Potential impact of stocking density on growth, carcass traits, indicators of biochemical and oxidative stress and meat quality of different broiler breeds

Mohammed A. F. Nasr,[*,1] Adel Q. Alkhedaide,[†] Amal A. I. Ramadan,[‡] Abd-El Salam E. Hafez,[‡] and Mohamed A. Hussein[‡]

[*]*Animal Wealth Development Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt;* [†]*Department of Clinical Laboratory Sciences, Turabah University College, Taif University, Taif, 21944, Saudi Arabia; and* [‡]*Food Control Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt*

ABSTRACT The aim of the current study to investigate the potential impact of different stocking densities on growth performance, carcass traits, indicators of biochemical and oxidative stress and meat quality of Arbor Acres and Ross-308 broiler breeds to recommend the better stocking density with low production cost simultaneously with high quality. A total of 312 one-day old of each Arbor Acres broiler and Ross-308 were randomly classified into 3 experimental groups with different stocking density, each of 6 replicates. The first group (SD_1) was 14 birds/m^2 (28 kg/m^2), while the second group (SD_2) was 18 birds/m^2 (36 kg/m^2) and the third group (SD_3) was 20 birds/m^2 (40 kg/m^2). The growth performance, carcass traits, meat quality hematological and biochemical parameters were measured. SD_3 group possessed the lowest body weight. Alanine transaminase in Arbor Acres was 15 and 14% higher in SD_3 when compared with SD_1 and SD_2, respectively. While, was 21 and 20% of Ross-308, respectively. SD_3 revealed the highest values of cholesterol, TG, MDA, and LDL of both breeds when compared with SD_1 and SD_2, with the lowest levels of HDL, GPX, and IGG. Birds of SD_3 was the nastiest carcass weight 873 ($P = 0.000$) and 1,411.60 g ($P = 0.000$); dressing percentage 63.07 ($P = 0.000$) and 75.83% ($P = 0.000$); breast weight 513.10 g ($P = 0.000$) and 885.50g ($P = 0.000$); thigh weight 359.90 g ($P = 0.000$) and 526.08 g ($P = 0.000$) when compared with SD_1 and SD_2 of Arbor Acres and Ross-308, respectively. The dressing % of SD_1 and SD_2 was approximately 19% better than that of SD_3 of Arbor Acres, while it was 4% of Ross-308. The cooking loss and drip loss of breast and thigh muscles were higher in SD_3 of both breeds. Moreover, SD_3 possessed the highest bacterial count. In conclusion birds reared in medium stocking density revealed better performance and welfare than high density but similar to low density. Therefore, from the economic point, medium density was the best.

Key words: stocking density, Ross 308, arbor acres, performance, biochemical parameters

2021 Poultry Science 100:101442
https://doi.org/10.1016/j.psj.2021.101442

INTRODUCTION

Recently, the poultry industry is successfully producing food with high superiority together with a reduction of the production cost as a result of improving the genes makeup and managemental condition. In the last decade, the need for broilers meat is increased because the consumers consideration of a high-quality food with low fat and high protein. Therefore, consumers are aware of animal welfare and quality. Stocking density is considered one of the main factors influencing birds welfare, physical activity, and product quality (Bessei, 2006; Deep et al., 2010; Gomes et al., 2014).

Stocking density is defined as the number of birds or the birds live weight (kg) reared in an exact space (m^2) (European Commission, 2007; Berg and Yngvesson, 2012). The crucial aim of global poultry industry is not only to amplify the production of broilers meat (kg) per m^2 with superior uniformity and quality but also to prevent production losses caused by overcapacity. High stocking density of broilers is a management routine intended for decreasing cost related with labor, fuel, housing, and equipments, but may have detrimental effect on poultry health, immune system, welfare, and productive performance (Shanawany, 1988; Houshmand et al., 2012).

Received April 23, 2021.
Accepted August 15, 2021.

[1]Corresponding author: Mohammed.nasr@zu.edu.eg, Nasr.maf@gmail.com (MAFN); a.khedaide@tu.edu.sa (AQA)

There is a considerable distinction related to the stocking density of broiler among different countries; that is, the stocking density in Netherlands is 45 to 54 kg/m^2, United Kingdom is 40 kg/m^2, and Switzerland is 30 to 36 kg/m^2 (Yuan, 2017). Additionally, the welfare guidelines of the National Chicken Council (NCC, 2005) advocate 41.5 kg/m^2 for broilers more than 2 kg in the United States as an ideal stocking density.

Intensive rearing (high stocking density) is assumed as a factor of stress. Increasing hens stocking density, decreased body weight (Davami et al., 1987). Scarcity of space available for birds has been linked with endocrinological and behavioral alterations pinpointing of stress and demoted well-being (Swanson, 1995). Stress triggers the hypothalamo−pituitary−adrenal pathways that participates a pertinent responsibility in the harmony of body physiological and immunological responses of laying hens (Dohms and Metz, 1991). There was a significant decrease in immune response of high stocking density in Japanese quails (Erisir and Erisir, 2002). Heterophil/lymphocyte (**H/L**) ratio is considered a marker of chronic stress related to immune function and welfare of laying hens (Gross and Siegel, 1983; Nicol et al., 2009).

Blood biochemical profiles are mainly used as indicators to the physiological and metabolic condition of broiler (Zhang et al., 2018). High stocking density in broilers displayed metabolic modifications of blood biochemical parameters including decrease lymphocyte, increased heterophile with augmented heterophile to lymphocyte ratio (Astaneh et al., 2018), elevated blood stress hormones (Najafi et al., 2015), decrease in immune response (Turkyilmaz, 2008; Mustafa et al., 2010), increase in oxidative stress (Gursu et al., 2004; Lan et al., 2004), increase vulnerability to infection as in Newcastle disease and necrotic enteritis (Mustafa et al., 2010; Tsiouriset al., 2015), increased plasma levels of glucose, corticosterone, and cholesterol during the adaptive phase of stress (Puvadolpirod and Thaxton 2000; Shakeri et al., 2014). Therefore, the aim of the current study to investigate the potential impact of different stocking densities on growth performance, carcass traits, indicators of biochemical and oxidative stress and meat quality of Arbor Acres and Ross-308 broiler breeds to recommend the better stocking density with low production coast simultaneously with high quality.

MATERIALS AND METHODS

Animals and the Experiment

The current investigation was authorized by the Animal Care and Welfare Committee (ZU-IACUC) of Zagzaig University, Egypt (ZU-IACUC/2/F/95/2019). The study has been performed at Research Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. A total of 312 one-day old of each Arbor Acres broiler (48.39 ± 0.0.07g) and Ross-308 chickens (41.68 ± 0.27) were randomly classified into 3 experimental groups with different stocking densities, each of 6 replicates. The first group (SD_1) was 14 birds/m^2 (28 kg/m^2), while the second group (SD_2) was 18 birds/m^2 (36 kg/ m^2) and the third one (SD_3) was 20 birds/m^2 (40 kg/m^2). Broilers of each group were kept in identical pens with room temperature maintained at 32°C during the first week of age and gradually decreased to 24°C toward the end of the third week and thereafter until the end of the study (40 d). One side of each pen was designed to be flexible and so we can adjust the space allowance for birds according to the body weight per each m^2. Each pen was supplemented with 2 tube feeders (0.60 m length × 0.07 m width × 0.06 m depth) and 2 adjustable water nipple systems (3 nipple water drinkers) hanging outside each pen. The birds were fed ad libitum (Table 1) (NRC, 1994). The experiment started at February in closed control house.

Table 1. Chemical composition of basal diets.

	Basal diet	Finisher diet
Ingredients (%)	1−21 d	22−42 d
Yellow corn	57.13	60.53
Gluten meal	6.50	6.10
Soybean meal	31.65	27.15
Limestone	1.24	1.15
Dicalcium phosphate	1.70	1.50
NaCl	0.30	0.30
Soybean oil	1.00	2.85
Vit- min premix*	0.30	0.30
L- Lysine	0.13	0.10
DL- methionine	0.05	0.02
Calculated analysis		
Metabolizable energy (MJ)	12.33	12.94
Crude protein %	23.00	21.00
Crude fibers %	3.56	3.31
Phosphorous (Available) %	0.45	0.40
Calcium %	1.00	0.90
Methionine + cysteine %	0.83	0.74
Lysine %	1.20	1.05

*Growth vitamin and mineral premix. Each 2.5 kg consists of: vitamin A, 12,000,000 IU; vitamin D3, 2,000,000 IU; vitamin E, 10 g; vitamin K3, 2 g; vitamin B1, 1,000 mg; vitamin B2, 49 g; vitamin B6, 105 g; vitamin B12, 10 mg; pantothenic acid, 10 g; niacin, 20 g, folic acid, 1,000 mg; biotin, 50 g; choline chloride, 500 mg, Fe, 30 g; Mn, 40 g; Cu, 3 g; Co, 200 mg; Si, 100 mg; and Zn, 45 g.

Growth Performance, Hematological, and Biochemical Parameters

All birds were weighed weekly for estimating the body weight and body gain. Feed intake and feed conversion ratio had been calculated. Blood samples were collected as soon as possible of initial disturbance. Two blood samples (50% of birds near to the average body weight of each replicate) were gently collected from the wing vein (50% of each replicate) under aseptic situation; one with and the other without anticoagulant. The sample with anticoagulant was used to determine lymphocyte and heterophil, while the samples without anticoagulant were centrifuged at 4,000 rpm for 15 min to collect serum. Total protein, globulin, albumin, aspartate transaminase (**AST**), alanine transaminase (**ALT**), creatinine, urea, triglyceride (**TG**), high-density lipoprotein (**HDL**) cholesterol (mg/dL), low-density

lipoprotein (**LDL**), and very low-density lipoprotein (**VLDL**) levels were determined with commercial kits (Wako Pure Chemical Industries, Osaka, Japan). The activity of superoxide dismutase (**SOD**) and glutathione peroxidase (**GSH**), levels of malondialdehyde (**MDA**) and reduced glutathione (GSH) were determined using commercial kits and a spectrophotometer (Shimadzu, Japan). Level of complement C and immunoglobulin G (**IgG**) was measured using Chicken Immunoglobulin G (IgG) ELISA Kit (Cat No. MBS260043) with sensitivity up to 5 ng/mL, intra-assay precision <= 8% and interassay precision <= 12%,

Carcass Traits

At the end of the study, all birds were weighed and slaughtered. The internal organs (gizzard, heart, liver, and intestine) were removed and weighed. The carcass (breast and thigh) was weighed and the dressing percentage was estimated. The internal organ weights were presented as a proportion to the preslaughter live weight (relative weight).

Meat Quality Measurements

Breast and thigh muscles (10 g of each) were cut from all the chilled carcass and the loose connective tissue were gently removed to determine the physical meat quality (pH, cooking loss, and drip loss) and bacteriological counts (from 50% of birds of each replicate). Ultimate pH (pHu) was evaluated after chilling for 24 h in a mixture of homogenized 1g of the muscle (breast/thigh) with 10 mL of 5 M iodoacetate for 30 s by a knick digital pH meter (Broadly Corp. Santa Ana, CA; calibrated to pH 4.01, 7.00, and 10.01 standard buffers) (Korkeala et al., 1986). Cooking loss was determined by putting approximately 20 g of the muscle in open aluminum pans and cooking in an electric oven (preheated to 200°C) to an internal temperature of 80°C for 15 min (Cyril et al., 1996). Then the muscle samples were left for 30 min for cooling to 15°C by drying their surface. Cooking loss percentage was the weight difference between the cooked and the initial samples. Thawing loss was calculated by the difference between frozen and thawing meat sample weight and blotting dry with filter paper.

Five grams of the muscle samples (of each breast and thigh) were transferred to a septic blender jar with 225 mL of 0.1% sterile peptone water. Each sample was homogenized using the blender at 2,000 rpm for 2 min and then tenth−fold serial dilutions were prepared. One mL from each dilution was transferred to 2 separate sterile Petri-dishes, and then 10 mL of the sterile standard plate count agar melted at 45°C was poured to each Petri-dish. The plates were incubated after mixing and solidifying at 37°C for 24 to 48 h. Colonies were recorded and counted as total bacterial count (cfu/g) (APHA, 2001). Three sterile MacConkey broth tubes (oxide CM5) with inverted Durham's tubes were inoculated separately with 1 mL of decimal dilution and then incubated at 37°C for 24 h and 48 h to be examined. Positive tubes showed acid and gas production. The most probable number of coliforms /gm was counted (ICMSF, 1978). One mL of the prepared dilution was transferred and spread on the surface of 2 sterile Petri-dishes that included Violet Red Bile Glucose Agar (**VRBG**). The plates were kept at room temperature for 15 min to dry and then incubated at 37°C for 25 h (ISO, 1974).

Statistical Analysis

Data were statistically analyzed with SAS statistical system Package V9.1 (SAS, 2009). Kolmogorov−Smirnov test was applied to guarantee the homogeneity and normality of variances among the different study groups. One-way ANOVA with the stocking density (SD1, SD2, and SD3) as the fixed effect and the random effect of replicates was carried out. The difference among means was applied with Tukey's test. Log geometric mean was estimated for bacteriological counts. The significant was established at $P < 0.05$.

RESULTS

Body weight of 1-day-old chick of Arbor Acres and Ross-308 breeds did not reveal significant difference among the different stocking density groups, while later on the different weeks of age the difference was significant. SD_3 group possessed the lowest body weight when compared with birds of SD_1 and SD_2. It was 41 and 38% lighter at 6 wk of age than SD_1 and SD_2 of Arbor Acres breed, respectively but it was approximately 13% lighter than SD_1 and SD_2 of Ross-308 breed. The average daily gain was the lowest at SD_3 especially at 6 wk of age when compared with SD_1 and SD_2 of both breeds. There was a significant difference among different stocking densities regarding feed intake of both breeds. SD_1 of Arbor Acres breed consumed 99 and 750 g higher feed than SD_2 and SD_3, respectively. While of Ross-308 breed, it was 15 and 350 g higher feed than SD_2 and SD_3, respectively. Feed conversion ratio was better in SD_1 and SD_2 of Arbor Acres breed when compared with SD_3. While in Ross-308 there was no significant difference regarding the feed conversion (Table 2)

There was a significant effect of stocking density on biochemical parameters. Creatinine, urea, ALT, and AST were increased by increasing the stocking density. They reached the highest levels at SD_3 than that of SD_1 and SD_2 in both breeds. ALT in Arbor Acres breed was 15 and 14% higher in SD_3 when compared with SD_1 and SD_2, respectively. While, was 21 and 20% of Ross-308 breed, respectively. Total protein and albumin were higher in SD_1 and SD_2 than SD_3 (Table 3). SD_3 revealed the highest values of cholesterol, TG, MDA, and LDL of both breeds when compared with SD_1 and SD_2, with the lowest levels of HDL, GPX, and IGG (Table 4).

Table 2. Growth performance of broilers reared with different stocking densities (n = 312/breed).

	Arbor acres breed					Ross-308 breed				
Parameters	SD_1	SD_2	SD_3	SEM[1]	*P*-value	SD_1	SD_2	SD_3	SEM	*P*-value
Body weight (g) at different weeks of age										
Day 1	48.43	48.50	48.25	0.69	0.98	41.36	42.22	41.45	0.62	0.83
1st wk	160.71[a]	159.72[a]	132.75[b]	4.00	0.002	152.93	151.67	144.50	2.95	0.45
2nd wk	465.36[a]	442.78[a]	386.25[b]	9.09	0.001	440.71[a]	437.22[a]	370.70[b]	8.52	0.000
3rd wk	737.86[a]	690.00[a]	626.75[b]	12.01	0.000	754.14[a]	750.56[a]	607.50[b]	15.59	0.000
4th wk	1,086.43[a]	1,013.33[b]	835.75[c]	20.55	0.000	1,088.57[a]	1,022.50[a]	867.20[b]	19.94	0.000
5th wk	1,445.64[a]	1,398.33[a]	1,022.25[b]	31.02	0.000	1,488.93[a]	1,426.11[a]	1,282.95[b]	17.39	0.000
6th wk	1,951.07[a]	1,903.61[a]	1,381.50[b]	40.24	0.000	2,109.07[a]	2,099.11[a]	1,859.05[b]	25.35	0.000
Average daily gain (g/d) at different weeks of age										
0–1 wk	16.04[a]	15.89[a]	12.07[b]	0.50	0.000	15.94	15.63	14.72	0.44	0.48
1–2 wk	43.52[a]	40.44[a]	36.21[b]	0.79	0.000	41.11[a]	40.79[a]	31.96[b]	1.19	0.001
2–3 wk	38.93[a]	35.32[b]	34.36[b]	0.56	0.002	44.78[a]	44.76[a]	34.08[b]	2.03	0.04
3–4 wk	49.80[a]	46.19[a]	29.86[b]	1.62	0.000	47.78	38.85	37.67	2.73	0.30
4–5 wk	51.32[a]	55.00[a]	26.64[b]	2.20	0.000	57.19	57.66	58.93	3.04	0.97
5–6 wk	72.20[a]	72.18[a]	51.32[b]	2.29	0.000	88.59	96.14	82.30	3.69	0.28
0–6 wk	45.30[a]	44.17[a]	31.74[b]	0.96	0.000	49.23[a]	48.97[a]	43.28[b]	0.61	0.000
Relative growth rate (%) at different weeks of age										
0–1 wk	106.69[a]	105.13[a]	92.57[b]	1.52	0.000	114.25	111.78	109.82	1.77	0.62
1–2 wk	97.65	94.67	97.82	0.90	0.27	96.44[a]	96.87[a]	87.04[b]	1.98	0.05
2–3 wk	45.47[a,b]	43.71[b]	48.09[a]	0.66	0.01	52.26	52.92	47.67	2.53	0.64
3–4 wk	37.94[a]	37.98[a]	28.86[b]	1.01	0.000	35.73	30.58	36.23	2.54	0.60
4–5 wk	28.35[a]	32.21[a]	20.30[b]	1.09	0.000	31.14	33.07	39.26	1.94	0.19
5–6 wk	29.82	30.70	30.14	0.97	0.94	34.61	37.99	36.38	1.40	0.65
Feed intake (g)										
1–42 d	3,050.00[a]	2,950.92[b]	2,300.40[c]	91.51	0.000	3,200.36 [a]	3,185.38[b]	2,850.2[c]	43.34	0.000
Feed conversion ratio (%)										
1–42 d	1.56[b]	1.55[b]	1.66[a]	0.01	0.000	1.52	1.52	1.53	0.002	0.06

SD_1: stocking density of 14 birds/m^2; SD_2: stocking density of 18 birds/m^2; SD_3: stocking density of 20 birds/m^2.
Relative growth rate (%) was calculated based on Broody (1945).
[1]SEM, standard error mean.
[a,b,c]Means within the same row with different superscripts letter was differ significantly.

Stocking density had a significant effect on carcass characteristics of Arbor Acres and Ross-308 breeds. Birds of SD_3 was the nastiest carcass weight 873 and 1411.60 g; dressing percentage 63.07 and 75.83%; breast weight 513.10 and 885.50 g; thigh weight 359.90 and 526.08 g when compared with SD_1 and SD_2 of Arbor Acres and Ross-308 breeds, respectively. While the internal organs were the heaviest in SD_3 of both breeds. On the other hand, both breeds kept at SD_1 and SD_2 revealed similar results (Table 3). The dressing % of SD_1 and SD_2 birds was approximately 19% better than that of SD_3 of Arbor Acres breed, while it was 4% of Ross-308 breed (Table 5). The cooking loss and drip loss of breast and thigh muscles were higher in SD_3 when compared

Table 3. Hematology parameters of broilers reared with different stocking densities (n = 156/breed).

	Arbor acres breed					Ross-308 breed				
Parameters	SD_1	SD_2	SD_3	SEM[1]	*P*-value	SD_1	SD_2	SD_3	SEM	*P*-value
Lymphocyte %	62.20[a]	60.97[b]	60.83[b]	0.09	0.000	66.76[a]	64.37[b]	61.06[c]	0.33	0.000
Heterophil %	23.97[c]	25.77[b]	26.93[a]	0.18	0.000	21.59[c]	22.17[b]	26.50[a]	0.32	0.000
H/L[2]	0.39[c]	0.42[b]	0.44[a]	0.004	0.000	0.32[c]	0.34[b]	0.43[a]	0.01	0.000
Creatinin (mg/dL)	0.86[c]	1.06[b]	1.52[a]	0.05	0.000	1.09[b]	1.19[b]	2.54[a]	0.13	0.000
Urea (mg/dL)	17.03[b]	18.10[b]	21.27[a]	0.40	0.000	14.49[b]	16.60[b]	24.70[a]	1.99	0.000
TP (g/dL)[3]	7.43[a]	7.48[a]	6.67[b]	0.10	0.000	8.01[a]	7.73[b]	6.75[c]	0.08	0.000
Albumin (g/dL)	4.21[a]	4.04[a,b]	3.92[b]	0.05	0.06	4.41[a]	4.32[a,b]	4.16[b]	0.04	0.03
Globulin (g/dL)	3.23[b]	3.44[a]	2.75[c]	0.05	0.000	3.60[a]	3.41[b]	2.59[c]	0.07	0.000
A/G[4]	1.30[b]	1.17[c]	1.43[a]	0.02	0.000	1.22[b]	1.27[b]	1.64[a]	0.04	0.000
ALT(U/L)[5]	25.23[b]	25.43[b]	29.53[a]	0.32	0.000	29.71[b]	30.03[b]	37.65[a]	0.66	0.000
AST(U/L)[6]	34.70[b]	35.07[b]	38.37[a]	0.29	0.000	45.13[b]	45.33[b]	51.40[a]	0.86	0.001

SD_1: stocking density of 14 birds/m^2; SD_2: stocking density of 18 birds/m^2; SD_3: stocking density of 20 birds/m^2.
[1]SEM, standard error mean.
[2]H/L, Heterophil/Lymphocyte ratio.
[3]TP, total protein.
[4]A/G, albumin/globulin ratio.
[5]ALT, alanine transaminase.
[6]AST, aspartate transaminase.
[a,b,c]Means within the same row with different superscripts letter was differ significantly.

Table 4. Biochemical, oxidative stress and immunological parameters of broilers reared with different stocking densities (n = 156/breed).

	Arbor acres breed					Ross-308 breed				
Parameters	SD_1	SD_2	SD_3	SEM[1]	*P*-value	SD_1	SD_2	SD_3	SEM	*P*-value
Cholesterol (mg/dL)	155.67[b]	173.67[b]	218.00[a]	7.90	0.003	172.36[b]	181.00[b]	248.85[a]	6.19	0.000
Triglyceride (mg/dL)	166.67[b]	183.00[b]	261.00[a]	8.27	0.000	216.71[b]	225.00[b]	320.20[a]	7.43	0.000
HDL (mg/dL)[2]	61.00[a]	57.00[a]	42.33[b]	1.90	0.000	65.07[a]	63.67[a]	40.50[b]	1.83	0.000
LDL (mg/dL)[3]	69.53[c]	80.07[b,b]	122.20[a]	3.73	0.000	75.73[b]	71.27[b]	151.38[a]	6.91	0.000
VLDL (mg/dL)[4]	40.00[b]	42.60[b]	56.47[a]	1.89	0.000	41.19[b]	42.33[b]	67.54[a]	1.75	0.000
SOD (U/mL)[5]	5.710[a]	5.48[a]	3.38[b]	0.22	0.000	5.76[a]	5.09[a,b]	4.32[b]	0.19	0.007
GSH (mmol/mL)[6]	1.313[a]	1.25[a]	0.86[b]	0.07	0.008	0.82[a]	0.78[a]	0.47[b]	0.03	0.000
GPX(U/mL)[7]	186.67[a]	172.00[a]	126.33[b]	5.66	0.000	192.07[a]	176.67[a]	145.55[b]	5.01	0.000
MDA(nmol/L)[8]	8.67[c]	10.30[b]	15.03[a]	0.43	0.000	5.81[b]	5.70[b]	9.08[a]	0.44	0.000
Total antioxidant capacity (mM/L)	1.70[a]	1.71[a]	0.56[b]	0.11	0.000	1.73[a]	1.71[a]	1.02[b]	0.06	0.000
C_3 (ug/mL)[9]	17.61[a]	18.37[a]	4.22[b]	1.05	0.000	16.48[a]	15.68[a]	9.40[b]	0.71	0.000
IGG (ng/mL)[10]	16.35[a]	12.47[b]	6.92[c]	0.84	0.000	20.97[a]	20.72[a]	9.88[b]	1.07	0.000

SD_1: stocking density of 14 birds/m^2; SD_2: stocking density of 18 birds/m^2; SD_3: stocking density of 20 birds/m^2.
[1]SEM, standard error mean.
[2]HDL, high-density lipoprotein.
[3]LDL, low-density lipoprotein.
[4]VLDL, very low-density lipoprotein.
[5]SOD, superoxide dismutase.
[6]GSH, reduced glutathione.
[7]GPX, glutathione peroxidase.
[8]MDA, malondialdehyde.
[9]C_3, complement 3.
[10]IgG, immunoglobulin G.
[a,b,c]Means within the same row with different superscripts letter was differ significantly.

with SD_1 and SD_2 of both breeds. Moreover, SD_3 possessed the higher bacterial count (Total bacteria, Coliforms, and Enterobacteriaceae) than of SD_1 and SD_2, while the bacterial counts of SD_1 and SD_2 were similar (Tables 6 and 7).

DISCUSSION

The current study investigated the potential impact of different stocking densities on growth performance, carcass traits, indicators of biochemical and oxidative stress and meat quality of Arbor Acres and Ross-308 broiler breeds to recommend the better stocking density with low production coast simultaneously with high quality. The reduction of growth performance in high stocking density may be attributed to several factors; a) increased temperature with decreased air circulation at bird level (Feddes et al., 2002), b) increased stress which accelerates corticosterone release that cause a restriction of glucose utilization, therefore growth in the form of protein accretion is reduced (Carsia, 2015), c) decrease body metabolic heat dissipation and releases ammonia (Dawkins et al., 2004; Yadgari et al., 2006), d) restrict the space for growth and feed access that may cause nutritional deficits and high energy expenditure which augment the stress, e) the body primarily uses energy for adapting the immune response, releasing antibodies and increasing heterophil production with decline in lymphocyte, causing a larger energy expenditure and poorer feed efficiency (McFarlane and Curtis, 1989; Carsia, 2015), f) stress enhances the metabolic rate causing an unfavorable effect on growth performances (Settar et al., 1999; Deeb et al., 2002). Chickens are more susceptible to stress especially at high temperatures, because they lack sweat gland and enhances the metabolic rate causing an adverse effect on growth performances (Ensminger et al., 1990; Settar et al., 1999; Deeb et al., 2002).

There have been conflicting outcomes on the effect of stocking density on the growth performance of broilers.

Table 5. Carcass traits and relative organs of broilers reared with different stocking densities (n = 312/breed).

	Arbor acres breed					Ross-308 breed				
Parameters	SD_1	SD_2	SD_3	SEM[1]	*P*-value	SD_1	SD_2	SD_3	SEM	*P*-value
Carcass weight	1,525[a]	1,470[a]	873[b]	45.05	0.000	1,663.00[a]	1,658.83[a]	1,411.60[b]	25.41	0.000
Dressing %	78.02[a]	77.20[a]	63.07[b]	1.02	0.000	78.77[a]	78.95[a]	75.83[b]	0.33	0.000
Breast %	62.14[a]	61.11[a]	58.75[b]	0.23	0.000	62.78[a]	62.76[a]	61.76[b]	0.19	0.03
Thigh %	37.86[b]	38.89[b]	41.25[a]	0.23	0.000	38.23[a]	38.24[a]	37.24[b]	0.19	0.03
Liver %	2.69[b]	2.76[b]	5.51[a]	0.20	0.000	2.29[b]	2.37[b]	4.05[a]	0.13	0.000
Heart %	0.50[b]	0.53[b]	0.89[a]	0. 03	0.000	0.32[b]	0.33[b]	0.39[a]	0.008	0.001
Spleen %	0.13[b]	0.15[b]	0.36[a]	0.01	0.000	0.08	0.08	0.10	0.005	0.25
Gizzard %	1.79[b]	1.82[b]	3.17[a]	0.10	0.000	1.71[b]	1.75[b]	2.25[a]	0.05	0.000

SD_1: stocking density of 14 birds/m^2; SD_2: stocking density of 18 birds/m^2; SD_3: stocking density of 20 birds/m^2.
[1]SEM, standard error mean.
[a,b,c]Means within the same row with different superscripts letter was differ significantly.

Table 6. Meat quality of breast muscle of broilers reared with different stocking densities (n = 312/breed).

	Arbor acres breed					Ross-308 breed				
Parameters	SD_1	SD_2	SD_3	SEM	*P*-value	SD_1	SD_2	SD_3	SEM	*P*-value
pH_u	6.24[a]	6.20[a]	6.11[b]	0.01	0.000	6.26[a]	6.27[a]	6.08[b]	0.02	0.000
Cooking loss %	18.88[b]	19.02[b]	21.25[a]	0.16	0.000	19.34[b]	19.89[b]	21.68[a]	0.17	0.000
Drip loss %	11.05[b]	10.92[b]	11.49[a]	0.07	0.009	10.66[b]	11.00[b]	12.24[a]	0.11	0.000
Total bacterial count (Log CFU/g) *	4.65[b]	4.56[b]	5.31[a]	0.04	0.000	4.45[b]	4.49[b]	5.18[a]	0.05	0.000
Enterobacteriaceae (Log CFU/g) *	3.00[b]	3.09[b]	3.82[a]	0.05	0.000	3.11[b]	3.20[b]	3.74[a]	0.05	0.000
Coliforms bacteria (Log CFU/g) *	2.92[b]	2.99[b]	3.18[a]	0.03	0.001	2.85[b]	2.88[b]	3.43[a]	0.04	0.000

SD_1: stocking density of 14 birds/m^2; SD_2: stocking density of 18 birds/m^2; SD_3: stocking density of 20 birds/m^2.
*Results were of Log transformed data (n = 156/breed).
[a,b,c]Means within the same row with different superscripts letter was differ significantly.

Several authors have detected a significant reduction of growth performance with increasing stocking density (Feddes et al., 2002; Guardia et al., 2011; Simitzis et al., 2012; Petek et al., 2014; Chegini et al., 2018). While others have stated that stocking density did not affect growth performance (Buijs et al., 2009; Mahrose et al., 2019a; Obeidat et al., 2019). Our results were comparable with the majority of studies that mentioned increasing stocking density had a detrimental impact on growth performance. Estevez et al. (1997) and Sørensen et al. (2000) reported a decrease in body weight when space per bird was lower than 0.066 m^2, moreover, the body weight gain was declined with increasing the stocking density from 25 to 40 kg/m^2 (Dozier et al., 2006) that supported the current results.

There was no significant difference regarding the growth performance between low and medium stocking density. The finest performance was realized with the medium (18 birds/m^2) and low (14 birds/m^2) densities, while the lowest performance was in high densities (22 birds/m^2) (Kryeziu et al., 2018b) which confirmed our results. Moreover, stocking density further than 35 kgBW/m^2 reduced the final BW (Dozier et al., 2006). Abo Alqassem et al. (2018) stated that medium stocking density (15 bird/m^2) was heavier 140 g of body weight when compared with high stocking density (20 bird/m^2), but it was comparable with the control group (12 bird/m^2). High stocking density of *Arbor Acres* (45 kg/m^2) resulted in a decreased growth performance and feed utilization on d 42 when compared with low stocking density (37.5 kg/m^2) (Li et al., 2019). On the other hand, Ligaraba et al. (2016) and Palizdar et al. (2017) detected that stocking densities of 30 and 40 kg body weight/m^2 did not influence the growth performance of broilers (Ross 308 and Avian 48) and also of 10 to 20 birds/m^2 (Pettit-Riley and Estevez, 2001).

The current results regarding FCR were similar to the range reported by Khalid et al. (2021). There was a conflict results regarding FCR. Some authors reported that high stocking density increased the FCR (Rambau et al., 2016; Astanch et al. 2018), that supported our findings. While, others did not detect any differences, in spite of the detrimental effect of high stocking density on growth performance (Abudabos et al., 2013; Bailie et al., 2018; Heidari and Toghyani, 2018; Li et al., 2019). FCR of Ross-308 breed did not reveal any significant difference which confirmed by others (Kim et al., 2021).

High stocking density causes stress of birds, therefore reducing growth performance which attributed to redirects blood flows from the gastrointestinal tract to the peripheral tissues, consequently smashes up the mucosal tight junction barrier of gastrointestinal tract and boosts intestinal permeability. This will encourage the transmission of luminal endotoxins (lipopolysaccharides) into the body that enhance the inflammatory reactions with a reduction of the nutrient and oxygen supply that harms gut and intestine health, performance (Lambert, 2009) and compromised nutrient absorption of broilers (Shakeri et al., 2014). Moreover, the reduction of body weight may be due to interfering with breast muscle hypertrophy and differentiation through regulating the expression of IGF-I, MyoD, and MSTN (Li et al., 2019).

The carcass performance is a crucial economic factor of the broiler business (Nasr et al., 2017, 2019). The current study clarified that low and medium stocking density had similar results regarding the carcass traits.

Table 7. Meat quality of thigh muscle of broilers reared with different stocking densities (n = 312/breed).

	Arbor acres breed					Ross-308 breed				
Parameters	SD_1	SD_2	SD_3	SEM	*P*-value	SD_1	SD_2	SD_3	SEM	*P*-value
pH_u	6.31[a]	6.26[a]	6.16[b]	0.02	0.000	6.31[a]	6.23[a]	6.09[b]	0.01	0.000
Cooking loss	19.37[b]	19.06[b]	21.47[a]	0.13	0.000	19.53[b]	19.85[b]	21.94[a]	0.17	0.000
Drip loss	10.51[b]	10.37[b]	11.45[a]	0.08	0.000	10.80[b]	11.05[b]	12.17[a]	0.11	0.000
Total bacterial count (Log CFU/g)*	4.67[b]	4.72[b]	5.30[a]	0.04	0.000	4.59[b]	4.59[b]	5.30[a]	0.05	0.000
Enterobacteriaceae (Log CFU/g)*	3.10[b]	3.10[b]	3.36[a]	0.03	0.000	3.09[b]	3.10[b]	3.65[a]	0.04	0.000
Coliforms bacteria (Log CFU/g)*	2.63[b]	2.45[b]	3.31[a]	0.04	0.000	2.82[b]	2.83[b]	3.20[a]	0.03	0.000

SD_1: stocking density of 14 birds/m^2; SD_2: stocking density of 18 birds/m^2; SD_3: stocking density of 20 birds/m^2.
*Results were of Log transformed data (n = 156/breed).
[a,b,c]Means within the same row with different superscripts letter was differ significantly.

They possessed the best carcass characteristics when compared with high stocking density. Our results were confirmed with others who mentioned that increasing stocking density affects the carcass traits and reduced carcass quality (Skomorucha et al., 2009; Sekeroglu et al., 2011) a lower breast fillet (Castellini et al., 2002; Dozier et al., 2006; Abo Ghanima et al., 2020), whole breast yield (Feddes et al., 2002; Škrbić et al., 2011; Abo Ghanima et al., 2020), and thigh (Abo Ghanima et al., 2020). Moreover, carcass traits of unsexed Arbor Acres broilers revealed that moderate stocking density (15 bird/ m^2) had the highest dressing weight and higher dressing percentage (75−77%) when compared with other groups (12 bird/m^2 and 20 bird/ m^2) but the difference was not significant (Abo Alqassem et al., 2018).

The carcass weights and breast % of Ross 308 reared on medium (18 birds/m^2) and low (14 birds/ m^2) stocking density were significantly higher when compared with the high stocking density (22 birds/ m^2) (Kryeziu et al., 2018a). High stocking density of male Arbor Acres (18 birds/m^2, 45.0 kg/m^2) resulted in a decreased carcass weight, thigh % when compared with low stocking density (15 birds/m^2, 37.5 kg/m^2) (Li et al., 2019). The reported dressing percentage in this study was comparable with others who reported an average of 75 to 77% (Abo Alqassem et al., 2018; Kryeziu et al., 2018a). There was an increase in the internal organs weigh in high stocking density of both breeds. These results confirmed by Chegini et al., 2018 who stated that spleen weight was increased in Ross 308 male reared on high stocking density when compared with low one and also on quails (Mahrose et al., 2019b). Stress may cause an increase of lymphoid organ to encourage the immune status of birds (Pope, 1991; Gore and Qureshi, 1997). Moreover, the increased liver weight may be due to high liver lipids of stressed broiler that caused high fat % in liver (Puvadolpirod and Thaxton, 2000).

Blood biochemical indices are considered crucial tool for diagnosis especially for metabolic diseases (Rotava et al., 2008). Broilers faced stress, revealed alterations of blood system that may be due to the thermoregulatory responses (Arieli et al., 1979). High stocking density can be stressful and has detrimental effects on broiler performance and physiological indices (Cengiz et al., 2015). Corticosterone is of low valuable indicator for long-term (chronic) stress and birds welfare (Cunningham et al., 1988), while heterophil-to-lymphocyte (H/L) ratio is considered a approachable indicator of chronic stress in laying hens (Gross and Siegel, 1983; Zulkifli et al., 2003) and as a welfare index of hens (Nicol et al., 2009). Corticoids restrain immune system function, decrease serum protein levels with an increase of blood glucose levels, that have a harmful effect on birds' performances (Bollengier-Lee et al., 1998). Our results supported this as the total protein and albumin was decreased by increasing the stocking density. Lymphocyte number was decreased, while the heterophil was increased as a result of increasing the stocking density which was comparable with others (Nathan et al., 1976; Mashaly et al., 2004; Ajakaiye et al., 2010). This may be attributed to a) the release of glucocorticoide which dissolute the lymphocytes in lymphoid tissues (Gross and Siegel, 1983), b) increase the level of corticosterone (Siegel, 1985), c) the release of adrenocorticotrophic hormone that stimulate the bone marrow to synthesis heterophil (Al-Murrani et al., 1997).

There have been conflicting outcomes on the effect of stocking density on H/L ratio of broilers. A number of authors have detected an increase of H/L ratio with increasing stocking density (Feddes et al., 2002; Zulkifli et al., 2003; Thaxton et al., 2006; Cengiz et al., 2015). While others have stated that stocking density did not affect H/L ratio (Heckert et al., 2002; Spinu et al., 2003; Turkyilmaz, 2008). Our findings were comparable with the majority of studies that mentioned increasing stocking density caused an increase of H/L ratio. But, on the other hand Chegini et al., 2018 stated that overcrowding stress decreased H/L ratio of Ross 308 male broilers. The H/L ratio of the current study was in accordance with the range (0.43−0.45) reported by Li et al. (2019) with the similar stocking density.

There was a significant increase of albumen/globulin ratio of broiler exposed to stress (Tollba and Hassan, 2003) that confirmed our findings. Moreover, Abudabos et al. (2013) detected a reduction of the total protein with increasing stocking density, which is comparable to the current results. Total protein and its fractions values provided the information that interprets the incidence of dehydration, infections, immune diseases, and inflammatory responses (Silva et al., 2007). Moreover, serum protein was positively correlated with the weight of body and protein synthesis consequently (Al-Attar and Rashd, 1985; Colse, 1986). High stocking density reduced the albumin level due to stress (Erisir and Erisir, 2002), total protein and globulin with an increase of the uric acid. Uric acid is an indicator for protein catabolism, and its increase exhibits high protein or amino-acid catabolism (Carsia 2015). Liver is a crucial organ that has a role in metabolic body processes. Serum transaminases activities (AST and ALT) were assessed the damage and recovery of the hepatic cells and birds' pathological condition (Jaensch, 2000; Atsafack et al., 2015), which are sensitive to toxic stuffs (Gudiso et al., 2019). High stocking density elevated serum ALT and AST compared to the low density of broilers that supported by others (Abudabos et al., 2013). This elevation may be attributed to hepatic or muscle injury, septicemia, and/or toxemia and their levels are correlated with the amount and severity of cell damage (Nobakht and Hosseini Fard, 2016). At high densities, birds struggle to eat more, consequently more risk of muscular injury that increased the levels of AST and ALT in the blood. But on contrary, Jobe et al. (2019) stated that stocking density did not affect on AST and ALT.

High stocking density increased cholesterol and HDL levels, this mainly due to HDL play an important role of

transporting the excess of cholesterol in body tissues to liver (Tall, 1998; Sahin and Kucukm, 2001). High stocking density escalated physiological and oxidative stress and encouraged the damage of intestinal mucosal. Consequently, they are more vulnerable to infectious diseases (Li et al., 2019). MDA and glutathione are the main indicator of lipid peroxidation used for evaluating the oxidative damage (Sevanian and Mcleod, 1997; Aengwanich and Suttajit, 2010). Glutathione protects cells and tissues from oxidative destruction. The reduced glutathione binds quickly with reactive oxygen species (**ROS**) and is converted to the oxidized form (GSSG). Therefore, the reduced glutathione is decreased with the presence of high amount of ROS (Bar-Peled et al., 1996). There was a balance between antioxidant defenses and pro-oxidant production in living animals at normal physiological condition. Inequity of this condition will initiate the elevation of ROS and generate oxidative stress that leads to oxidation and damage of lipids and proteins in the cell and its compartments (Zhang et al., 2012). Consequently, living organisms are capable of survive with the oxidative stress through antioxidant enzymes production and restore the physiological system. Antioxidant enzymes (SOD, GSH, and GPx) perform an important role of antioxidant defenses (Seven et al., 2009).

In the current investigation high stocking density induced oxidative stress status of broilers, as its augmented MDA and decreased the activity of SOD and GPx in the serum. Our results were confirmed by the majority of researchers who investigating the deleterious effect of high stocking density of broilers (Simitzis et al., 2012; Zhang et al., 2015; Abo Ghanima et al., 2020). This may be attributed to crowding increasing fights among birds and causing metabolic disturbances, therefore instigating stress, higher lipid peroxidation and high production of ROS, increased oxidative destruction and generating MDA due to the and reduced the antioxidant enzymes activity (Droge, 2002; Yun-Zhong et al., 2002; Simsek et al., 2009).

Regarding the immunity, there was a reduction of complement 3 and IgG at high stoking density which supported by other researchers (Heckert et al., 2002; Mashaly et al., 2004; Palizdar et al., 2017) who stated that high stocking density suppresses the broilers immunity. Energy is intended for preserving the stress response in the chronic stressor conditions and less energy is offered for the innate immune system (McFarlane et al., 1989; McFarlane and Curtis, 1989; Carsia, 2015). Moreover, releasing somatostatin and adrenal corticosteroid hormones are responsible for decreasing the production of immunoglobulin (Herman et al., 2004). On the other hand, Li et al. (2019) detected an increase of IgG and IgM with increasing the stocking density. Moreover, overcrowding is amplified IgA and IgM levels of Ross 308 male broilers that may be attributed to enhance the protection of immune cells as a result of suppressing ROS and lipid peroxidation (Chegini et al., 2018).

Cooking loss and drip loss percentage of this study was in accordance with the results of Moreira et al. (2004) who reported that broilers reared at density between 10 and 16 birds/m^2 did not influence broilers meat quality of Ross 308, Cobb 500 and Hybro PG commercial strains. Ultimate muscle pH (pHu) was decreased with increased in cooking loss and drip loss at SD_3 when compared with SD_1 and SD_2. This may be attributed to pHu was the high negatively correlated with drip loss (van Laack et al., 2000) and cooking loss (Huff-Lonergan and Lonergan, 2005; Lafuente et al., 2013) caused by hastening lactate deposit and successively rises meat toughness (Lynch and Frei, 1993). The high drop loss caused high loss of soluble nutrients and flavor constituents (Liu et al., 2011). Low pH of the meat is associated with decreased glycogen deposit in meats (Castellini et al., 2002) with distorted meat quality due to protein denaturation (Wilhelm et al., 2010) and increased cooking loss (Jeong et al., 2020). Additionally, this may be attributed to the myofibrillar proteins lose their capability to hold water due to the disturbance of the collagen and myofibrillar protein matrix through the ageing and water pushed out from myofibrils to channels formed between the muscle fiber and the cell membrane as a result of contraction at rigor mortis and then water may move out as drip (Lawson 2004).

Most of cooking loss is due to temperature prompted denaturation of meat proteins. Myosin denatured at 40 to 53°C with terrible reduction of collagen fibers onsets at 53 to 63°C (Brüggemann et al., 2010). While, actin denatured at 66 to 80°C (Martens et al., 1982; Tornberg, 2005; Dominguez-Hernandez et al., 2018). Recently, Pang et al. (2021) stated that the cooking loss mainly arises from protein denaturation and decrease in intramyofibrillar water.

Stress may alter the number of leucocytes and reduced humoral immunity, consequently decrease the birds immunity to overcome the bacterial and viral infections (Mench et al., 1986). Regarding the bacterial counts of breast and thigh muscles of both breeds revealed that high stocking density possessed the highest bacterial count and infection. These results were supported by others (Kristensen and Wathes, 2000; Bessei, 2006; Burkholder et al., 2008; Guardia et al., 2011). Poultry litter is a combination of poultry manure and bedding materials that considered an environmental ecosystem with the presence of some microbial populations (Lovanh et al., 2007). High stocking density boosts ammonia and moisture level in the litter as a result of higher deposits of fecal matter, spilled water out, and insufficient ventilation, consequently reducing the litter quality and enhances the bacterial growth. Physicochemical characteristics and microbiota of poultry litter affect the intestinal ecosystem as they influence the colonization of enteric pathogens in broiler (*C. perfringens* and *Eimeria* spp.) that ease horizontal transmission and multiply the shedding of pathogen (Kristensen and Wathes, 2000; Bessei, 2006; Burkholder et al., 2008; Guardia et al., 2011).

CONCLUSIONS

The need for broilers meat is increased because the consumers consideration of a high-quality food with low fat and high protein. Therefore, consumers are aware of animal welfare and quality. Stocking density is considered one of the main factors influencing birds welfare, physical activity, and product quality. The current study concluded that high stocking density has a detrimental effect on broilers performance and welfare of both breeds. It possessed the lowest body weight, carcass weight, dressing percentage, breast weight, thigh weight, HDL, GPX, and IGG with the highest levels of ALT, cholesterol, TG, MDA, LDL, cooking loss, drip loss of breast and thigh muscles, and bacterial count of both breeds. Therefore, the current study recommended that the best stocking density of Arbor Acres and Ross-308 breed is SD_2 (18 birds/m^2).

ACKNOWLEDGMENTS

The authors extend their appreciation to the Taif University Researchers supporting Project number (TURSP-2020/104) Taif University, Taif, Saudi Arabia, for funding this work.

DISCLOSURES

None of the authors have any conflict of interest to declare.

REFERENCES

Abo Ghanima, M. M., M. E. Abd El-Hack, A. E. Taha, V. Tufarelli, V. Laudadio, and M. A. E. Naiel. 2020. Assessment of stocking rate and housing system on performance, carcass traits, blood indices, and meat quality of French Pekin ducks. Agriculture 10:273.

Abo Alqassem, Z., S. Ahmed, E. Ahmed, and K. El Iraqi. 2018. Effect of different stocking densities as an environmental stressing factor on broiler behavior and performance. Benha Vet. Med. J. 34:51–65.

Abudabos, A. M., E. M. Samara, E. O. S. Hussein, M.a. Q. Al-Ghadi, and R. M. Al-Atiyat. 2013. Impacts of stocking density on the performance and welfare of broiler chickens. Ital. J. Anim. Sci 12:e11.

Aengwanich, W., and M. Suttajit. 2010. Effect of polyphenols extracted from tamarind (Tamarindus indicaL.) seed coat on physiological changes, heterophil/lymphocyte ratio, oxidative stress and body weight of broilers (Gallus domesticus) under chronic heat stress. Anim. Sci. J. 81:264–270.

Ajakaiye, J. J., A. Perez-Bello, and A. Mollineda-Trujillo. 2010. Impact of vitamins C and E dietary supplementation on leukocyte profile of layer hens exposed to high ambient temperature and humidity. Acta Vet. Brno 79:377–383.

Al-Attar, A. H., and M. A. Rashd. 1985. Electrophoretic serum protein fraction in dwarf and normal layer hens and their correlation with productive traits. Anim. Breed. Abst. 53:6710.

Al-Murrani, W. K., A. Kassab, H. Z. Al-Sam, and A. M. K. Al-Athari. 1997. Heterophil/lymphocyte ratio as a selection criterion for heat resistance in domestic fowls. Br. Poult. Sci. 38:159–163.

American Public Health Association (APHA) 2001. Pages 365-366 in Compendium of Methods for Microbiological Examination of Foods, 4th ed. Washington, DC

Arieli, A., A. Meltzer, and A. Berman. 1979. Seasonal acclimatization in the hen. Brit. Poult. Sci. 20:505–513.

Astaneh, I. Y., M. Chamani, S. N. Mousavi, A. A. Sadeghi, and M. A Afshar. 2018. Effects of stocking density on performance and immunity in Ross 308 broiler chickens. Kafkas Univ. Vet. Fak. Derg. 24:483–489.

Atsafack, S. S., J.-R. Kuiate, R. S. Mouokeu, M. L. Koanga Mogtomo, A. T. Tchinda, T. J. De Dieu, H. Magnifouet Nana, R. M. Ebelle Etame, L. Biyiti, and R. A. Ngono Ngane. 2015. Toxicological studies of stem bark extract from Schefflera barteri Harms (Araliaceae). BMC Complement. Altern. Med. 15:1–9.

Bailie, C. L., C. Ijichi, and N. E O'Connell. 2018. Effects of stocking density and string provision on welfare-related measures in commercial broiler chickens in windowed houses. Poult. Sci. 97:1503–1510.

Bar-Peled, O., E. Korkotian, M. Segal, and Y. Groner. 1996. Constitutive overexpression of Cu/Zn superoxide dismutase exacerbates kainic acid-induced apoptosis of transgenic-Cu/Zn superoxide dismutase neurons. Proc. Natl. Acad. Sci. 93:8530–8535.

Berg, C., and J. Yngvesson. 2012. Optimal stocking density for broilers Optimal for whom? In XXIVWorld Poultry Congress, Area: Poultry Welfare and Environment 5−9 August, Salvador, Bahia, Brazil.

Bessei, W. 2006. Welfare of broilers: a review. Worlds Poult. Sci. J. 62:455–466.

Bollengier-Lee, S., M. A. Mitchell, D. B. Utomo, P. E. V. Williams, and C. C. Whitehead. 1998. Influence of high dietary vitamin E supplementation on egg production and plasma characteristics in hens subjected to heat stress. Br. Poult. Sci. 39:106–112.

Broody, S. 1945. Bioenergetics and Growth. Reinhold Pub. Crop., NY, NY.

Brüggemann, D. A., J. Brewer, J. Risbo, and L. Bagatolli. 2010. Second harmonic generation microscopy: a tool for spatially and temporally resolved studies of heat induced structural changes in meat. Food Biophys. 5:1–8.

Buijs, S., L. Keeling, S. Rettenbacher, E. Van Poucke, and F. A. M. Tuyttens. 2009. Stocking density effects on broiler welfare: identifying sensitive ranges for different indicators. Poult. Sci. 88:1536–1543.

Burkholder, K. M., K. L. Thompson, M. E. Einstein, T. J. Applegate, and J. A. Patterson. 2008. Influence of stressors on normal intestinal microbiota, intestinal morphology, and susceptibility to salmonella enteritidis colonization in broilers. Poult. Sci. 87:1734–1741.

Carsia, R. V. 2015. Chapter 26 - Adrenals. Pages 577-611 in Sturkie's Avian Physiology. (6th ed.) ed. C.G. Scanes. Acad. Press, San Diego, CA.

Castellini, C., C. Mugnai, and A. Dal Bosco. 2002. Effect of organic production system on broiler carcass and meat quality. Meat Sci 60:219–225.

Cengiz, Ö., B. H. Köksal, O. Tatlı, Ö. Sevim, U. Ahsan, A. G. Üner, P. A. Ulutaş, D. Beyaz, S. Büyükyörük, A. Yakan, and A. G. 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.

Chegini, S., A. Kiani, and H. Rokni. 2018. Alleviation of thermal and overcrowding stress in finishing broilers by dietary propolis supplementation. Ital. J. Anim. Sci. 17:377–385.

Colse, E. H. 1986. Vet. Clinical Pathology. 4th ed W.B. Saunders Company, Philadelphia, PA.

Cunningham, D. L., A. van Tienhoven, and G. Gvaryahu. 1988. Population size, cage area, and dominance rank effects on productivity and well-being of laying hens. Poult. Sci. 67:399–406.

Cyril, H. W., C. Castellini, and A. Dal Bosco. 1996. Comparison of three cooking methods of rabbit meat. Italian J. Food Sci. 8:337–342.

Davami, A., M. J. Wineland, W. T. Jones, R. L. Ilardi, and R. A. Peterson. 1987. Effects of population size, floor space, and feeder space upon productive performance, external appearance, and plasma corticosterone concentration of laying hens. Poult. Sci. 66:251–257.

Dawkins, M., C. A. Donnelly, and T. A. Jones. 2004. Chicken welfare is influenced more by housing conditions than by stocking density. Nature 427:342–344.

Deeb, N., A. Shlosberg, and A. Cahaner. 2002. Genotype-by-environment interaction with broiler genotypes differing in growth rate. 4. Association between responses to heat stress and to cold-induced ascites. Poult. Sci. 81:1454–1462.

Deep, A., K. Schwean-Lardner, T. G. Crowe, B. I. Fancher, and H. L. Classen. 2010. Effect of light intensity on broiler production, processing characteristics, and welfare. Poult. Sci. 89:2326–2333.
Dohms, J. E., and A. Metz. 1991. Stress—mechanisms of immunosuppression. Vet. Immunol. Immunopathol. 30:89–109.
Dominguez-Hernandez, E., A. Salaseviciene, and P. Ertbjerg. 2018. Low-temperature long-time cooking of meat: eating quality and underlying mechanisms. Meat Sci. 143:104–113.
Dozier, W. A., J. P. Thaxton, J. L. Purswell, H. A. Olanrewaju, S. L. Branton, and W. B. Roush. 2006. Stocking density effects on male broilers grown to 1.8 kilograms of body weight. Poult. Sci. 85:344–351.
Dröge, W. 2002. Aging-related changes in the thiol/disulfide redox state:implications for the use of thiol antioxidants. Exp. Gerontol. 37:1333–1345.
Ensminger M. E. and Oldfield J. E., *Feeds and nutrition digest: formerly, feeds and nutrition—abridged,* 1990, Ensminger Publishing Company; Clovis, CA.
Erisir, M., and Z. Erisir. 2002. Yerleflim sıklı.ı arttırılan bıldırcınların (Coturnix coturnix japonica) bazı biyokimyasal kan parametrelerindeki de.ifliklikler. Turkish J. Vet. Anim. Sci. 26:491–496.
Estevez, I., R. C. Newberry, and L. Arias de Reyna. 1997. Broiler chickens: a tolerant social system? Etologia 5:19–29.
European Commission. 2007. Council Directive of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production, 2007/43/EC. In:. Off. J. Europ. Uni. L 182:19–28.
Feddes, J. J., E. J. Emmanuel, and M. J. Zuidhoft. 2002. Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. Poult. Sci. 81:774–779.
Gomes, A. V. S., W. M. Quinteiro-Filho, A. Ribeiro, V. Ferraz-de-Paula, M. L. Pinheiro, E. Baskeville, A. T. Akamine, C. S. Astolfi-Ferreira, A. J. P. Ferreira, and J. Palermo-Neto. 2014. Overcrowding stress decreases macrophage activity and increases Salmonella Enteritidis invasion in broiler chickens. Avian Pathol. 43:82–90.
Gore, A. B., and M. A. Qureshi. 1997. Enhancement of humoral andcellular immunity by vitamin E after embryonic exposure. Poult. Sci. 76:984–991.
Gross, W. B., and H. S. Siegel. 1983. Evaluation of the Heterophil/Lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27:972.
Guardia, S., B. Konsak, S. Combes, F. Levenez, L. Cauquil, J. F. Guillot, C. Moreau-Vauzelle, M. Lessire, H. Juin, and I. Gabriel. 2011. Effects of stocking density on the growth performance and digestive microbiota of broiler chickens. Poult. Sci. 90:1878–1889.
Gudiso, X. C., V. A. Hlatini, C. N. Ncobela, M. Chimonyo, and P. L. Mafongoya. 2019. Serum metabolites and weights of internal organs of broilers fed on varying levels of Acacia angustissima leaf meal. Can. J. Anim. Sci. 99:475–481.
Gursu, M. F., M. Onderci, F. Gulcu, and K. Sahin. 2004. Effects of vitamin c and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress in vivo. Nutr. Res. 24:157–164.
Heckert, R. A., I. Estevez, E. Russek-Cohen, and R. Pettit-Riley. 2002. Effects of density and perch availability on the immune status of broilers. Poult. Sci. 81:451–457.
Heidari, S., and M. Toghyani. 2018. Effect of stocking density and methionine levels on growth performance and immunity of broiler chicks. Iranian J. Appl. Anim. Sci. 8:483–489.
Herman, J. P., N. K. Mueller, and H. Figueiredo. 2004. Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. Ann. N. Y. Acad. Sci. 1018:35–45.
Houshmand, M., K. Azhar, I. Zulkifli, M. H. Bejo, and A. Kamyab. 2012. Effects of prebiotic, protein level, and stocking density on performance, immunity, and stress indicators of broilers. Poult. Sci. 91:393–401.
Huff-Lonergan, E., and S. M Lonergan. 2005. Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. Meat Sci. 71:194–204.
ICMSF "International Commission on Microbiological Specifications for Foods". 1978. Microorganisms in Foods, Their Significance and Method of Enumeration. 2nd ed. Univ. of Toronto Press, Toronto and Buffalo's Canada.
ISO "International Standard Organization" 1974. Meat and meat products: measurement of pH (reference method): 1917-1974. ISO, Geneva, Switzerland.
Jaensch, S. 2000. Diagnosis of avian hepatic disease. Semin. Avian Exotic Pet. Med. 9:126–135.
Jeong, S., Y. B. Kim, J. W. Lee, D. H. Kim, B. H. Moon, H. H. Chang, Y. H. Choi, and K. W. Lee. 2020. Role of dietary gamma-aminobutyric acid in broiler chickens raised under high stocking density. Anim. Nutr. 6:293–304.
Jobe, M. C., C. N. Ncobela, N. W. Kunene, and A. R. Opoku. 2019. Effects of Cassia abbreviata extract and stocking density on growth performance, oxidative stress and liver function of indigenous chickens. Trop. Anim. Health Prod. 51:2567–2574.
Khalid, N., M. M. Ali, Z. Ali, Y. Amin, and M. Ayaz. 2021. Comparative productive performance of two broiler strains in open housing system. Adv. Life Sci. 8:124–127.
Kim, H. J., J. J. Jeon, H. S. Kim, J. Son, K. Y. Kim, R. S. You, E. C. Hong, B. S. Kang, and H. K Kang. 2021. Effects of stocking density on the growth performance, immune status and breast meat quality of broiler. Korean J. Poult. Sci. 48:13–22.
Korkeala, H., O. Mäki-Petäys, T. Alanko, and O. Sorvettula. 1986. Determination of pH in meat. Meat Sci. 18:121–132.
Kristensen, H. H., and C. M. Wathes. 2000. Ammonia and poultry welfare: a review. Worlds Poult. Sci. J. 56:235–245.
Kryeziu, A. J., N. Mestani, Sh. Berisha, and M. A. Kamberi. 2018a. The European performance indicators of broiler chickens as influenced by stocking density and sex. Agron. Res. 16:483–491.
Kryeziu, A. J., M. Kamberi, S. Muji, N. Mestani, and S. Berisha. 2018b. Carcass traits of broilers as affected by different stocking density and sex. Bulg. J. Agric. Sci. 24:1097–1103.
Lafuente, R., M. López, J. Blackshaw, N. Mach, A. Bach, A. Velarde, and V. Muchenje. 2013. Effects of lairage temperature and holding time on pig behaviour and carcass and meat quality. Meat Sci. 96:116–129.
Lambert, G. P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J. Anim. Sci. 87:E101–E108.
Lan, P. T., 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.
Lawson, M. A. 2004. The role of integrin degradation in post-mortem drip loss in pork. Meat Sci. 68:559–566.
Li, X. M., M. H. Zhang, S. M. Liu, J. H. Feng, D. D. Ma, Q. X. Liu, Y. Zhou, X. J. Wang, and S. Xing. 2019. Effects of stocking density on growth performance, growth regulatory factors, and endocrine hormones in broilers under appropriate environments. Poult. Sci. 98:6611–6617.
Ligaraba, T. J., K. Benyi, and J. J. Baloyi. 2016. Effects of genotype and stocking density on broiler performance under three feeding regimes. Trop. Anim. Health Prod. 48:1227–1234.
Liu, B. Y., Z. Y. Wang, H. M. Yang, J. M. Wang, D. Xu, R. Zhang, and Q. Wang. 2011. Influence of rearing system on growth performance, carcass traits, and meat quality of Yangzhou geese. Poult. Sci. 90:653–659.
Lovanh, N., K. L. Cook, M. J. Rothrock, D. M. Miles, and K. Sistani. 2007. Spatial shifts in microbial population structure within poultry litter associated with physicochemical properties. Poult. Sci. 86:1840–1849.
Lynch, S. M., and B. Frei. 1993. Mechanisms of copper- and iron-dependent oxidative modification of human low-density lipoprotein. J. Lipid Res. 34:1745–1753.
Mahrose, K. M., M. E. Abd Elhack, and S. A. Amer. 2019a. Influences of dietary crude protein and stocking density on growth performance and body measurements of ostrich chicks. Anais Acad. Bras. Ciências 91:e20180479.
Mahrose, K. M., M. Alagawany, M. E. Abd Elhack, S. A. Mahgoub, and F. A. M Attia. 2019b. Influences of stocking density and dietary probiotic supplementation on growing Japanese quail performance. Anais Acad. Bras. Ciências 91:e20180616.
Martens, H., E. Stabursvik, and M. Martens. 1982. Texture and colour changes in meat during cooking related to thermal denaturation of muscle proteins. J. Texture Stud. 13:291–309.
Mashaly, M. M., G. L. Hendricks, M. A. Kalama, A. E. Gehad, A. O. Abbas, and P. H. Patterson. 2004. Effect of heat stress on

production parameters and immune responses of commercial laying hens. Poult. Sci. 83:889–894.
McFarlane, J. M., and S. E. Curtis. 1989. Multiple concurrent stressors in chicks. Poult. Sci. 68:522–527.
McFarlane, J. M., S. E. Curtis, R. D. Shanks, and S. G. Carmer. 1989. Multiple concurrent stressors in chicks. Poult. Sci. 68:501–509.
Mench, J. A., A. van Tienhoven, J. A. Marsh, C. C. McCormick, D. L. Cunningham, and R. C. Baker. 1986. Effects of cage and floor pen management on behavior, production, and physiological stress responses of laying hens. Poult. Sci. 65:1058–1069.
Moreira, J., A. A. Mendes, R. O. Roca, E. A. Garcia, I. A. Naas, R. G. Garcia, I. C. Lima, and A. Paz. 2004. Effect of stocking density on performance, carcass yield and meat quality in broilers of different commercial strains. Rev. Bras. Zoot. 33:1506–1519.
Mustafa, M. Y., M. A. Muneer, A. A. Anjum, and M. Ahamd. 2010. Influence of stocking density on immune response of broilers against newcastle disease virus. Pak. J. Life Soc. Sci. 8:7e10.
Najafi, P., I. Zulkifli, N. A. Jajuli, A. S. Farjam, S. K. Ramiah, A. A. Amir, E. O'Reily, and D. Eckersall. 2015. Environmental temperature and stocking density effects on acute phase proteins, heat shock protein 70, circulating corticosterone and performance in broiler chickens. Int. J. Biometeorol. 59:1577–1583.
Nasr, M. A. F., E. M. R. Ali, and M. A Hussein. 2017. Performance, carcass traits, meat quality and amino acid profile of different Japanese quails strains. J. Food Sci. Technol. 54:4189–4196.
Nasr, M. A. F., H. Mohammed, R. A. Hassan, A. A. Swelum, and I. M Saadeldin. 2019. Does light intensity affect the behavior, welfare, performance, meat quality, amino acid profile, and egg quality of Japanese quails? Poult. Sci. 98:3093–3102.
Nathan, D. B., E. D. Heller, and M. Perek. 1976. The effect of short heat stress upon leucocyte count, plasma corticosterone level, plasma and leucocyte ascorbic acid content. Br. Poult. Sci. 17:481–485.
National Chicken Council. 2005. National Chicken Council Animal Welfare Guidelines and Audit Guidelines. National Chicken Council, Washington, DC.
National Research Council. 1994. Nutrient Requirements for Poultry. 9th rev. edn. Natl. Acad. Press, Washington, DC.
Nicol, C. J., G. Caplen, J. Edgar, and W. J. Browne. 2009. Associations between welfare indicators and environmental choice in laying hens. Anim. Behav. 78:413–424.
Nobakht, A., and B. Hosseini Fard. 2016. The effects of using rice bran, enzyme and probiotic on performance, egg quality traits and blood metabolites in laying hens. Iranian J. Anim. Sci. 46:417–427.
Obeidat, M. D., H. Al-Tamimi, B. Nusairat, and B. S. Obeidat. 2019. Effect of breed and density on growth performance and carcass traits of two broiler breeds. International Congress on Agriculture and Forestry research, 8-10 April 2019, Marmaris, Turkey.
Palizdar, M. H., M. K. Daylami, and M. R. Pourelmi. 2017. Effects of high stocking density on growth performance, blood metabolites and immune response of broilers (ROSS 308). J. Livest. Sci. 8:196–200.
Pang, B., X. Yu, B. Bowker, J. Zhang, Y. Yang, and H. Zhuang. 2021. Effect of meat temperature on moisture loss, water properties, and protein profiles of broiler pectoralis major with the woody breast condition. Poult. Sci. 100:1283–1290.
Petek, M., H. ÜStÜNer, and D. YeŞİLbaĞ. 2014. Effects of stocking density and litter type on litter quality and growth performance of broiler chicken. Kafkas Univ. Vet. Fakultesi Dergisi 20:743–748.
Pettit-Riley, R., and I. Estevez. 2001. Effects of density on perching behavior of broiler chickens. Appl. Anim. Behav. Sci. 71:127–140.
Pope, C. R. 1991. Pathology of lymphoid organs with emphasis onimmunosuppression. Vet. Immunol. Immunopathol. 30:31–44.
Puvadolpirod, S., and J. P Thaxton. 2000. Model of physiological stress in chickens. 1. Response parameters. Poult. Sci. 79:363–369.
Rambau, M. D., M. L. Mudau, S. D. Makhanya, and K. Benyi. 2016. Effects of stocking density and daily feed withdrawal periods on the performance of broiler chickens in a semi-arid environment. Trop. Anim. Health. Prod. 48:1547–1554.
Rotava, R., I. Zanella, A. K. Karkow, A. P. Dullius, L. P. da Silva, and C. C. Denardin. 2008. Bioquímica sanguínea de frangos de corte alimentados com subprodutos da uva. Agrarian 1:91–104.
Sahin, R., and O. Kucukm. 2001. A simple way to reduce heat stress in laying hens as judged by egg laying, body weight gain and biochemical parameters. Acta Vet. Hung. 49:421–430.
SAS. 2009. SAS Statistical System Package-Jmp 8 User's Guide. 2nd ed SAS Institute Inc., Cary, NC ISBN 978-1-60764-301-2.
Sekeroglu, A., M. Sarica, M. S. Gulay, and M. Duman. 2011. Effect of stocking density on chick performance, internal organ weights and blood parameters in broilers. J. Anim. Vet. Adv. 10:246–250.
Settar, P., S. Yalcin, L. Turkmut, S. Ozkan, and A. Cahanar. 1999. Season by genotype interaction related to broiler growth rate and heat tolerance. Poult. Sci. 78:1353–1358.
Sevanian, A., and L. Mcleod. 1997. Pages 47−70 in Formation of Biological Reactivity of Lipid Peroxidation Products. Taylor and Francis, Washington, DC.
Seven, P. T., S. Yılmaz, I. Seven, I. H. Cercı, M. A. Azman, and M. Yılmaz. 2009. Effects of propolis on selected blood indicators and antioxidant enzyme activities in broilers under heat stress. Acta Vet. Brno. 78:75–83.
Shakeri, M., I. Zulkifli, A. F. Soleimani, E. L. O'Reilly, P. D. Ecker-sall, A. A. Anna, S. Kumari, and F. F Abdullah. 2014. Responseto dietary supplementation of L-glutamine and L-glutamate inbroiler chickens reared at different stocking densities under hot, humid tropical conditions. Poult. Sci. 93:2700–2708.
Shanawany, M. M. 1988. Broiler performance under high stocking densities. Br. Poult. Sci. 29:43–52.
Siegel, H. S. 1985. Immunological responses as indicators of stress. Worlds Poult. Sci. J. 41:36–44.
Silva, P. R. L., O. C. Freitas Neto, O. M. Laurentiz, A. C. Junqueira, and J. J Fagliari. 2007. Blood serum components and serum protein test of Hybro-PG broilers of different ages. Braz. J. Poult. Sci. 9:229–232.
Simitzis, P. E., E. Kalogeraki, M. Goliomytis, M. A. Charismiadou, K. Triantaphyllopoulos, A. Ayoutanti, K. Niforou, A. L. Hager-Theodorides, and S. G. Deligeorgis. 2012. Impact of stocking density on broiler growth performance, meat characteristics, behavioural components and indicators of physiological and oxidative stress. Br. Poult. Sci. 53:721–730.
Simsek, U. G., B. Dalkilic, M. Ciftci, and A. Yuce. 2009. The influences of di_erent stocking densities on some welfare indicators, lipid peroxidation (MDA) and antioxidant enzyme activities (GSH, GSH-Px, CAT) in broiler chickens. J. Anim. Vet. Adv. 8:1568–1572.
Skomorucha, I., R. Muchacka, E. Sosnowka-Czajka, and E. Herbut. 2009. Response of broiler chickens from three genetic groups to different stocking densities. Ann. Anim. Sci. 9:175–184.
Škrbić, Z., Z. Pavlovski, and D. Milić. 2011. The effect of rearing conditions on carcass slaughter quality of broilers from intensive production. Afr. J. Biotechnol. 10:1945–1952.
Sørensen, P., G. Su, and S. C. Kestin. 2000. Effects of age and stocking density on leg weakness in broiler chickens. Poult. Sci. 79:864–870.
Spinu, M., S. Benveneste, and A. A. Degen. 2003. Effect of density and season on stress and behaviour in broiler breeder hens. Br. Poult. Sci. 44:170–174.
Swanson, J. C. 1995. Farm animal well-being and intensive production systems. J. Anim. Sci. 73:2744.
Tall, A. R. 1998. An overview of reverse cholesterol transport. Eur. Heart J. 19(Suppl):A31e5.
Thaxton, J. P., W. A. Dozier, S. L. Branton, G. W. Morgan, D. W. Miles, W. B. Roush, B. D. Lott, and Y. Vizzier-Thaxton. 2006. Stocking density and physiological adaptive responses of broilers. Poult. Sci. 85:819–824.
Tollba, A. A. H., and M. S. H. Hassan. 2003. Using some natural additives toimprovephysiological and productiveperformanceofbroilerunder high temperature condition. Egypt Poult. Sci. 23:327–340.
Tornberg, E. V. A. 2005. Effects of heat on meat proteins-implications on structure and quality of meat products. Meat Sci. 70:493–508.
Tsiouris, V., I. Georgopoulou, C. Batzios, N. Pappaioannou, R. Ducatelle, and P. Fortomaris. 2015. High stocking density as a predisposing factor for necrotic enteritis in broiler chicks. Avian Pathol. 44:59e66.
Turkyilmaz, M. K. 2008. The effect of stocking density on stress reaction in broiler chickens during summer. Turk. J. Vet. Anim. Sci. 32:31–36.
Van Laack, R. L. J. M., C. H. Liu, M. O. Smith, and H. D Loveday. 2000. Characterization of pale, soft, exudative broiler breasts. Poult. Sci. 79:1057–1061.
Wilhelm, A. E., M. B. Maganhini, F. J. Hern_andez-Blazquez, E. I. Ida, and M. Shimokomaki. 2010. Protease activity and the

ultrastructure of broiler chicken PSE (pale, soft, exudative) meat. Food Chem. 119:1201e4.

Yadgari, L., R. Kinereich, S. Druyan, and A. Cahaner. 2006. The effects of stocking density under hot conditions on growth, meat yield and meat quality of featherless and feathered broilers. Worlds Poult. Sci. J. 62(Suppl):603–604.

Yuan, J. M. 2017. Advances in density stress and nutrition regulation of poultry. China Poult. 39:1–5 (in Chinese).

Yun-Zhong, F., Y. Sheng, and W. Guoyao. 2002. Regulation of physiological systems by nutrients: free radicals, antioxidants, and nutrition. Nutrition 18:872–879.

Zhang, M., X. T. Zou, H. Li, X. Y. Dong, and W. Zhao. 2012. Effect of dietary g-aminobutyric acid on laying performance, egg quality, immune activity and endocrine hormone in heat-stressed Roman hens. Anim. Sci. J. 83:141e7.

Zhang, C., J. Q. Luo, B. Yu, J. L. Chen, and D. W Chen. 2015. Effects of resveratrol on lipid metabolism in muscle and adiposetissues: a reevaluation in a pig model. J. Funct. Foods 14:590–595.

Zhang, Y. R., L. S. Zhang, Z. Wang, Y. Liu, F. H. Li, J. M. Yuan, and Z. F. Xia. 2018. Effects of stocking density on growth performance, meat quality and tibia development of Pekin ducks. Anim. Sci. J. 89:925–930.

Zulkifli, I., P. K. Liew, D. A. Israf, A. R. Omar, and M. Hair-Bejo. 2003. Effects of early age feed restriction and heat conditioning on heterophil/lymphocyte ratios, heat shock protein 70 expression and body temperature of heat-stressed broiler chickens. J. Therm. Biol. 28:217–222.