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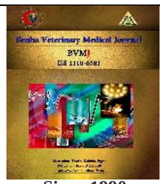

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Effect of stocking density on behavioral patterns, growth performance, blood hormones, and carcass parameters of Sasso broiler chickens

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ABSTRACT

This study was conducted to evaluate the impact of stocking density on behavioral patterns, growth performance, blood hormones, and carcass quality of Sasso broilers within 60 days of rearing cycle. A total number of 150 one day old unsexed Sasso (first generation) chicks were allocated randomly according to their stocking density to 3 groups 10,13 and 15 birds per m^2in different floor spaces5, 3.8, and 3.3 m^2 respectively. Growth performance and behavioral patterns were estimated throughout the study period. Blood samples for cortisol and thyroid hormones estimation were collected every two weeks. At the end of the study 3 birds from each group were slaughtered to check the effect of stocking density on carcass parameters. The results of the current study revealed that stocking density affect significantly ($P = 0.05$) on final body weight, feed consumption, and organs weight, but had no significant effect on feed conversion rate (FCR). There was no significant effect of stocking density on blood cortisol or thyroid hormones concentrations. It was concluded that rearing Sasso broiler at stocking density 10 birds /m^2 has the best effect as it did not affect negatively either on growth performance, blood parameters, or behaviors.

1. INTRODUCTION

Poultry industry in the developing countries has significant economic, social, cultural advantages and plays an important role in family nutrition. By 2020, the proportional contribution of poultry to the world's total production of animal protein is projected to rise to 40%, with the largest increase being in the developing world (Delgado et al., 1999).

Broilers like other animal have basic requirements to maintain its well-being. The proper management of broilers is necessary to express the normal behavior and performance. Broilers should have the ability to run freely, peck, scratch ,wing flap , groom their feather, rest and sleep (Broom, 2001). Using of local breeds as an alternative system in poultry industry has significant advantages, as these breeds are closely linked to the environment and contribute to the maintenance of biodiversity and effective agricultural production especially in deprived areas (Franco et al., 2012). Fast-growing poultry with high efficient feed conversion rates has resulted in undesirable side effects on the health, meat quality and welfare (da Silva et al., 2017; Hartcher and Lum, 2020), but slower-growing broilers, relative to fast-growing meat poultry hybrids, are also considered to suffer less from physical limitations and health problems (Bergmann et al., 2017).

Stocking density (SD) means that optimizing floor space by increasing the number of birds per unit of space to reduce overhead costs associated with poultry house maintenance and increase feed utilization efficiency to compromise bird performance (Nahashon et al., 2011). Stocking density can have adverse effects on the output and welfare of broilers (Estevez et al., 2007).

Increased stocking density in broilers leads to a decrease in their efficiency and growth due to heat stress induced by issues with heat dissipation, rather than causing physical restriction and leg problems such as increased the development of footpad lesions (Sørensen et al., 2000; Hongchao et al., 2014). High SD causes changes in behaviors and risky on the welfare and performance of broiler production (Estevez et al., 2007). Also, has negative effect on welfare of birds through decrease of feeder space apportionment, which induce aggression, stress and mortality (Thogerson et al., 2009).

This study was conducted to evaluate the effect of stocking density on behavioral patterns, growth performance, blood hormones, and carcass quality of Sasso broilers.

2. MATERIAL AND METHODS

The study procedures were conducted in compliance with the recommendations of the guidelines for the care and use of animals at the college of Veterinary Medicine, Benha University. This study started in February and continued for 60 days.

2.1. Birds and management

A total number of 150 unsexed one day old Sasso (first generation) chicks were purchased from private farm in province of Al-Qualubia. Chicks were reared in brooding area for 15 days before their distribution in different floor space pens.

Chicks were received in February in previously prepared house. Floor was covered with a layer of wood shaving (10 cm) and warmed pens at 33ºc then gradually decrease by 2 º C every week and relative humidity was ranged from 50-70 % during brooding period. This brooding period

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continued for 2 weeks then birds were randomly distributed in pens with different floor spaces, the study procedures were started from the 3rd week.

Feed and water were provided ad libitum via feeders and water troughs at temperature 33ºc at chicks arrival. A starter diet (23% CP and 2988 kcal/kg ME) was supplied from 0-21 day old, while grower diet (21% CP and 3083 kcal/kg ME) was fed from 22- 42 day old and finally the finisher diet (19% CP and 3200 kcal/kg ME) was supplied from 43 till the end of experimental period at the 60th day.

Table 1 The contents of diet

Nutrient	Starter (1-21 days)	Grower (22-42 days)	Finisher (43-60 days)
Metabolizable Energy (kcal\kg)	2988	3083	3200
Crude Protein%	23	21	19
Calcium%	1.1	1.2	1.2
Phosphorus%	0.45	0.5	0.5
Crude Fat%	3.5	4	4
Lysine%	1.25	1.20	1.15
Methionine%	0.55	0.5	0.5

*Each 3 Kg of premix contains: vitamins: A: 12000000 IU; D3: 2000000 IU; E: 1000000 mg; K3: 2000 mg; B1: 1000 mg; B2: 5000 mg; B6: 1500 mg; B12: 10 mg; Biotin: 50 mg; Choline chloride: 250000 mg; Pantothenic acid: 10000 mg; Nicotinic acid: 30000mg; Folic acid: 1000 mg; Minerals: Mn: 60000 mg; Zn: 50000 mg; Cu: 10000mg; I: 1000mg; Se: 100 mg and Co: 100mg. This composition of diet produced by NEW HOPE company.

2.2. Vaccination and drugs program

All chicks were vaccinated against Marek's disease in hatchery. Along experimental period, Birds were vaccinated against Newcastle disease with Hitchiner IB vaccine via drinking water at the age of 7 days and booster dose with Colona vaccine at the age of 21 days. Colona vaccine was repeated every 10 days during rearing cycle. Birds were vaccinated against avian influenza (H5 and H9) via I/M injection in breast muscle at10 days, also against infectious bursal disease 2 doses of vaccination, the first dose at the age of 14 days while, the booster dose after two weeks.

2.3. Experimental design

After the brooding periods, chicks were allocated randomly to 3 equal groups according to the floor space, the available floor space was 5, 3.8, and 3.3 m^2 for the first, second and third group respectively, which means that the first group (G1) had 10 birds per m^2, the second group (G2) had 13 birds per m^2,and the third group (G3) had 15 birds per m^2.

2.4. Growth performance parameters

2.4.1. Average feed intake per bird

By subtracting the weight of feed left from original amount of feed offered to birds in grams three times per week then divided on number of birds (Dagaas and Claveria, 2008)

2.4.2. Average weight gain

It is the difference between average body weight and the average body weight of the previous week (Yalcin et al., 1998)

2.4.3. Feed conversion rate

By dividing average feed intake on average weight gain (Dagaas and Claveria, 2008).

2.5. Blood sampling and hormonal analysis

To determine changesof stress level and metabolic rate, three birds were chosen randomly from each group for blood samples collection. Blood samples were collected 3 times during the study at 30, 45, and 60 days from wing vein. 3 ml of blood was collected from each bird and centrifuged at 3000 rpm for 10 minutes then serum samples were collected and labeled. Samples were preserved at (-20^0 C) till analysis by using ELISA (Alm et al., 2014),to measure cortisol level as indicator for stress and thyroid hormones (T_3 and T_4) as indicator for metabolism.

2.6. Slaughtering and carcass quality

At the end of the study, 3 birds from each group were chosen randomly slaughtered and dressed then carcass weights, fat, and organs percentage were recorded.

2.7. Behavioral observations

Behavioral patterns of birds were observed by focal observation for three days per week in three times daily (8.00 – 9.00 am , 12.00 – 1.00 noon , and 5.00 – 6.00 pm) each period was one hour that's mean 20 minutes for each pen (Ventura et al., 2012 and Cornetto and Estevez, 2001).

All observations were carried out by one observer who was present at all measurement points in the experiment to familiarize the chickens with the presence of humans in order to avoid the effect of the observer on the actions of chickens.

Table 2 Behavioral patterns through experiment.

Feeding	Bird putting its beak inside the feeder and pecking.
Drinking	Bird touching the drinker with its beak and raising its head.
Breast rest	Bird sitting with its head retracted, eyes opened, and its breast in contact with the litter.
Lateral rest	Bird sitting with its head retracted, eyes opened or closed, and lateral part of chick is in contact with the litter.
Leg and wing stretch	Unilateral extension of one leg and \ or wing towards bird sides.
Lateral leg and wing stretch	Unilateral extension of one leg and \ or wing in lateral rest position
Sleep	Bird sitting with its head retracted, eyes closed.
Preening	Bird is using its beak to peck, stroke or comb plumage.
Litter picking	Bird is scratching at the ground with feet and take one or two steps backwards and pecks at litter.
Wing flap	bird stretches to its full height and flaps its wings repeatedly.
Dust bathing	Bird is lying on the ground and tossing dirt onto its back/wings by ruffling and shaking its feathers.
Body shake	Bird shakes its feathers vigorously

Ethogram of broiler chicken behaviors used in the trail , based on former studies (Cornetto and Estevez, 2001; Shields et al., 2005; Mahmoud et al., 2015)

2.8. Statistical analysis

Data analysis was performed using SPSS program version 22. Data was analyzed using analysis of variance (ANOVA). Values were presented as means ± SE means Data was declared different at ($P \leq 0.05$).

3. RESULTS

3.1. Live body weight

Results of the effect of stocking density on body weight of Sasso broilers were shown in table (3). During the rearing phase, there was a highly significant effect of stocking density on broilers (p = 0.05) and the highest body weight was observed in stocking density 10 followed by 13,while the inferior body weight was recorded in 15 birds /m^2.

3.2. Feed consumption

Results of the effect of stocking density on feed consumption of Sasso broilers were shown in **table (4)**. There was high significance effect of stocking density on broilers feed consumption ($p \leq 0.001$)**,** from fourth week till 8th week, the highest feed intake was observed in stocking density 10 and 13birds \$m^2$ while the lowest feed consumption was recorded in 15 birds \$m^2$.

Table 3 Effect of stocking density on body weight of Sasso broilers.

Age	Body weight (BW) (g)			
	G1	G2	G3	P-value
3rd week	566.5±16.36[a]	538.9±14.53[a]	454.7±15.88[b]	0.001
4th week	983.5±42.74[a]	870.0±39.05[ab]	792.5±47.57[b]	0.01
5th week	1345.5±57.45[a]	1258.0±62.69[ab]	1113.5±75.75[b]	0.05
6th week	1856.0±58.30[a]	1785.0±49.77[a]	1533.0±82.71[b]	0.001
7rd week	2237.5±90.27[a]	2142.0±84.49[ab]	1948.0±101.38[b]	0.09
8th week	2578.9±82.10[a]	2421.0±101.81[a]	2127.5±103.96[b]	0.001
Final BW (60 d)	2896.66±102.68[a]	2700.0±120.55[ab]	2395.0±123.79[b]	0.05

Least Square Means (± SE) with different superscript letters in the same row are significantly different at P≤0.05. G1 was 10 birds/m^2, G2 was 13 birds/m^2, and G3 was 15 birds/m^2.

Table 4 Effect of stocking density on feed consumption of Sasso broilers.

Age	Feed consumption (g)			
	G1	G2	G3	P-value
4th week	579.97 ± 30.93	545.99 ± 16.99	516.14 ± 4.85	0.10
5th week	754.21 ± 31.62[a]	726.42 ± 18.69[a]	635.55 ± 12.89[b]	<0.001
6th week	1017.66 ± 6.86[a]	1021.45 ± 31.97[a]	838.35 ± 25.92[b]	<0.001
7rd week	959.55 ± 34.35[a]	958.03 ± 34.35[a]	794.38 ± 12.49[b]	<0.001
8th week	969.77 ± 38.83[a]	951.85 ± 5.99[a]	713.24 ± 7.29[b]	<0.001

Least Square Means (± SE) with different superscript letters in the same row are significantly different at P≤0.05. G1 was 10 birds/m^2, G2 was 13 birds/m^2, and G3 was 15 birds/m^2.

3.3. Feed conversion ratio (FCR)

Results of the effect of stocking density on feed conversion ratio of sasso broilers were shown in table (5). Stocking density has no effect on feed conversion ratio of broilers throughout the experimental period.

3.4. Blood hormones

Results of the effect of stocking density on cortisol and thyroid hormones (T_3 and T_4) in Sasso broilers were shown in table (6). Blood corticosterone concentration always been widely used as a detect for environmental stress in broilers and in this study stocking density did not cause a recognizable trend in cortisol concentrations, also no significant change in T_3 and T_4over all stocking densities.

Table 5 Effect of stocking density on feed conversion ratio of Sasso broilers.

Age	Feed conversion ratio (FCR)			
	G1	G2	G3	P-value
4th week	1.52±0.16	2.10±0.45	1.93±0.39	0.51
5th week	2.70±0.43	3.78±1.55	3.25±0.85	0.77
6th week	2.35±0.31	4.00±2.07	2.82±0.48	0.63
7rd week	3.70±0.97	6.06±2.12	2.58±0.52	0.21
8th week	3.34±0.54	10.26±4.42	8.38±4.60	0.40

Least Square Means (± SE) with different superscript letters in the same row are significantly different at P≤0.05. G1 was 10 birds/m^2, G2 was 13 birds/m^2, and G3 was 15 birds/m^2.

Table 6 Effect of stocking density on hormones of Sasso broilers.

	Hormone type	Hormone level(μg/dL)			
		G1	G2	G3	P-value
At 30 days	cortisol	0.20±0.00	0.28±0.08	0.25±0.04	0.57
	T3	1.80±0.50	2.00±0.63	2.04±0.49	0.94
	T4	0.82±0.13	1.51±0.23	1.12±0.29	0.19
At 45 days	cortisol	0.29±0.05	0.29±0.04	0.35±0.07	0.69
	T3	3.56±0.08	4.06±0.37	3.18±0.40	0.23
	T4	1.62±0.32	2.30±0.35	1.63±0.63	0.52
At 60 days	Cortisol	0.44±0.14	0.38±0.09	0.42±0.10	0.92
	T3	1.70±0.22	1.47±0.53	2.92±0.96	0.31
	T4	1.94±0.65	1.00±0.07	1.55±0.42	0.40

Least Square Means (± SE) with different superscript letters in the same row are significantly different at P≤0.05. G1 was 10 birds/m^2, G2 was 13 birds/m^2, and G3 was 15 birds/m^2.

3.5. Carcass parameters

Results of the effect of stocking density on slaughter weight, carcass weight, and carcass quality of Sasso broilers slaughtered and dressed on the final day of the rearing period (60thday) were shown in table (7). there was no significant effect of stocking density on carcass weight and fat percentage in body (P>0.05) but there was a significant effect of different stocking densities on organs weight (p< 0.05).

3.6. Behavioral patterns

The effect of stocking density on behaviors of Sasso broilers were shown in the table (8).Stoking density has no effect on ingestive behavior of broiler (P>0.05), also their were non significance in resting or grooming behaviors. While has high significance on comfort behavior, as stretching of leg and wing either during standing was (p< 0.001). or during resting was (p< 0.05).

Table 7 Effect of stocking density on carcass parameter of Sasso broilers.

Carcass parameters	Stocking density			
	G1	G2	G3	P-value
Live body weight(g)	2896.66±102.68[a]	2700.0±120.55[ab]	2395.0±123.79[b]	.05
Carcass weight(g)	2230.0±59.23	2063.33±117.34	1950.0±91.69	.18
Organs %	16.95±1.51[a]	17.56±0.57[a]	13.02±0.67[b]	.03
Fat %	16.73±0.21	17.21±0.69	16.56±1.06	.82

Least Square Means (± SE) with different superscript letters in the same row are significantly different at P≤0.05. G1 was 10 birds/m^2, G2 was 13 birds/m^2, and G3 was 15 birds/m^2.

Table 8 Effect of stocking density on behavioral pattern of Sasso broilers.

Behaviors	Stocking density			
	G1	G2	G3	P-value
Feeding	1.53±0.04	1.44±0.05	1.54±0.04	0.29
Drinking	1.36±0.04	1.34±0.04	1.42±0.05	0.43
Breast Rest	2.51±0.04	2.50±0.04	2.53±0.04	0.86
Lateral Rest	1.45±0.12	1.41±0.17	1.42±0.15	0.93
Lateral leg and wing stretch	1.26±0.08[a]	1.00±0.10[b]	1.21±0.08[ab]	0.05
Leg and wing stretch	1.31±0.05[a]	1.35±0.05[a]	1.14±0.05[b]	<0.001
Sleep	1.69±0.08	1.68±0.06	1.61±0.07	0.93
Preening	2.07±0.08	1.98±0.08	2.04±0.07	0.64
Wing flap	1.46±0.10	1.59±0.11	1.64±0.12	0.63
Aggression	1.48±0.19	1.00±0.30	1.50±0.21	0.13
Dust path	3.64±0.52	3.40±0.87	2.38±0.78	0.70
Body shake	1.00±0.04	1.11±0.05	1.09±0.04	0.22

Least Square Means (± SE) with different superscript letters in the same row are significantly different at P≤0.05. G1 was 10 birds/m^2, G2 was 13 birds/m^2, and G3 was 15 birds/m^2.

4. DISCUSSION

Regarding the live body weight, during the rearing phase, there was a highly significant effect of stocking density on final body weight of Sasso broilers. This result may be explained by increasing number of birds/m^2, they faced difficulties to reach feeders and drinkers that lead to decrease in feed intake and body weight.

The current results are in the same line with Skrbic et al., (2009) who observed negative effect of high stocking densities on final body mass of broiler chickens and Uzum and Toplu., (2013) who found that the final body weight of broilers reared at 18 broilers/m^{2}was 219 gram lower than those reared at 12 broilers/m^2 (P<0.001). there was notable decreases in body weight at densities of 15, 19 and 23 birds/m^2 (Velo and Ceular, 2017). Increasing stocking density from 14 to 18 birds/m^2 result in decreasing cumulative BW and feed consumption by 3.6 and 3.2%, respectively (Dozier et al., 2006).

In contrast, Skrbic et al, (2009) who established insignificance results in final body mass of broilers in stocking densities of 10 and 13 bird/m^{2}per floor surface and Benyi et al, (2015) who reported that stocking density influenced feed consumption with a progressive reduction in feed consumption with density but had no effect on 35day body weight, body weight gain, feed conversion ratio or mortality rate. Also Rambau et al., (2016) showed that the broilers reared at 35 and 40 kg BW/m^2 despite of reduction floor space and decreasing feed intake they were able to have similar body weights in entire study periods and reach equal slaughter weights as birds reared at 30 kg BW/m2.

Concerning feed consumption there was a highly significant effect of stocking density on Sasso feed consumption. Decreasing of feed consumption with increasing stocking density was probably the result of decrease feeding space on feeders. The current results are in the same line with Dozier et al. (2006) found that feed intake did not affected at first 15 days of study but by the time there was adverse effect of increasing stocking density on feed consumption. Also Rambau et al. (2016) found that birds reduced their feed intake as stocking density increased. Also Benyi et al. (2015) reported that there was a progressive reduction in feed intake with increasing stocking density and that similar result to Uzum and Toplu. (2013), who said that increasing stocking density affect negatively on feed consumption.

Feed conversion ratio (FCR)

Stocking density has no effect on feed conversion ratio of Sasso broilers at the finisher and during entire experimental period. The current results are in the same line with Rambau et al. (2016) who observed that insignificant effects of high stocking density on 42-day feed conversion ratio, and mortality rate during the finisher and entire study period. Uzum and Toplu. (2013) reported that feed conversion ratio was unaffected by stocking density.

Furthermore, the current findings contradict those of Nahashon et al. (2009), who reported that at two, three, and six week of age there were no difference in feed conversion ratio in birds reared in 13.6, 12, and 10.7 birds\m^2 but there were significantly lower than those birds reared at 15.6 birds/m^2. Also Cengiz et al. (2015) found that the feed consumption, feed conversion ratio, and weight gain were negatively affected in stocking density 20 birds/m^2 but not affect at 10 or 16birds/m^2.

Regarding blood hormones changes, stocking density is one of the stress factors that affects serum hormones parameters. Cortisol and thyroid hormones (T_3 and T_4) are a sensitive indicators of stress condition. In this study stocking density did not cause a recognizable trend in cortisol concentrations. This result may be explained by rearing birds at 13 and 15 (birds/m^2) stocking density not causing stress condition on birds that due to birds in these stocking densities did not were over weighted as in 10 birds/m^2 sticking density that allowed air exchange and heat dissipation between birds, so they avoided from heat stress, specially this study applied in winter.

The current results support Tabeekh. (2016) who reported that stocking density had no significant effect (P>0.05) on serum cortisol in different densities, 12, 15,and 18 birds/m2. Also Dozier et al. (2006) revealed that corticosteroid levels not significantly affected by stocking density and that agreed with (Uzum and Toplu, 2013; Tabeekh, 2016). stocking density(20, 25, 30, 35, 40, 45, 50, and 55 kg/m2) did not result in a recognizable trend in corticosterone concentrations, according to (Thaxton et al., 2006), that agreed with Dozier et al. (2006), who reported that stocking density (30 to 45 kg of BW/m2) did not influence plasma corticosterone.

Thyroid hormones in circulation T_3 and $T_4$are effective growth promoters in broilers and they play a role in both growth inhibition and compensatory growth acceleration (Tabeekh., 2016). Stocking density did not affect cortisol concentrations during and at ending of rearing cycle. The significance was (P>0.05) over all densities. Also thyroid hormones concentrations (T_3 and T_4) in blood plasma were not significantly affected by different stocking densities12, 15,and 18 birds/m2 according to (Tabeekh, 2016).

About carcass parameter, there was no significant effect of stocking density on carcass weight and fat percentage in body, but organs weights were significantly different between groups. The current results agreed with Cengiz et al. (2015), who reported that the overall relative carcass yield not affected significantly by different stocking densities. Also Uzum and Toplu (2013) reported that abdominal fat not affected by stocking density, The amount of abdominal fat not influenced by high stocking density (Dozier et al., 2006).

In contrast, Rearing broilers in lower stocking density provides more intensive growth and higher absolute yield of processed carcass (Skrbic et al., 2009). Furthermore Dozier et al. (2006) reported that Carcass weight decreased linearly when stocking density increased, that also disagree with Zuowei et al. (2011), who found that stocking density had a significant influence on the abdominal fat content.

Relating to behavioral patterns, stoking density has no effect on ingestive, resting, and preening behaviors of broilers. While has high significance on comfort behaviors as Leg, wing stretch(P<0.001). The current result agreed with Abo. Alqassem et al. (2018), who reported that there were no significant differences between stocking density 12, 15, and 20 birds /m^2 in ingestive and preening behaviors, but there were significant differences in comfort behaviors specially leg and wing stretch. There was low aggression level in different stocking density 5, 10,15, and 20 birds /m^2, and non-significance in percent of eating and drinking behavior (P>0.05)(Thomas et al., 2011). Furthermore Ventura et al. (2012) revealed that different stocking density 8, 13, and 18 birds /m^2 did not affect feeding, drinking, preening, or aggressive behaviors.

In contrast, stocking density 12, 15, and 20 birds /m^2 had significant effect on grooming behaviors (Ventura et al., 2012; Abo. Alqassem et al., 2018).

5. CONCLUSION

It is concluded that stocking density 10 birds /m^2 has significant effect on the final body weight, feed intake, and organs weight, on the other hand it has not negative effect on feed conversion rate, cortisol, thyroid hormones, and fat percentage. Furthermore, it did not affect negatively on feeding, drinking, resting, and grooming behaviors of broilers, but has significance on comfort behaviors as leg, wing stretch

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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