55
Probability	0.0232	0.0054	0.4120	0.0321	
			Jejunum		
	Villous fusions	Dilated Blood capillaries	Haemorhage	Epithelial defect	Total lesion score
NC	2.4±0.40	1.4±0.24	0.6±0.24	3.0±0.34	7.4
PC	3.2±0.58	2.0±0.31	0.8±0.31	3.4±0.60	9.4
TUCh	1.6±0.81	1.2±0.20	0.2±0.20	2.2±0.37	5.2
UCh	1.6±0.67	1.4±0.40	0.4±0.24	2.8±0.48	6.2
Probability	0.0891	0.5241	0.6897	0.4588	
			Ileum		
	Villous fusions	Dilated Blood capillaries	Haemorhage	Epithelial defect	Total lesion score
NC	1.7±0.47^{a}	1.0±0.00^{b}	0.0±0.00^{b}	1.2±0.25^{bc}	4
PC	1.8±0.20^{a}	1.8±0.20^{a}	0.6±0.36^{a}	2.0±0.00^{a}	6.25
TUCh	0.6±0.20^{b}	0.5±0.20^{c}	0.0±0.00^{b}	0.4±0.20^{c}	1.52
UCh	0.7±0.47^{ab}	1.0±0.00^{b}	0.0±0.00^{b}	1.7±0.18^{b}	3.46
Probability	0.0098	0.0001	0.0259	0.0001	

*Means followed by different, superscripts, within trait, are statistically significant different (P≤0.05).

Table 7: Effects of high stocking density and *Saccharomyces cerevisiae boulardii* dietary treatments on histomorphology of Jujenum and ileum

Groups	Jejunum			Ileum		
	Villi height	Crypt depth	V/C ratio	Villi height	Crypt depth	V/C ratio
NC	1361±54.8^{d*}	185±24.6^{a}	7.58±0.9	538±12.9^{b}	141±6.2^{a}	3.85±0.2^{b}
PC	918±12.0^{c}	123±8.2^{b}	7.67±0.4	533±23.1^{b}	140±7.2^{a}	3.84±0.3^{b}
TUCh	1527.7±41.0^{a}	150±4.5^{ab}	11.44±0.4	658±30.3^{a}	106±3.8^{b}	6.43±0.5^{a}
UCh	1189±28.1^{c}	179±3.5^{a}	6.82±0.23	615±7.8^{a}	137±5.5^{a}	4.50±0.2^{b}
Probability	0.0001	0.0088	0.4621	0.0002	0.0001	0.0001

*Means followed by different, superscripts, within trait, are significantly different (P≤0.05).

Table 8a: Effects of *high stocking density and S.C. boulardii* dietary treatments on fecal total *Lactic acid bacteria* and total *Coliforms* (10^4 cfu/g feces)

	Fecal samples							
Chicken	*Lactic acid bacteria*				*Coliforms*			
groups	14 ds	28 ds	35 ds	42 ds	14 ds	28 ds	35 ds	42 ds
NC	7.0±1.5^{c*}	7.0±1.5^{c}	80.0±5.8^{c}	333.3±120.2^{c}	800.0±57.8^{b}	2300.0±0.0^{c}	1466.67±88.2^{d}	190000.0±0.0^{a}
PC	20.0±0.0^{c}	20.0±0.0	1400.0±152.8^{b}	500.0±115.5^{c}	140.0±5.8^{c}	11000.0±0.0^{a}	8000.0±577.6^{c}	24000.0±0.0^{c}
TUCh	150.0±5.8^{b}	76.7±8.8^{b}	1100.0±57.7^{b}	8666.7±881.9^{a}	1433.3±120.2^{a}	800.0±57.7^{d}	15666.7±881.9^{b}	26666.7±6666.7^{c}
UCh	223.3±8.8^{a}	600.0±57.7^{a}	3000.0±577.4^{a}	7000.0±0.0^{b}	1366.7±120.19^{a}	5333.3±333.33^{b}	30000.0±0.0^{a}	53333.3±3333.3^{b}
Probability	0.0001	0.0001	0.0009	0.0001	0.0001	0.0001	0.0001	0.0001

*Means followed by different, superscripts between treatment groups, within trait and age, are significantly different (P≤0.05).

Table 8b: Effects of *high stocking density and S.C. boulardii* dietary treatments on cecal total *Lactic acid bacteria* and total *Coliforms* (10^4 cfu/g feces)

	Cecal samples							
Chicken groups	*Lactic acid bacteria*				*Coliforms*			
	14 ds	28 ds	35 ds	42 ds	14 ds	28 ds	35 ds	42 ds
NC	25±0.0^{c*}	1500±100.0^{b}	500±57.7^{b}	180±5.8^{b}	146±14.5^{b}	2500±115.5	12000±1154.7	15000±577.4^{b}
PC	17±1.2^{c}	90±0.0^{d}	2400±0.0^{a}	400±57.7^{b}	120±11.6^{b}	2266±290.6	9000±1154.7	10666±881.9^{c}
TUCh	800±0.0^{b}	500±57.7^{c}	600±152.8^{b}	1100±57.7^{b}	190±5.8^{a}	1366±233.3	15000±1732.1	14000±577.4^{b}
UCh	1366±145.3^{a}	12000±0.0^{a}	400±100.0^{b}	15000±577.4^{a}	213±8.8^{a}	2166±440.9	12000±577.4	18000.0±577.4^{a}
Probability	0.0001	0.0001	0.0001	0.0001	0.0001	0.1087	0.0519	0.0004

*Means followed by different, superscripts between treatment groups, within trait and age, are significantly different (P≤0.05).

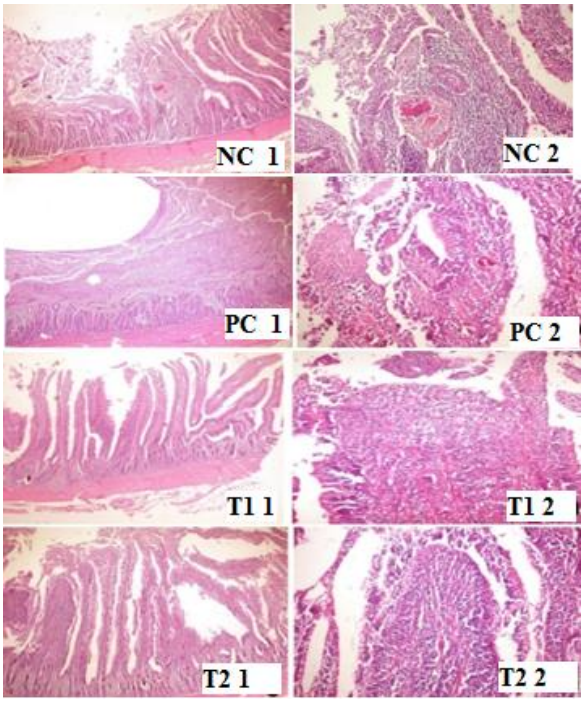

Fig. 1: Histopathological lesions of intestinal tract post vVND challenge. NC-1) Negative control group showing villous fusion with disruption of intestinal villi (100X). NC-2) Negative control group showing sever necrosis and desquamation of intestinal epithelium with intense lymphocytic infiltration associated with edema and congestion (400X). PC-1) Positive control group showing massive destruction of intestinal villi with severe inflammatory reaction involving the intestinal propria (100X). PC-2) Positive control group showing necrosis of intestinal epithelium, hyperemia of blood capillaries and edema with lymphoctic infiltration (400X). TUCh-1) Unchallenged treated group showing mild inflammatory reaction involving the intestinal wall. TUCh-2) Unchallenged treated group showing focal apical desquamation of intestinal villi with lymphocytic infiltration in the lamina propria associated with mild congestion (400X). UCh-1) Challenged treated group showing villous fusion (100X). UCh-2) Challenged treated group showing sloughing of intestinal epithelium with intense lymphocytic infiltration in the lamina propria (400X).

Table 5 illustrates the macroscopic lesion scores PI with vVND. A numerically higher lesion score was observed in PC vs. NC groups (+2.4 at 7 and 14 days PI). Reduced lesion score was detected in TUCh vs. NC groups (-4 and -0.8 at 7 and 14 days PI respectively). While UCh showed 3.2 and 1.2 reduction vs. PC groups (at 7 and 14 days PI respectively).

Microscopic (histopathologic) lesion scores post vVND infection of the intestinal tract (duodenum, jejunum and ileum) are shown in Table 6 and Fig. 1. All intestinal segments showed the same pattern of total lesion score. HSD challenge resulted in higher scores in challenged groups (PC and T2). T1 group showed the lowest score as compared with other different studied groups and T2 group showed less score than PC group. Our obtained results clarify the immune modulation of the used probiotic which is on line with findings of other investigators (Lan *et al.*, 2005; Apata, 2008).

Gut integrity investigated by histomorphological examination of jejunum and ileum is summarized in Table (7). V/C ratio of the jejunum and ileum revealed significant increase in T1 group vs. NC group respectively (P≤0.05). Based on HSD challenge; there was significant decrease in Jejunum villi height in PC vis. NC birds (P≤0.05). *S. c. boulardii* supplementation alone caused significantly the highest jejunal villi length than any of all other 3 investigated groups (P≤0.05). The significant increase (P≤0.05) in ileum villus height in unchallenged treated group (T1) was accompanied with a significant reduction in crypt depth (P≤0.05) that indicates better histomorphological structure. On the other hand; the jejunal villus length was significantly decreased (P≤0.05) in PC (challenged controls) group accompanied with significant reduction in their crypt depth when compared with T2 (challenged treated) group (P≤0.05). This indicates less regenerative capacity of intestinal crypts as compared with challenged *S. c. boulardii* supplemented group. Kabir *et al.* (2005a) reported that probiotics can change intestinal histology. Bradley *et al.* (1994) found lower goblet cells number and crypt depth in ileal mucosa of broilers supplemented with *S. cerevisiae* which demonstrate reduction of mucosa turnover. Santin *et al.* (2001) reported on a significant improvement in FCR for broilers receiving diet with cell wall of *S. cerevisiae*. They suggested that the increase of villus height observed in intestinal mucosa, in that study was a possible explanation for these results.

The structure of the intestinal mucosa is an important determinant of intestinal function (digestive and absorptive) affecting growth performance of poultry. Generally, increases in villus height and V/C ratio increases the absorption of nutrients due to a larger surface area (Afsharmanesh and Sadaghi, 2014). Probiotics in poultry diets can affect the histology of the intestinal mucosa. The villus height and the V/C ratio in the intestinal mucosa were increased by *B. subtilis* (Afsharmanesh and Sadaghi, 2014), *B. coagulans* (Hung *et al.,* 2012). Also, the probiotic reconstituted the normal structure of chicken intestinal villi distorted and damaged by stress factors (Jayaraman *et al.,* 2013).

Results of microbial population are shown in Table 8(a and b). Fecal examination revealed that total *LAB* significantly increased in T1 group vs. NC group during the entire period of the trial (42 days) (P≤0.05). Based on HSD challenge; there was numerical increase in *LAB* in PC vs. NC groups at 14, 28 and 42 days of age with only significant increase at 35 days (P≤0.05). *S. c. boulardii* supplementation with HSD challenge caused significant increase in *LAB* in T2 group (challenged treated group) over all other 3 groups at 14, 28 and 35 days of age (P≤0.05). Caecal examination indicated significant (P≤0.05) increase in total *LAB* in T1 group vis. NC group at 14 days and numerical increase at 35 and 42 days of age. HSD challenge resulted in significant and numerical increase in *LAB* in PC vs. NC group at 35 and 42 days of age respectively (P≤0.05). *S. c. cerevisiae* supplementation with HSD challenge resulted in significant increase of T2 (challenged treated) group vis. PC (challenged controls) at 14, 28 and 42 days of age (P≤0.05). On the other hand; the total coliforms count gave variable results at different investigated intervals in both fecal samples and caecal contents as well. Mountzouris *et al*. (2007) concluded that probiotics can modulate the intestinal microbiota.

Regarding aforementioned findings; generally speaking; both HSD challenge and *S. c. boulardii* supplementation increased *LAB* in either fecal samples or caecal contents. *LAB* plays a major role in increasing the acidity of the gut ecosystem which helps in obtaining a balanced gut microbiota. This increase in *LAB* is expected due to addition of the probiotic *S.c. boulardii*, however an association between HSD challenge and the increase in such bacteria counts might be a defense mechanism by the host to overcome the adverse effect produced under challenging conditions, an assumption which needs further investigation.

One of the major determinants of a healthy GIT is the composition of the microbial population. Probiotics can change the microbial population dynamics in the GIT eventually creating a more favorable microbial population due to a shift in the balance of beneficial and harmful microbes (Mountzouris *et al.,* 2009). Mountzouris *et al.* (2007) concluded that probiotics can modulate the intestinal microbiota. The most common modulation of the GIT microflora by probiotics (for example in chickens) is the increase in the populations of *Lactobacillus* and *Bifidobacteria* while, populations of coliforms particularly *Escherichia coli* decreased (Mookiah *et al.,* 2014; Cao *et al.,* 2013) Healthy microbial populations in the GIT are often associated with enhanced animal performance, reflecting more efficient digestion and improved immunity (Hung *et al.,* 2012). The reduction in pathogenic microorganisms in the GIT may be attributable to the adhesion of the probiotic microbes to the intestinal epithelium, thereby excluding pathogens competitively or by inducing immune system response (Shim *et al.,* 2012). Mulder (1996) mentioned that probiotics are able to inhibit the growth of potentially pathogenic microorganisms by lowering the pH through production of lactate, lactic acid and volatile fatty acids. Our results and taking into consideration results of Joan Jeffrey (1998) suggests the use of competitive exclusion products to restore a protective microflora following disruption of the intestinal bacteria by a disease or alterations of intestinal flora due to stress. Application of such policy in controlling intestinal infections likely to be the body's first line of defense against harmful microorganisms and this bacterial army prevents a range of illnesses.

Conclusions

Based on our results, it could be concluded that broiler's productive performance appear to be negatively influenced by increasing the stocking density. In conclusion; dietary supplementation of the probiotic *S.c. boulardii* improved the BW, FI, FCR and the mortality rate. This improvement in productive performance variables might be related to the improving of the microbial population balance in GIT which has an important role in the health and performance of broiler chickens (Koc *et al.,* 2010). The present study eventually proved that dietary supplementation of *S. c. boulardii* is able to enhance broiler chicken performance and gut integrity in either balanced or disturbed intestinal microbiota due to HSD stress. So, it could be recommended that supplementation of *S. c. boulardii* can alleviate the negative effect of the high stocking density on the productive performance of broilers.

Acknowledgments

The authors acknowledge Lallem and SAS, Blagnac., France for supplying of the material of treatment and for sponsorship and financial support of this research. They also acknowledge Animal Production Department, Faculty of Agriculture, Cairo University, Egypt, for carrying out the experimental work.

REFERENCES

Abudabos AM, EM Samara, EO Hussein, MAQ Al-Ghadi and RM Al-Atiyat, 2013. Impacts of stocking density on the performance and welfare of broiler chickens. Italian J Anim Sci, 12: 66-71.

Afsharmanesh M and B Sadaghi, 2014. Effects of dietary alternatives (probiotic, green tea powder, and Kombucha tea) as antimicrobial growth promoters on growth, ileal nutrient digestibility, blood parameters, and immune response of broiler chickens. Compar Clinic Pathol, 23: 717-724.

Apata DF, 2008. Growth performance, nutrient digestibility and immune response of broiler chicks fed diets supplemented with a culture of *Lactobacillus bulgaricus.* J Sci Food Agric, 88: 1253-1258.

Berchieri A, E Sterzo, J Paiva, C Luckstadt and R Beltran, 2006. The use of a defined probiotic product (Biomin® PoultryStar) and organic acids to control Salmonella enteritidis in broiler chickens. 9th International Seminar on Digestive Physiology in the Pig, 2: 217-219. Biochem Biophys Res Com, 85: 1551-1559.

Bozkurt M, N Aysul, K KüçüKyilmaz, S Aypak, G Ege, AU Catli, *et al.,* 2014. Efficacy of in-feed preparations of anticoccidia, multienzyme, prebiotic, probiotic and herbal essential oil mixture in healthy and Eimeria spp. infected broilers. Poult Sci, 93: 389-399.

Bradley GL, TF Savage and KI Timm, 1994. The effects of supplementing diets with *Saccharomyces cerevisiae* var. boulardii on male poultry performance and ileal morphology. Poult Sci, 73: 1766-1770.

Brady WL, 1968. Measurement of some poultry performance parameters. Vet Rec, 88: 245-260.

Cao GT, XF Zeng, AG Chen, L Zhou, L Zhang, YP Xiao and CM Yang, 2013. Effects of a probiotic, Enterococcus faecium, on growth performance, intestinal morphology, immune response, and cecal microflora in broiler chickens challenged with Escherichia coli K88. Poult Sci, 92: 2949-2955.

Cengiz O, BH Köksal, OTÖ Sevim, U Ahsan AG, Üner PA Ulutaş, D Beyaz, S Büyükyörük, A Yakan and AG Önol, 2015. Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. Poult Sci, 94: 2395–2403.

Duncan DB, 1955. Multiple range and multiple F testes. Biometrics, 11: 7-42.

Feddes JJR, EJ Emmanuel and MJ Zuidhof, 2002. Broiler performance, body weight variance, feed and water

intake, and carcass quality at different stocking densities. Poult Sci, 81: 774–779.

Ghadban GS, 2002. Probiotics in broiler production-A review. Arch Für Geflügelkunde, 66: 49-58.

Gopinger E, VR Silveira, D Perondi, A Aparecida, EL Krabbe and V Fernando, 2015. Performance, carcass characteristics and litter moisture in broilers housed at two densities. Acta Scientiarum Animal Sci, 37: 35–39.

Hung AT, SY Lin, TY Yang, CK Chou, HC Liu, JJ Lu, B Wang, SY Chen, and TF Lien, 2012. Effects of Bacillus coagulans ATCC 7050 on growth performance, intestinal morphology, and microflora composition in broiler chickens. Anim Prod Sci, 52: 874-879.

Huyghebaert G, R Ducatelle and F Van Immerseel, 2011. An update on alternatives to antimicrobial growth promoters for broilers. Vet J, 187: 182-188.

Jayalakshmi T, R Kumararaj, T Sivakumar, TT Vanan, and D Thiagarajan, 2009. Influence of stocking densities on litter moisture, microbial load, air ammonia concentration and broiler performance. Tamilnadu J Vet Anim, Sci, 5: 80-86.

Jayaraman S, G Thangavel, H Kurian, R Mani, R Mukkalil and H Chirakkal, 2013. Bacillus subtilis PB6 improves intestinal health of broiler chickens challenged with Clostridium perfringens-induced necrotic enteritis. Poult Sci, 92: 370-374.

Joan S Jeffrey, 1998. University of California Poultry Symposium-University of California-Davis.

Kabir SML, 2009c. The Role of Probiotics in the Poultry Industry. Int J Mol Sci, 10: 3531-3546.

Kabir SML, MM Rahman, and MB Rahman, 2005b. Potentiation of probiotics in promoting microbiological meat quality of broilers. J. Bangladesh Soc Agric Sci Technol, 2: 93-96.

Kabir SML, MM Rahman, MB Rahman, MZ Hosain, MSI Akand and SK Das, 2005a. Viability of probiotics in balancing intestinal flora and effecting histological changes of crop and caecal tissues of broilers. Biotechnology. 4: 325-330.

Koc F, H Samli, A Okur, M Ozduven, H Akyurek and N Senkoylu, 2010. Effects of saccharomyces cerevisiae and/or mannanoligosaccharide on performance, blood parameters and intestinal microbiota of broiler chicks. Bulgarian J Agric Sci, 16: 643-650.

Kumprechtová D, P Zobač and I Kumprecht, 2000. The effect of *Saccharomyces cerevisiae* Sc47 on chicken broiler performance and nitrogen output. Czech J Anim Sci, 45: 169-177.

Lan PT, M Sakamoto and Y Benno, 2004. Effects of two probiotic *Lactobacillus* strains on jejunal and cecal microbiota of broiler chicken under acute heat stress condition as revealed by molecular analysis of 16S rRNA genes. Microbiol Immunol, 48: 917-929.

Lan Y, MWA Verstegen, S Taminga and BA Williams, 2005. The role of the commensal gut microbial community in broiler chickens. World Poult Sci J 61: 95-104.

Mookiah S, CC Sieo, K Ramasamy, N Abdullah and YW Ho, 2014. Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. J Sci Food Agric, 94: 341-348.

Mountzouris KC, C Balaskas, I Xanthakos, A Tzivinikou and K Fegeros, 2009. Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with *Salmonella enteritidis*. Br Poult Sci, 50: 467-478.

Mountzouris KC, H Beneas, P Tsirtsikos, E Kalamara and K Fegeros, 2006. Efficacy of a new multi-strain probiotic product in promoting broiler performance and modulating the composition and activities of cecal microflora. Int Poult Sci Forum Atlanta, Georgia, pp: 59.

Mountzouris KC, P Tsirtsikos, E Kalamara, S Nitsch, G Schatzmayr and K Fegeros, 2007. Evaluation of the efficacy of probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult Sci, 86: 309-317.

Mulder RWAW, 1996. Probiotics competitive exclusion microflora against salmonella. World Poult, pp: 30-32.

North MO, 1984. Broiler, Roaster, and Capon management Ch.20, P.387. "In commercial chicken production Manual". 3rd Ed. by the AVI publishing Company Inc. West Port Connecticut.

Pandurang L, G Kulkarni, G Gangane, P More, K Ravikanth Maini S *et al.*, 2011. Overcrowding stress management in broiler chicken with herbal antistressor. Iran J Appl Anim Sci, 1: 49-55.

Pelicano ERL, PA Souza, HBA Souza, A Oba, EA Norkus, LM Kodawara and TMA Lima, 2003. Effect of different probiotics on broiler carcass and meat quality. Braz J Poult Sci, 5: 207–214.

Perumalla AVS, NS Hettiarachchy and SC Ricke, 2011. Current perspectives on probiotics in poultry preharvest food safety. In T. R. Callaway, & S. C. Ricke (Eds.), Direct-Fed Microbials and Prebiotics for Animals (pp: 89-120), USA: Springer.

Qaid MI, HI Albatshan, TI Shafey, EI Hussei, and AM Abudabos, 2016. Effect of Stocking Density on the Performance and Immunity of 1- to 14-d- Old Broiler Chicks. Braz J Poult Sci, 18: 683-692.

Rashidi N, MR Ghorbani, A Tatar and S Salari, 2019. Response of broiler chickens reared at high density to dietary supplementation with licorice extract and probiotic. J Anim Physiol Anim Nutr, 103: 100-107.

Sainsbury D, 1984. Systems of management Ch.9 P. 102. In "Poultry health and Management". 2nd Ed. by D. Sainsbury. Granada Publishing Ltd. 8 Grafton Street, London WIX 3 LA.

Samanya M and K Yamauchi, 2002. Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto. Comp. Bioch. Physiol Part A: Mol Integ Physiol, 133: 95-104.

Santin E, A Maiorka, M Macari, M Grecco, JC Sanchez, TM Okada and AM Myasaka, 2001. Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. J Appl Poult Res, 10: 236-244.

SAS®, 2000. User's guide, Release 8th ed. (Cary, NC, SAS Institute).

Shareef AM and ASA Al-Dabbagh, 2009. Effect of probiotic (Saccharomyces cerevisiae) on performance of broiler chicks. Iraq J Vet Sci, 23: 23-29.

Shim Y, S Ingale, J Kim, K Kim, D Seo, S Lee, B Chae and I Kwon, 2012. A multi-microbe probiotic formulation processed at low and high drying temperatures: effects on growth performance, nutrient retention and caecal microbiology of broilers. Br Poult Sci, 53: 482-490.

Sohail MU, ZU Rahman, A Ijaz, MS Yousaf, K Ashraf, T Yaqub and H Rehman, 2011. Single or combined effects of mannanoligosaccharides and probiotic supplements on the total oxidants, total antioxidants, enzymatic antioxidants, liver enzymes, and serum trace minerals in cyclic heatstressed broilers. Poult Sci, 90: 2573–2577.

Thaxton J, W Dozier, S Branton, G Morgan, D Miles, W Roush, B Lott and Y Vizzier-Thaxton, 2006. Stocking density and physiological adaptive responses of broilers. Poult Sci, 85: 819-824.

Xavier PS, AR Donatti, MCC Souza, and G Alves, 2011. Supplementation of the diet of broilers with pectin and probiotics. Higieni Alimentar, 25: 158-165.

Yadav SB, 2017. Impact of *Bacillus amyloliquefaciens* Probiotic Strain H57 on the Intestinal Microbiota and Broiler Performance. A thesis submitted for the degree of Doctor of Philosophy at The University of Queensland. pp: 53-65.

Zhang HY, XS Piao, Q Zhang, P Li, JQ Yi, JD Liu and GQ Wang, 2013. The effects of Forsythia suspensa extract and berberine on growth performance, immunity, antioxidant activities, and intestinal microbiota in broilers under high stocking density. Poult Sci, 92: 1981-1988.