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EFFECT OF STOCKING DENSITY ON BROILERS BEHAVIOUR AND WELFARE INDICES

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ABSTRACT

The purpose of the research was to determine how stocking density affected the behaviour, growth, physiological responses, and leg condition of broilers. 195 five-day old, unsexed Ross strain chicks were randomly allotted into 3 treatments. low stocking density (10 birds/m^2, LSD), medium stocking density (15 birds/m^2, MSD) and high stocking density (18 birds/m^2, HSD). Each treatment had 3 pens (1.5 m^2), and approximately four weeks were spent on the study. The HSD demonstrated reduced rates ($P < 0.05$) of laying, locomotion, eating, preening and longer periods of tonic immobility. Final body weight and total body weight gain were significantly ($P < 0.05$) reduced in the HSD. Also, HSD group showed increased ($P < 0.05$) heterophil/lymphocyte ratio, serum corticosterone, glucose and cholesterol, and significantly increased gait issues and footpad and hock burns. However, no significant ($P > 0.05$) difference was found in litter quality (moisture, ASH, pH) and bone quality (tibiae and femurs measurements) between all treatments. Conclusion, the results indicate that HSD had a negative effect on broiler's behaviour and welfare indicators, therefore it should be avoided in poultry farms and further investigations are still required to figure out the best methods for its control.

Keywords: Stocking density, broilers, behaviour, leg health.

INTRODUCTION

Production of broiler chicken is a capital-intensive industry with a good return on investment, as a result, the most expensive element of the broiler chicken industry is the cost of obtaining land and constructing a broiler house, so broiler producers try to make their business as efficient as possible to get a reasonable economic return by rearing broilers in large numbers per square meter (Ghosh *et al.,* 2012). Another problem is the urgent need to provide a source of low-price animal protein to cover the increasing human population needs all over the world. As a result, poultry producers throughout the world must optimize the number of kilos of chicken produced per square meter of land while minimizing production losses due to overcrowding (Thaxton *et al.,* 2006; Ghosh *et al.,* 2012).

Stocking density has been demonstrated to impact a range of welfare indicators in

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broilers, including behaviour (Kierim, 2013). When stocking densities were increased, chickens showed reduced resting (Hall, 2001) and mobility and foraging behaviours (Ventura *et al.,* 2012; Knierim, 2013). Physiological stress markers in broilers, such as blood corticosterone, glucose, cholesterol, and the heterophil: lymphocyte (H:L) ratio, are unclear and controversial (Heckert *et al.,* 2002; Thaxton *et al.,* 2006; Estevez, 2007).

Leg health, gait scores, hock burns and footpad lesions are good indicators of overall poultry wellbeing (Sanotra *et al.,* 2001; Škrbić *et al.,* 2009; Khosravinia, 2015), were harmed by increasing density from 14 to 18 chicks per m^2 of floor space (Khosravinia, 2015).

However, several research studies have been published on the effect of stocking density on broiler production, well-being, and its economic importance around the world. There is currently no standard definition for broiler stocking density during rearing. The European Union (Council Directive 2007/43/EC) suggests keeping the allowed stocking density for advanced broiler chickens at 33 kg/m^2, but raising it to 39 kg/m^2 if fatality is managed below a certain level and climatic parameters are suitably regulated. As well as considering consumers' opinion changes about poultry well-being, welfare standards, and product quality all over the world, and lack of information in Egypt due to the limited studies about stocking density effects on broiler behavioral changes and welfare with problems that were carried out resulted in an essential call to establish high density as a strategy to increase broiler production. As a result, the current research was created to evaluate the impact of varying stocking density on broiler growth parameters, behavioural components, stress response indicators, and leg health.

MATERIALS AND METHODS

This research was carried out in the animal and poultry behaviour and management research unit in the hospital of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, from November to December 2021.

1. Birds and housing

1.1. Incubation of birds

From a business hatchery in the governorate of Assiut, 195 day-old, unsexed Ross chicks were bought. Chicks were incubated in a separate room within the same facility to adapt to the place for four days.

1.2. Experimental design

Five-day-old, unsexed Ross strain chicks of total 195 were weighed individually and divided into 9 pens (100 cm x 150 cm), with the broilers being distributed into 3 treatments, each of which had 3 replicates. Low stocking density (LSD) had 10 birds, medium stocking density (MSD) had 15 birds, while high stocking density (HSD) had 18 birds per meter (Ventura *et al.,* 2010).

The experiment's pens were constructed using metal and plastic wire on all four sides. On the floor of the pen, there was bedding made of wood shavings that was 10 cm high.

2. Diet and nutrition

During their first two weeks of life, chicks were given access to a commercial starter feed (23% CP and 3027 kcal ME/kg diet), a growing diet (21%CP and 2950 k cal ME/kg diet) during the two following weeks of life (15 to 28 days), and a finisher diet (18% CP and 3228 k cal ME/kg diet) till the end of the experiment, industrial diets were produced by Ront Vet, Egypt. The ration formulation is presented in Table 1**.**

3. Temperature and humidity

The temperature and humidity in the room were recorded every hour for 33 days using a testo data logger (Germany) placed 30 cm above the litter surface.

4. Behavioural observation

Throughout the study, direct observation of chicks' behaviour using the scanning

technique described by Mahmoud *et al.* (2015) was carried out and presented in Table 2. Observations were conducted three times per week for three days per week (from Tuesday to Thursday) during the experiment: 8.0 - 9.0 am, 12.0 - 1.0 pm, and 4.0 - 5.0 pm (from 5 to 33 days of age). There were observations of behaviours including standing, sitting, moving, eating, drinking, grooming, stretching, pecking at walls and pecking at feathers. The proportion of birds engaging in a certain behaviour was calculated.

5. Welfare indicator: Tonic immobility test (TI)

On the 21st and 33rd days, within the same facility, the tonic immobility test (TI) was carried out in a different room. The chicks were restrained on their right side and wings for 15 seconds after being brought into the test room to make the tonic immobility. The observer stood silently 1 meter away from the test table. Durations were recorded using a stopwatch till the chicken rose by itself (Taskin, 2009). If the chicken stood up in 10 seconds, it was caught, and the operation was repeated (Yildirim, 2017).

6. Growth performance

From five to thirty-three days of age, the growth performance of the experimental birds was monitored weekly, including body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR).

7. Blood biochemistry

At the age of 33 days, 2 ml of blood was taken from each selected bird (2 birds per replication, 6 birds per treatment), which was then euthanized by severing the jugular vein in accordance with Islamic slaughtering practices (Ahmed *et al.,* 2018) and allowed to bleed for approximately 2 min. The blood sample was collected into an EDTA test tube to determine the heterophil / lymphocyte (H/L) ratio. Additionally, a 5 mL blood sample was obtained into a serum separator tube without anticoagulant and permitted to clot for 2 to 3 hours before being centrifuged at 3000 rpm for 10 minutes to separate the serum. The isolated serum was then transferred to another Eppendorf tube using a micropipette and stored at -20 °C until it was sent to the lab for the assessment of serum corticosterone, glucose, cholesterol, total protein, albumin, globulin, phosphorus and calcium by commercial spectrophotometry kits.

8. Litter quality

Litter samples were collected from the identical sites in each pen at 33 days of age for pH (1:10 litter per distillated water using AD 12 pH meter made in Romania) and moisture content (for 24 hours at 105 °C in an oven) (Farhadi *et al.*, 2016). Each litter sample consisted of six litter subsamples taken away from drinking and feeding equipment (Farhadi *et al.*, 2016). In addition, ASH was determined by dividing the sample weight from the moisture content.

9. Leg health measurements

Every chicken in every pen was checked for hock burn, footpad dermatitis, and gait score on the 21st and 33rd days, together with their body weight. All of the chicks were handled gently throughout the test to reduce any potential stress reactions. The Bilgili *et al.* (2006) described a four-point scale that was used to grade footpad dermatitis. Chickens were placed in one of the following categories: 0, no abnormalities; 1, moderate lesion measuring less than 0.75 cm (diameter); 2, large lesions measuring more than 1.5 cm; or 3, severe lesions measuring more than 1.5 cm. According to Srensen *et al.* (2000), each chicken was examined for the prevalence of hock burns, and the total scores for both legs were given a score between 0 and 3, with 3 indicating extensive burn and inflammation.

A modified approach proposed by Garner *et al.* (2002) and Dozier *et al.* (2006) was used to assess the gait score. A human walked slowly behind each chicken in each pen to encourage it to walk, and the bird was scored each time it moved. The birds were given one of four scores: 0, normal walking with

no noticeable impairment in moving ability; 1, apparent walking capacity disorder; 2, noticeable walking ability impairment that influenced the bird's capacity to move; and 3, unwilling walks, with severe walking ability disorder causing unwillingness to rise up and move.

10. Bone measurements

At the end of the experiments, two birds from each pen had their tibiae and femurs removed, sealed in plastic bags, and kept at -20 °C for later analysis. The bone's fresh weight, length, and width were measured after the cartilage, fat, muscle, and other tissues were properly removed.

11. Statistical analysis

The results have been displayed as the mean ± standard error of the mean (SEM). The pens served as the statistical components. Due to the limited sample size (3 pens/treatment group), the Kruskal-Wallis test was utilized for multiple comparisons between groups, and if the data were significant, the Mann-Whitney test had been used. All statistical studies were carried out using the SPSS for Windows software, version 16.0. (SPSS, Inc., Chicago, IL, USA). A probability (P) value of 0.05 was statistically significant.

RESULTS

1. Behaviour parameter

The findings presented in Table 3 indicated that the percentage of broilers resting in LSD and MSD was markedly higher than HSD ($P < 0.05$). Furthermore, LSD and MSD were substantially greater than HSD in walking ($P < 0.05$). During eating behaviour, LSD was substantially greater than MSD and HSD ($P < 0.05$). LSD was substantially higher than MSD and HSD for comfort behaviour (preening) ($P < 0.05$). Standing, drinking, stretching, feather pecking, and wall pecking behaviours showed no significant difference between all treatments ($P > 0.05$).

2. Welfare test

The effects of stocking density on the welfare test showed that the tonic immobility test at 21 days in LSD and MSD had significantly lower duration time than HSD ($P < 0.05$). However, no significant changes ($P > 0.05$) were seen between any treatments at day 33 (table 3).

3. Performance

The effects of stocking density on growth performance showed that final BW and total body weight in LSD and MSD increased considerably ($P < 0.05$) greater than in HSD. Furthermore, there were no significant changes ($P > 0.05$) in FI and FCR among all treatments (table 4).

4. Blood biochemistry

The effects of stocking density on blood parameters showed that there were no significant variations in (total protein, albumin, globulin, calcium, and phosphorus) across all treatments ($P > 0.05$). However, corticosterone, glucose, and cholesterol concentrations in HSD were considerably ($P < 0.05$) greater than in LSD and MSD (table 5).

Also, Table 5 shows that the H/L ratio was considerably ($P < 0.05$) greater in HSD compared to LSD and MSD.

5. Leg health measurements

The prevalence and severity of FPD for broilers submitted to MSD and HSD exhibited a higher severity and frequency of FPD at days 21 ($P > 0.05$ for scores 0 and 1) and 33 ($P > 0.05$ for scores 0 and 3) than those subjected to LSD (table 6)

Also, broilers submitted to MSD and HSD exhibited a higher rate and severity of HB at day 21 ($P > 0.05$ for scores 0 and 2) than those subjected to LSD (table 6).

In contrast to those put in LSD and HSD showed a higher severity and frequency of HB at day 33 than MSD ($P > 0.05$). In addition, HSD had a higher occurrence and severity of HB at day 33 ($P > 0.05$ for score 3) than LSD and MSD.

In addition to previously mentioned results, broilers submitted to MSD and HSD exhibited a higher occurrence and severity of

GS at day 21 (P 0.05 for scores 0 and 2) than those subjected to LSD (table 6).

6. Litter analysis

The effects of stocking density on litter parameters revealed that there was no considerable variation in (moisture, ASH, pH) among all treatments (P > 0.05) (table 7).

7. Bone measurements

Table 7 shows the effects of stocking density on bone measures. There were no significant changes in (tibia and femur measurements) across all treatments (P > 0.05).

Table 1: Ration formulation

Chemical analysis	Starter diet	Grower diet	Finisher diet
Raw protein %	23	21	19
Raw fat %	4.93	4.52	10
Crude fiber	3.63	3.58	3.71
Energy kcal	3027	2950	3228
Component			
Yellow corn %	55	59	55.03
Soya bean meal %	(48) 30	(44) 28.7	33.5
Soya bean oil %	2.2	1.7	7.5
Di calcium phosphate %	---	1.8	18
Mono calcium phosphate %	1.6	---	---
Limestone %	1.5	1.3	1.3
Food salt %	0.42	0.35	0.4
A mixture of vitamins and minerals salts %	---	(8665) 0.3	0.27
Choline %	0.30	---	0.3
DL-methionine %	0.10	0.05	0.27
L Lysine hydrochloride %	0.10	---	---
Gluten	(62) 8.8	(60) 6.8	---

Table 2: Behavioural ethogram. Mahmoud *et al.* (2015).

Behaviour	Definition
Standing	The floor is in contact with both feet; no other body part is in contact with the floor.
Laying	The floor is in touch with the majority of the ventral area of the bird's body. There is no space between the floor and the bird.
Walking	Bird is taking several steps, including "walking in place."
Feeding	The bird's head is within the feeder.
Drinking	The bird's beak has made contact with the drinker.
Preening	Pecking or scratching its own feathers gently.
Stretching	Extending a leg or wing
Wall pecking	Pecking at non - edible things or the ground
feather pecking	A bird pecks or pulls another bird's feathers.

Table 3: Effect of stocking density on behaviour and welfare indicators of broiler chickens.

			LSD	MSD	HSD	SEM	Chi square	Df	Asymp. sig
Behavioural activities%	Posture%	Laying	64.56[a]	63.29[ab]	61.65[b]	1.08	6.59	2	0.037
		Standing	1.73	2.12	2.64	0.47	3.72	2	0.156
		Walking	2.75[a]	2.19[ab]	1.92[b]	0.46	6.23	2	0.044
	Ingestive%	Feeding	7.77[a]	6.37[ab]	5.82[b]	0.61	8.31	2	0.016
		Drinking	5.95	8.15	8.91	0.92	5.06	2	0.08
	Grooming%	Stretching	5.85	5.47	5.73	0.84	0.25	2	0.881
		Preening	6.65[a]	5.82[ab]	5.75[b]	0.49	6.09	2	0.048
	Pecking%	wall pecking	4.07	5.67	5.8	0.90	4.41	2	0.111
		feather pecking	0.66	0.92	1.78	0.35	2.43	2	0.297
welfare test	Tonic immobility	At 21 days (sec)	73.33[b]	82.83[b]	154.67[a]	18.74	11.45	2	0.003
		At 33 days (sec)	55.33	83.5	175.67	34.53	5.98	2	0.05

Effect of stocking density on behavior and welfare indicators. LSD: low stocking density; MSD: medium stocking density; HSD: high stocking density. [a,b,c]Mean ± SEM with different letters indicate significant differences at $P < 0.05$ (Kruskal-Wallis test followed by Mann-Whitney test).

Table 4: Effect of stocking density on performance of broiler chickens.

		LSD	MSD	HSD	SEM	Chi square	Df	Asymp.sig
Body weight (Kg) /week	At 5 day	0.18	0.18	0.18	0.002	7.2	2	0.146
	1st week	0.5	0.51	0.5	0.01	5.42	2	0.43
	2nd week	1.05	1.06	1.02	0.02	3.32	2	0.19
	3rd week	1.76	1.76	1.65	0.02	1.69	2	0.066
	4th week	2.43[a]	2.32[a]	2.22[b]	0.03	3.85	2	0.027
Body weight gain (Kg) /week	1st week	0.32	0.32	0.32	0.01	6.49	2	0.491
	2nd week	0.55	0.55	0.52	0.01	4.36	2	0.066
	3rd week	0.7	0.69	0.64	0.01	6.01	2	0.05
	4th week	0.67	0.56	0.57	0.05	5.42	2	0.113
	Total body weight gain	2.25[a]	2.13[a]b	2.04[b]	0.04	1.42	2	0.039
Feed intake (Kg) /week	1st week	0.52	0.51	0.48	0.03	5.42	2	0.393
	2nd week	0.84	0.84	0.8	0.02	1.69	2	0.066
	3rd week	1.12	1.13	1.03	0.02	5.60	2	0.061
	4th week	1.26	1.26	1.24	0.01	5.42	2	0.43
	Total feed intake	6.67	6.33	2	0.05	1.87	2	0.066
Feed conversion ratio	1st week	1.63	1.57	0.51	0.10	1.87	2	0.393
	2nd week	1.51	1.52	1.54	0.03	0.62	2	0.733
	3rd week	1.6	1.63	1.61	0.02	1.42	2	0.491
	4th week	1.87	2.29	2.18	0.18	5.42	2	0.066
	Average	1.65	1.75	1.71	0.06	5.42	2	0.066

Effect of stocking density on performance. LSD: low stocking density; MSD: medium stocking density; HSD: high stocking density. [a,b,c]Mean ± SEM with different letters indicate significant differences at $P < 0.05$ (Kruskal-Wallis test followed by Mann-Whitney test).

Table 5: Effect of stocking density on blood biochemistry of broiler chickens.

		LSD	MSD	HSD	SEM	Chi square	Df	Asymp.sig
H/L ratio		0.42b	0.62b	0.69a	0.26	2.31	2	0.001
Corticosterone	(ug/dl)	0.07b	0.12ab	0.22a	0.03	10.16	2	0.006
Glucose	(mg/dl)	151.67b	157.00b	172.50a	6.98	6.48	2	0.039
Cholesterol	(mg/dl)	82.17b	81.33b	93.00a	4.21	7.09	2	0.029
Phosphorus	(mg/dl)	6.28	6.07	6.09	0.38	0.89	2	0.642
Calcium	(mg/dl)	10.26	10.27	10.43	0.30	0.31	2	0.856
Total protein	(g/dl)	3.12	3.03	2.96	0.21	1.22	2	0.544
Albumin	(g/dl)	2.43	2.28	2.33	0.08	2.61	2	0.271
Globulin	(g/dl)	0.68	0.75	0.63	0.20	0.59	2	0.744

Effect of stocking density on blood biochemistry. LSD: low stocking density; MSD: medium stocking density; HSD: high stocking density. [a,b,c]Mean ± SEM with different letters indicates significant differences at $P < 0.05$ (Kruskal-Wallis test followed by Mann-Whitney test).

Table 6: Effect of stocking density on leg health measurements of broiler chickens.

		Score	LSD	MSD	HSD	SEM	Chi square	Df	Asymp.sig
Foot pad dermatitis (FPD)	At 21 day	0	100.00[a]	92.75[b]	83.64[c]	2.44	15.91	2	0
		1	0[b]	7.25[a]	12.96[a]	2.23	13.18	2	0.001
		2	0	0	0	0	0	2	1
		3	0	0	0	0	0	2	1
	At 33day	0	63.25[ab]	73.59[a]	56.09[ab]	7.19	7.87	2	0.02
		1	15.87	12.3	10.27	2.20	3.69	2	0.158
		2	18.01	11.5	20.01	4.06	4.73	2	0.094
		3	2.78[b]	2.54[b]	13.63[a]	2.07	11.21	2	0.004
Hock burn (HB)	At 21 day	0	78.33[a]	67.75[ab]	54.63[b]	6.30	8.94	2	0.011
		1	21.67	26.09	33.02	5.16	2.90	2	0.235
		2	0.00[b]	6.16[b]	12.35[c]	2.10	11.99	2	0.002
		3	0	0	0	0	0	2	1
	At 33 day	0	43.73	50.03	33.78	7.48	2.51	2	0.285
		1	26.87	24.47	15.55	5.87	5.43	2	0.066
		2	26.63[a]	18.74[b]	26.81[a]	1.93	10.96	2	0.004
		3	2.78[b]	6.76[b]	23.8[a]	3.31	12.93	2	0.002
Gait score (GS)	At 21 day	0	90.00[a]	80.43[b]	80.25[b]	3.23	8.18	2	0.017
		1	10	14.49	14.2	2.81	2.93	2	0.231
		2	0.00[b]	5.07[a]	5.56[a]	0.90	12.27	2	0.002
		3	0	0	0	0	0	2	1
	At 33day	0	90.48	92.27	94.38	5.75	0.27	2	0.874
		1	5.95	3.11	4.17	3.63	0.16	2	0.923
		2	3.57	4.62	1.45	3.87	1.11	2	0.575
		3	0	0	0	0	0	2	1

Effect of stocking density on leg health. LSD: low stocking density; MSD: medium stocking density; HSD: high stocking density. [a,b,c]Mean ± SEM with different letters indicate significant differences at $P < 0.05$ (Kruskal-Wallis test followed by Mann-Whitney test).

Table 7: Effect of stocking density on bone measurements and litter analysis of broiler chickens.

		LSD	MSD	HSD	SEM	Chi square	Df	Asymp.sig
Femur measurements	Length (cm)	6.33	6.68	6.45	0.17	4.39	2	0.185
	Width (cm)	0.8	0.85	0.83	0.04	1.69	2	0.431
	Weight (gm)	14.17	16.83	13.67	1.05	3.38	2	0.111
Tibia measurements	Length (cm)	9.68	10.1	9.97	0.30	0.70	2	0.424
	Width (cm)	0.83	00.88	0.87	0.04	1.56	2	0.459
	Weight (gm)	20.5	22.17	21.17	1.61	1.72	2	0.704
Litter analysis	Ash (gm)	3.17	2.83	2.9	0.24	1.16	2	0.424
	Moisture (gm)	1.83	2.17	2.1	0.24	1.72	2	0.424
	pH	7.87	8.16	8.25	0.45	1.72	2	0.561

Effect of stocking density on bone measurements and litter quality. LSD: low stocking density; MSD: medium stocking density; HSD: high stocking density. [a,b,c]Mean ± SEM with different letters indicates significant differences at $P < 0.05$ (Kruskal-Wallis test followed by Mann-Whitney test).

DISCUSSION

Many behavioural parameters can provide appropriate indicators of broiler well-being and health. Behaviour often shows animals' contentment with their surroundings (Erasmus, 2017). According to Beerda *et al.* (2000), behaviour is a key indicator of how well an animal has adapted to its physical surroundings and social environment. In our study, broilers raised at HSD were significantly lower in laying, walking, feeding, and comfort behaviour (preening). On the other hand, there was no significant difference in standing, drinking, stretching, wall pecking, and feather pecking between all treatments. This conclusion is in line with previous research findings that have shown the proportion of broiler chickens feeding, moving, and preening was clearly lower in HSD (Ma *et al.*, 2020), which was consistent with the findings of Buijs *et al.* (2010), who discovered that broiler chicken periods of preening and resting were significantly lower in the HSD than in the LSD. This conclusion can be explained in part by the knowledge that HSD can cause poor leg conditions, like lameness, which inhibits mobility and hence lowers grooming. According to certain research (Feddes *et al.*, 2002; Dozier *et al.*, 2005), lameness may restrict physical access to feeders, suggesting that the behaviour of broilers to feed declines as stocking density rises. Furthermore, stocking density had no effect on drinking and pecking behaviour (Son, 2013). These findings contradict the findings of Ma *et al.* (2020), who observed that the HSD group had such a greater proportion of drinking than the LSD group. Housing conditions, according to Dawkins *et al.* (2004), are especially critical in heavy rearing, because increasing density may limit air circulation and diminish body heat dissipation, which may be the reason for the increased drinking habit. When broilers are housed at a high stocking density, aggressive behaviour, feather pecking, and cannibalism have been noted (Türkyilmaz, 2008; de Jong *et al.*, 2012). Also, Bandyopadhyay *et al.* (2006) observed that higher stocking density results in more unstable social and aggressive behaviour.

In the current study, broilers raised at HSD significantly decreased in total body weight gain and end body weight. but no significant change in FI and FCR between groups, these results agree with studies that showed that HSD of broilers had been associated with slower rates of growth (Heidari and

Toghyani, 2018; Goo *et al.,* 2019; Jope *et al.,* 2019). According to the research, HSD has a harmful impact on broiler intestine functioning, causing damaged intestinal mucosa and reduced digestive and absorptive activities (Li *et al.,* 2019). Furthermore, HSD was observed to decrease growth factor networks (i.e., reduced insulin-like growth factor 1 (IGF-1) and (MyoD) (myoblast determination protein-1). However, few published studies have shown that density has little influence on chicken production. Henrique *et al.* (2017) discovered that elevating the density to 10 or 12 to 14 birds/m^2 has no detrimental effect on broiler growth performance, in the first two weeks of the study, there was no evident influence of stocking density on weight gain, feed consumption, or fatality, but weight gain and feed consumption were substantially higher in the LSD group than the HSD group at 4-to-5-weeks ages.

Furthermore, we found that HSD significantly increased the duration of tonic immobility. This result is in line with prior research findings that shown, HSD resulted in an inadequate growing area, which may result in lameness and animal health problems (Buijs *et al.,* 2012), fear behaviours and elevated tonic immobility (El-Lethey *et al.,* 2001). So, tonic immobility duration on HSD was prolonged (Onbaşlar *et al.,* 2008).

On the other hand, this result was related to a lower age of 21 than 35 days but was unaffected by density (Son. 2013). In principle, increased density has varying impacts on broiler stress response and metabolism (Estevez, 2007; Ravindran *et al.,* 2006). We observed that HSD raised stress markers in broilers, as evidenced by an increase in the H/L ratio, serum corticosterone, glucose, and cholesterol concentrations. This result is in line with prior research findings that overcrowding increased H/L ratio (Thaxton *et al.,* 2006; Onbaşlar *et al.,* 2008; Selvam *et al.,* 2017), serum corticosterone (Türkyilmaz, 2008; *Law et al.,* 2019), and serum glucose (Dozier *et al.,* 2006; Onbaşlar *et al.,* 2008; Zuowei *et al.,* 2011; Silas *et al.,* 2014). High stocking densities are believed to enhance aggression and stress in the chicks, which raises glucocorticoid levels (Ravindran *et al.,* 2006). Stress alters body metabolic activity by producing glucose for the energy necessary to maintain homeostasis in the presence of the stressor, which is a vital task (Virden and Kidd, 2009). (Puvadolpirod and Thaxton, 2000). Sources of stress raise the amount of cholesterol in chicken plasma (Dozier *et al.,* 2006). However, these findings contradicted the findings of Houshmand *et al.* (2012), who discovered that stocking density had no effect on the blood levels of corticosterone, glucose, cholesterol, and the H / L ratio of broiler chickens. While we did not find a significant difference in total protein, albumin, globulin, phosphorus and calcium concentrations in blood between all treatments.

In this study, litter quality did not find a significant difference (moisture, ASH, pH) between all treatments. These results agreed with Coufal *et al.* (2006) and Farhadi *et al.* (2016), who discovered that increasing density had no impact on litter pH; however, it appears that density has less impact on litter pH. (Zhang *et al.,* 2011). Increasing stocking densities had no impact on litter moisture (Farhadi *et al.,* 2016). In contrast, Thomas *et al.* (2004) and Dozier *et al.* (2005) found that when stocking density rose, litter wetness increased.

In broiler chickens, greater stocking density aggravated gait problems, footpad and hock burns. In agreement, Thomas *et al.* (2004), de Jong *et al.* (2012), and Rashidi *et al.* (2019) discovered that as stocking density rose, moving capability decreased and hock burn, and foot pad dermatitis increased in broilers. As our result, broilers spend less time walking and move less often and poorly (Hall 2001; Srensen *et al.,* 2000; Buijs *et al.,* 2009). However, no significant differences in gait score were discovered across the varied stocking densities (Hongchao *et al.,* 2014). This conclusion is similar to the findings of Dozier *et al.* (2005, 2006), who

found that increasing stocking density had no effect on gait score.

Furthermore, we documented that there was no significant difference in bone measurements (measurements of tibiae and femurs) between all treatments. Henrique *et al.* (2017) discovered that increasing density to 10 or 12 to 14 birds/m^2 had no significant effect on broiler bone quality. This finding differs from previous studies that found tibial length, width, and weight of broilers were considerably decreased in the HSD group (Li *et al.,* 2019), similar to the findings of Kestin *et al.* (1992), who discovered common leg problems in chickens fed at high densities, and Sanotra *et al.* (2001), who found elevated tibial dysplasia with rising stocking density. According to a recent study, layer breeder males housed at HSD have shorter tibiae (Li *et al.,* 2019).

Furthermore, greater stocking density enhanced morphological features of the tibiae, such as weight, length, and volume. Moreover, the HSD group had larger vertical internal and horizontal exterior diameters of the mid-shaft.

CONCLUSION

Finally, increasing the stocking density from 10 to 18 birds/m^2 had a negative impact on behaviour components, growth performance, welfare indicators, and physiological state. There were major changes in final body weight, total body weight gain, H:L ratio, serum corticosterone, and glucose levels between the three stocking densities. While bone quality features and litter analysis did not change between the three densities, we also found significant variations in leg health.

ETHICAL CONSIDERATION

The Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, authorized the protocol and procedures.

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تأثير الكثافة على سلوكيات ومؤشرات الرفاهية فى دجاج التسمين

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كان الغرض من البحث هو تحديد تأثير مختلف الكثافات على السلوك ، والنمو ، والاستجابات الفسيولوجية ، وحالة أرجل دجاج التسمين . تم تخصيص ١٩٥ فرخا من فصيلة روس غير مجنسة يبلغ عمرها خمسة أيام وتم توزيعها بشكل عشوائى إلى ٣ مجموعات علاجية. كثافة تربية منخفضة (١٠ طيور/ متر مربع LSD) ، كثافة تربية متوسطة (١٥ طائر / متر مربع ، MSD) وكثافة تربية عالية (١٨ طائر / متر مربع ، HSD) . كان لكل مجموعة علاجية ٣ أقفاص (١,٥ متر مربع)، استغرقت الدراسة أربعة أسابيع . أظهرت مجموعة HSD معدلات منخفضة ($P < 0.05$) فى سلوكيات الراحة والحركة والاكل والتنمق وفترات اطول فى اختبار يشير الى قلة الرفاهية للطيور. ايضا انخفض وزن الجسم النهائى واكتساب الوزن الكلى للجسم بشكل معنوى ($P < 0.05$) فى HSD. كما أظهرت مجموعة HSD زيادة ($P < 0.05$) فى نسبة الخلايا الليمفاوية ، وتركيز كورتيكوستيرون والجلوكوز والكوليسترول ، وزيادة ملحوظة فى مشاكل المشى والتهابات الوسادة والعرقوب . ومع ذلك ، لم يتم العثور على فرق معنوى ($P < 0.05$) فى جودة الفرشة (الرطوبة ، ASH ، الاس الهيدروجينى) وجودة العظام (قياسات عظم الساق وعظم الفخد) بين جميع المجموعات العلاجية . فى الختام تشير النتائج الى أن HSD كان له تأثير سلبى على سلوك الطيور ومؤشرات الرفاهية ، لذلك يجب تجنبها فى مزارع الدواجن ولا تزال هناك حاجة لمزيد من التحقيقات لمعرفة أفضل الطرق للتعامل مع مختلف الكثافات.