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Effect of stocking density on behavior, performance and some blood parameters of Muscovy ducks

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Abstract

The aim of this experiment was to examine the effect of different stocking densities on behavior, performance, and some blood parameters of Muscovy ducks. One hundred thirty-five-1-day old male Muscovy ducklings were divided into 3 treatments with 5 replicates per treatment for a 60-day trial. Birds were housed at 6 birds per m^2 (Control), 9 birds per m^2 (Moderate stocking density) or 12 birds per m^2 (High stocking density). The stocking density effects on duckling's behavior, performance, and some blood parameters were investigated. Instantaneous scan sampling technique was used in measuring behavior. At the end of the experiment, production and blood parameters were examined. The results showed that, stocking density birds exhibited less feeding, crouching, standing, walking and feather ruffling but more drinking, huddling, sitting, preening, feather pecking and wall pecking behaviors compared to control ducklings ($P < 0.05$). The stocking density ducklings also had lower body weight, feed intake, body weight gain, eviscerated weight, and dressing % compared to control birds ($P < 0.05$). At day 60, the serum calcium, phosphorus, total protein, thyroxine (T4) and total antioxidant of stocking density birds were lower, however, malondialdehyde and corticosterone were higher than that of control ducklings ($P < 0.05$); but there were no effects on globulin, albumin/globulin ratio, T3/T4 ratio, yawning behavior and spleen weights ($P > 0.05$). T3 and total albumin showed significant decrease only in high stocking density compared to control one ($P < 0.05$). These findings propose that rearing Muscovy ducks at 9 or 12 birds per m^2 during fattening phase led to harmful influence on duck behaviors, performance, carcass characteristics and physiological parameters, with the potential to rear six birds on one square meter.

Keywords: Stocking density, Muscovy ducks, behavior, production, blood parameters

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Introduction

Poultry production and welfare are affected by high stocking density. By rising the bird number per m^2, high economic returns can be gained, but the economic revenue may lead to impaired health and welfare of birds if the stocking density is increased (Xie et al., 2014). Previous reports have reviewed the effect of high stocking density on duck and these unfavorable consequences involved, linear reduction in feed consumption by birds (Puron et al., 1995), increase prevalence of leg injuries and limit the birds' ability for walk (De Buisonjé, 1999), reduction in egg production, fearfulness, cannibalism and feather pecking and high mortality (Keeling and Gonyou, 2001).

Nowadays, rearing of Muscovy ducks in indoor bird houses like broiler chickens has replaced the open water outdoors and conventional free range. Rearing of Muscovy ducks in indoor bird houses is completely different from ducks in open water outdoors in using different types of solid floors (i.e., wire floor or wood shaving) or water cups and nipples. Consequently, using high stocking densities in duck houses in this condition leads to many problems compared with rearing ducks in conventional free-range systems (Krunt et al., 2022).

Heckert et al. (2002) reported that, lowering floor space of broiler chickens significantly decreased the size of spleen and weight of bursa of Fabricius. Furthermore, Appleby et al., (2004) summarized that, rearing ducks in high stocking density leads to an increase in the feather pecking that occasionally leads to destruction of the skin and then cannibalism if the ducklings with such injuries are left with others. At the same time, Onbasilar et al., (2008) found that, lowering floor space in broiler chickens related to reduce feed intake, carcass traits and dressing percentage. Hypothalamic-pituitary-adrenal (HPA) axis stimulation is one of the essential physiological mechanisms related with stressors. Hypothalamus secretes arginine vasotocin and corticotropin releasing factor and pituitary gland secrete adrenocorticotropic hormone and then adrenals secrete glucocorticoids, especially corticosterone as a result of the stimulation of hypothalamic-pituitary-adrenal axis (Canoine et al., 2002).

The response to oxidative stress can be utilized as welfare parameters in birds. Methods for evaluating oxidative stress include the determination of the total antioxidant capacity of plasma or serum (Guan et al., 2004). Natural protective substances deficiencies result of stress may lead to oxidative stress, defined as an imbalance between oxidants and antioxidants levels (Finkel and Holbrook 2000).

Also, the circulating triiodothyronine (T3) hormone concentration appears to be correlated with feed intake and temperature as tri-iodothyronine effects much more optimal at condition of the bird, that resulted in higher growth rate (Yahav 2000 and Decuypere and Buyse 2005).

Rearing of Pekin ducks at a floor space of 8 ducklings per m^2 leads to feather damage than ducklings reared at floor space of 5, 6, or 7 per m^2. Moreover, feather-pecking was not detected in Muscovy ducks reared at floor space 6.3 ducklings/m^2, however sever injuries were detected in birds reared at floor space 11.6 ducklings/m^2 (Rodenburg et al., 2005).

Modern Muscovy ducks and broilers have been reared continuously for quick

growth rate, higher carcass yield, good feed conversion ratio and maximum growth rate efficiency over 6–8 weeks. Consequently, these parameters may be affected by reducing the stocking density of modern ducks (Hartcher et al., 2020). However, the data on the stocking density has not been refreshed; this study was designed in a small scale to examine the effects of different stocking densities on behavior, performance, and some blood parameters of Muscovy ducks.

Materials and Methods

Animals and housing

All steps were confirmed by the Animal Care and Use the Ethical Committee, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, according to the OIE standards under the No. 06/2023/0065.

One hundred thirty-five-1-day-old male Muscovy ducklings (French group Inc., Cairo, Egypt) were divided into 3 treatments with 5 pens (110 cm × 110 cm per pen) with similar average body weight in a temperature monitored room at the behavior and management of animal, poultry and aquatics research unit of Faculty of Veterinary Medicine, Assiut University for a 60-day trial. Birds were housed at 6 birds per m^2 (Control), 9 birds per m^2 (Moderate stocking density) or 12 birds per m^2 (High stocking density). Management of the ducklings was done according to the guidelines of Philippine recommendations for duck rearing (PCARRD, DOST LDC Technical Bulletin on Duck Raising). Ducklings were fed from one day to 45 days old on starter diet with 21-24% crude protein and from 45 day to 60 days old on grower diet with 16% crude protein, the diet was provided *ad libitum* (Table 1). The ducklings were reared at 34°C at day 1 with a progressive decrease to 27°C on day 14 till 60 days. The light program was fixed at 30 lx as continuous lighting program; the experimental compartment was equipped with 15 plastic feeders and 15 plastic drinkers. Fresh and dry wood shaving was used as bedding at a depth of 10 cm. This study was performed in strict accordance with the relevant guidelines and ethical regulations of Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt (no. 17300893).

Table 1. Components of the basal diet.

Physical composition	Starter%	Grower%
Yellow corn	38.67	51.75
Soya bean meal	36	18.35
Wheat bran	15	19.6
Sunflower oil	7.25	7.00
Sodium phosphate dibasic	1.12	1.3
Lime stone, Ground	1.42	1.40
Common salt	0.3	0.3
Methionine	0.04	0.00
Premix	0.3	0.3
Total	100	100
Chemical composition		
Cp (%)	22.05	16.02
ME (mcal/kg)	2908	2999.6
Ca (%)	0.65	0.6

TP (%)	**0.4**	**0.3**
CF (%)	**4.87**	**4.51**

Behavior

Number of 5 ducklings per replicate (25 total per treatment) were selected for behavioral data. The birds were painted with livestock spray marker on their wings (Livestock green sharp-mark spray paint marker, Cotran Corporation, Portsmouth, RI). Behavioral recording was performed two times per day from 09:00 to 10:00 and 15:00 to 16:00, three times per week (Sunday—Tuesday) from day 14 till the end of the rearing period. The observation was performed using an ethogram (Table 2), by instantaneous scan sampling (Altmann, 1974; Engel, 1996). The data was presented as a proportion of recorded behaviors out of the total behaviors possible (Kristensen et al., 2007).

Performance

Body weight, body weight gain, feed intake and feed conversion ratio were estimated at day 60 (Mohammed et al., 2018).

Carcass characteristics and weight of some internal organs

At the termination of the rearing period, 5 ducklings from each treatment (1 bird per replicate) were chosen randomly. Ducklings were killed by cutting off the carotid artery and jugular veins. After bleeding, each duckling was put in a hot water container to remove the feathers. After the separation of head, evisceration of carcass was done to estimate some carcass traits including dressing % and internal organs weight % (gizzard, liver, spleen, proventriculus and heart).

Dressing % was measured as following:

$$\textbf{Dressing \%} = \frac{\textbf{Eviscerated carcass weight + giblets (heart, empty gizzard and liver) weight x100}}{\textbf{Live body weight at slaughter}}$$

Internal organs weights were calculated as relative weight proportionate to pre-slaughter live body weight**.**

Blood sampling

Before determining the carcass characteristics and during the bird-sanguinary, five ml of blood samples from each duckling were taken in test tube without anticoagulant to estimate the chemical blood parameters and hormones (1 bird / replicate). The tubes were left at room temperature for half an hour then put in a refrigerator for an hour and then centrifuged at 3000 r.p.m for 10 minutes. Another Eppendorf's tube was used to put the separated serum using micropipette. The Eppendorf's tubes were kept at –20ºC, until analysis.

Blood parameters

Serum total proteins, albumin, inorganic phosphorus, and calcium were estimated by Digital spectrophotometer (Cecil instrument, Cambridge, England). Serum total proteins and albumin (g/ dl): were estimated by using a commercial kit manufactured by Egyptian company for biotechnology, Cairo, Egypt. Serum globulin (g/dl) = Serum total protein (g/dl) – Serum Albumin (g/dl) (Nadia, 2003). Albumin/globulin ratio = Serum albumin (g/dl) / Serum globulin (g/dl) (Nadia, 2003). Inorganic phosphorous (mg/dl), calcium (mg/dl), malondialdehyde and

total antioxidant capacity were estimated by using a commercial kit (Egyptian company for biotechnology, Cairo, Egypt).

Also, serum triiodothyronine (T3) and Thyroxin (T4) were estimated by using commercial ELISA kits (Bio Tina GmbH, Bugweg, Hagen, Germany). Serum corticosterone was assessed by Assay Max corticosterone ELISA kits (Assay Max, St. Charles. MO, USA).

Statistical analysis

The design of the study was performed in a randomized block design. Pen was used as an experimental unit (n = 5). Analysis of different stocking densities effects on Muscovy ducks' behavior, performance and some blood parameters were done by one way ANOVA. PROC MIXED model with SAS 9.4 software (SAS Institute Inc., Cary, NC) was utilized in analysis of the data means. Normality of the data was performed by the Shapiro-Wilk test. Comparison of means was done by Tukey-Kramer test ($P < 0.05$). Expression of the results is expressed as the mean±SE.

Table 2. Ethogram of Muscovy duck behaviors collected at different stocking densities

Behavior	Definition
Ingestive behavior	
Feeding	Pecking at feed on feed trough
Drinking	Obtaining water at the drinkers
Resting behavior	
Crouching	Lying or sitting with breast on the floor
Huddling	Three or more birds over lapped in a crouching position in an allelomimetic pattern
Movement activities	
Standing	The feet only are in contact with the ground
Walking	Taking at least two successive steps
Sitting	The ventral part of the bird is in contact with the ground
Comfort behavior	
Feather ruffling	Fluffing the feathers
Shaking	Shaking the body
Yawning	Opening the mouth without panting
Preening	Manipulating its own feathers with beak gently
Non aggressive pecking behavior	
Feather pecking	Pecking acts directed to another birds
Wall pecking	Pecking at inedible objects like the wall

Results

Behavioral observations

The overall relevance between the different stocking densities and behavioral patterns are shown in Table 3. Birds in the stocking density groups spent less time in feeding, crouching, standing, walking, and feather ruffling, but more time in huddling, sitting, feather pecking, wall pecking and preening ($P < 0.05$). There was no difference between the control and the moderate stocking density in the drinking and shaking behaviors ($P > 0.05$). The difference was disappeared between all the groups in the yawning behavior ($P > 0.05$). There was no difference between the moderate and the high stocking densities in feeding, standing, sitting, walking, feather ruffling, shaking and feather pecking ($P > 0.05$).

Table 3. Effect of different stocking densities on behaviors of Muscovy ducks.

Treatment	Control	Moderate stocking density	High stocking density
Ingestive behavior			
Feeding(%)	15.55 ± 1.89 [a]	8.45 ± 1.13 [b]	4.77 ± 1.4 [b]
Drinking(%)	5.46 ± 0.73 [b]	7.17 ± 0.76 [b]	14.63 ± 0.48 [a]
Resting behavior			
Crouching(%)	11.94 ± 3.79[a]	8.47 ± 1.12[b]	7.36 ± 1.41[c]
Huddling(%)	7.36 ± 1.01 [c]	10..28 ± 3.91 [b]	14.67 ± 1.04 [a]
Movement activities			
Standing(%)	62.87 ± 1.73 [a]	37.07± 1.87[b]	41.14 ± 1.21 [b]
Walking(%)	8.35 ± 1.2 [a]	3.71 ± 1.63 [b]	2.13± 1.3 [b]
Sitting(%)	37.13 ± 1.78 [b]	62.93 ± 1.88 [a]	58.86 ± 1.5 [a]
Comfort behavior			
Feather ruffling(%)	7.22 ± 0.68 [a]	4.88 ± 0.67 [b]	5.14 ± 0.61 [b]
Shaking(%)	13.72 ± 1.04 [a]	12.34 ± 1.01 [a b]	10.55 ± 1.05 [b]
Yawning(%)	4.59± 0.68	5.98 ± 0.52	5.21 ± 0.69
Preening(%)	9.74 ± 1.06[c]	14.45 ± 1.11[b]	17.78± 1.03[a]
Non aggressive pecking behavior			
Feather pecking(%)	2.78 ± 0.4 [b]	10.82 ± 0.7 [a]	11.39 ± 0.8 [a]
Wall pecking(%)	4.47 ± 0.61 [c]	6.67± 0.42 [b]	9.49± 0.61[a]

[a,b,c]Mean ± SE with different superscripts in the same row differ ($P < 0.05$). (n=5 per treatment; and the data were collected from 25 birds/treatment; 5 birds/pen x 5 pens/treatment).
Control, birds were housed at 6 birds per m^2, 9 birds per m^2 (Moderate stocking density) or 12 birds per m^2 (High stocking density).

Production performance, weight of some internal organs and carcass characteristics

The overall relationships between the different stocking densities and production performance, carcass characteristics and weight of some internal organs are shown in Table 4. The Muscovy ducks reared under high and moderate stocking densities had lower feed intake, body weight, body weight gain, eviscerated weights, and dressing % in comparison with controls ($P < 0.05$), the birds reared in the high stocking density are the lowest in the previously mentioned parameters except in eviscerated weights. The differences were disappeared between the moderate and high stocking densities in the feed conversion ratio ($P > 0.05$). Regarding the weights of the internal organs, the weights of heart, liver, proventriculus were reduced in the birds housed in the high stocking density compared with the control and the moderate stocking density ones ($P < 0.05$), the differences were disappeared between all the treatments in the weights of the spleen ($P > 0.05$).

Table 4. Effect of different stocking densities on production performance, carcass characteristics and weight of some internal organs of Muscovy ducks at the end of the experiment (day 60).

Treatment	Control	Moderate stocking density	High stocking density
Production performance			
Body weight (gm)	5549 ± 236.8 [a]	4421 ± 277 [b]	2439 ± 192.8 [c]
Body weight gain (gm)	5499± 151.94 [a]	4375 ± 151.94 [b]	2389 ± 151.94 [c]
Feed intake (gm)	15099± 114.1 [a]	12175 ± 114.10 [b]	11389 ± 114.10 [c]
Feed conversion ratio	3.32 ± 0.71[b]	3.95 ± 0.97[a]	4.05 ± 0.83[a]
Carcass characteristics			
Eviscerated weights (gm)	4583 ± 159.6 [a]	3503 ± 150.4[b]	2011 ± 111.2[c]

Dressing %	**86.36 ± 4.66 [a]**	**82.12 ± 2.96 [b]**	**80.34 ± 5.12 [b]**
Weights of internal organs			
Heart (gm)	**37.19 ± 3.12[a]**	**29.57 ± 2.87[a]**	**19.35 ± 2.17[b]**
Liver (gm)	**63.16 ± 2.3[a]**	**57.23 ± 0.9[a]**	**34.90 ± 1.6[b]**
Spleen (gm)	**2.50 ± 0.8**	**2.10 ± 0.7**	**1.50 ± 0.9**
Proventriculus (gm)	**12.41 ± 0.99 [a]**	**13.81 ± 1.41 [a]**	**7.79 ± 0.93 [b]**
Gizzard (gm)	**123.48 ± 4.36[a]**	**117.20 ± 2.99[b]**	**58.80 ± 1.82[c]**

[a,b,c]Mean ± SE with different superscripts in the same row differ (*P* < 0.05). (n=5 per treatment; and the data (Carcass characteristics and internal organs weights) were collected from 5 birds/treatment; 1 birds/pen x 5 pens/treatment).
Control, birds were housed at 6 birds per m^2, 9 birds per m^2 (Moderate stocking density) or 12 birds per m^2 (High stocking density).

Chemical blood parameters

The overall relationships between the different stocking densities and the chemical blood parameters are presented in Table 5. The Muscovy ducks housed under high and moderate stocking densities had lower total proteins compared to controls, but the total albumin was reduced in the high stocking density group in comparison with the moderate stocking density and the control groups (P < 0.05). The differences were disappeared between all the groups in the total globulin and albumin/globulin ratio (P > 0.05). The serum calcium and phosphorus concentrations were reduced in the stocking density groups in comparison with controls, the high stocking density group had the highest decrease (P < 0.05). The calcium/phosphorus ratio was reduced in the high stocking density group in comparison with the control one but not the moderate stocking density group.

Table 5. Effect of different stocking densities on chemical blood parameters of Muscovy ducks at the end of the experiment (day 60).

Treatment	Control	Moderate stocking density	High stocking density
Total proteins (g/dl)	**3.90 ± 0.2[a]**	**3.26 ± 0.12[b]**	**2.8 ± 0.11[b]**
Albumin (g/dl)	**2.22 ± 0.6[a]**	**1.98 ± 0.63[a]**	**1.52 ± 0.97[b]**
Globulin (g/dl)	**1.68 ± 0.15**	**1.28 ± 0.16**	**1.28 ± 0.12**
Albumin/Globulin ratio	**1.32 ± 0.24**	**1.54 ± 0.29**	**1.18 ± 0.25**
Calcium (mg/dl)	**11.44 ± 0.28[a]**	**8.90 ± 0.27[b]**	**7.40 ± 0.12[c]**
Phosphorus (mg/dl)	**7.70 ± 0.15[a]**	**6.36 ± 0.13[b]**	**5.52 ± 0.1[c]**
Calcium/Phosphorus ratio	**1.48 ± 0.034[a]**	**1.39 ± 0.029[ab]**	**1.34 ± 0.038[b]**

[a,b,c]Mean ± SE with different superscripts in the same row differ (*P* < 0.05). (n=5 per treatment; and the data were collected from 5 birds/treatment; 1 birds/pen x 5 pens/treatment).
Control, birds were housed at 6 birds per m^2, 9 birds per m^2 (Moderate stocking density) or 12 birds per m^2 (High stocking density).

Serum T3, T4 and corticosterone

The overall relationships between the different stocking densities and the serum hormones are presented in Table 6. The serum concentration of T3 was reduced in the high stocking density group in comparison with the control one (P < 0.05) but not the moderate stocking density group (P > 0.05).

The serum level of T4 was reduced in the stocking density groups in comparison with the control; the high stocking density group had the highest decrease (P < 0.05). The differences were disappeared between all the treatments in the T3/T4 ratio (P >

0.05). On the other hand, the serum corticosterone levels were increased in the stocking density groups compared to the controls; the high stocking density group had the highest increase (P < 0.05).

Table 6. Effect of different stocking densities on serum hormones of Muscovy ducks at the end of the experiment (day 60).

Treatment	Control	Moderate stocking density	High stocking density
T3 (nmol/L)	1.88 ± 0.2a	1.54 ± 0.25ab	1.24 ± 0.21b
T4 (nmol/L)	23.36 ± 0.18a	20.74 ± 0.15b	16.84 ± 0.15c
T3/T4 ratio	.080 ± 0.0070	0.074 ± 0.007	.073 ± 0.0130
Corticosterone (ng/ml)	10.12 ± 0.19c	13.52 ± 0.11b	22.16 ± 0.13a

[a,b,c]Mean ± SE with different superscripts in the same row differ (*P* < 0.05). (n=5 per treatment; and the data were collected from 5 birds/treatment; 1 birds/pen x 5 pens/treatment).
Control, birds were housed at 6 birds per m^2, 9 birds per m^2 (Moderate stocking density) or 12 birds per m^2 (High stocking density).

Antioxidant Activity

The overall relationships between the different stocking densities and the antioxidant activity are presented in Table 7. The serum concentration of malondialdehyde was increased in the stocking density groups compared to controls; however, the serum concentration of total antioxidant was decreased in the stocking density groups compared to controls (P < 0.05). The differences were disappeared between the high and the moderate stocking density groups (P > 0.05).

Table 7. Effect of different stocking densities on antioxidant activity of Muscovy ducks at the end of the experiment (day 60).

Treatment	Control	Moderate stocking density	High stocking density
Malondialdehyde (nmol/mL)	10.20 ± 1.47[b]	17.20 ± 1.35[a]	22 ± 1.41[a]
Total antioxidant (Mm/L)	0.07 ± 0.006[a]	0.02 ± 0.003[b]	0.01 ± 0.007[b]

[a,b]Mean ± SE with different superscripts in the same row differ (*P* < 0.05). (n=5 per treatment; and the data were collected from 5 birds/treatment; 1 birds/pen x 5 pens/treatment).
Control, birds were housed at 6 birds per m^2, 9 birds per m^2 (Moderate stocking density) or 12 birds per m^2 (High stocking density).

Discussion

Stocking density is a serious part of any rearing system, and it is crucial for duck welfare and health. Increasing the duckling's number per m^2 leads to higher economic returns, but the health and welfare of the birds may be affected if the increasing in densities is excessively elevated (Bai et al., 2020). The current findings indicate that, stocking density birds exhibited less feeding, crouching, standing, walking and feather ruffling but more drinking, huddling, sitting, feather pecking, wall pecking, and preening behaviors compared to control birds.

Ingestive behavior (feeding and drinking) results were in consistent with the general trend observed by Feddes et al., (2002); Minka et al. (2007); Mtileni et al. (2007); Onbasilar et al. (2008) and Huque

et al., (2011) and could be attributed to less feeder space as well as the birds cannot do the normal postural adjustments to reach the feeder (Thomas et al., 2004), but the increase in the drinking behavior in the high stocking density group in comparison with the other treatments could be returned to heat stress accompanied to high stocking density (Lucas and Rostango, 2013).

The finding of huddling behavior agreed with that of Singh and Sharma (2003) and Beg et al., (2011). It may be attributed to the fact that birds reared in high stocking densities are more fearful compared with that reared in low stocking densities so that large group size increases the resting behavior including the crouching and huddling behaviors with consequent high fear level among birds.

Walking behavior findings were in accordance with the result of Rodenburg et al. (2005) and Saleque (2010). It may be related to leg and foot problems due to bad management of litter floors that comes from rearing of ducks at high stocking densities which leads to difficulty in standing and walking behaviors and increase in sitting behavior. Furthermore, these results in consistent with the general trend of Joy (2005) who indicated that, wetter nature of duck feces compared to that of broilers and turkeys makes floor litter more slippery and affects the balance of ducks, this leads to leg disorders such as splay leg (Raud and Faure, 1994).

Regarding the comfort behavior, similar results were obtained by Albentosa and Cooper (2004); Defra (2004); Elwinger et al. (2004); Decuypere and Buyse (2005) and U.S. fish and waterfowl (2013). Improvement in the comfort behavior in the control group in comparison with stocking density groups could be related to the decrease in the discomfort associated with high stocking density. In addition, feather ruffling and shaking were noticed more in small stocking densities than in large ones as the lower stocking densities had smaller ultimate space than the higher ones to perform these behaviors (Decuypere and Kuhn, 1984).

The increase in the preening behavior may be related to the need to clean the body as increasing the stocking density leads to higher levels of wastes which boosts the incidence of skin problems and foot pad dermatitis (Bilgili et al., 2009).

Pekin ducks reared at a small space area have reportedly been found to experience more feather damage than ducklings reared at lower density. At the same time, feather-pecking was not detected in Muscovy ducks reared at low densities but sever injuries were found in those reared at high densities. The results of the feather pecking were in accordance with that of Appleby et al. (2004); Rodenburg et al. (2005); Gustafson et al. (2007a, b) and Riber and Mench (2008). On the contrary, Stanislaus (2006) found that, high incidence of feather pecking occurred in the small flock density. A potential explanation our results could be return to the reduction in the light intensity at the floor level which could in turn encourage the attractiveness of feathers as a pecking stimulus (Kjaer and Vestergaard, 1999). Another potential explanation may be due to these ducklings were trying to shape a social hierarchy but recognizing and remembering individuals was difficult (Campderrich et al., 2017).

From the previously mentioned findings it could be said that the high stocking density leaded to increased wall pecking. This finding was in consistent with Joy (2005); Rodenburg et al. (2005)

and Beg et al. (2011). These results could be attributed to less space and immovability that force the birds to be close to the walls and increase preference to special portions of the house such as walls and corners (Decuypere and Buyse, 2005).

Managerial stressors, including stocking density, are responsible for impaired health and welfare and linked with unfavorable meat quality and carcass characteristics including eviscerated carcass weight percentage and dressing percentage (Fouad et al., 2016). Under high stocking densities, ducks reduce their feed consumption and activities (Onbasilar et al., 2008). Consequently, metabolism is declined and reduced body weight, feed intake, and impaired feed conversion ratios are monitored (Onbasilar et al., 2008). Our findings indicated that body weight, feed intake, body weight gain, eviscerated weight, dressing % were decreased in stocking density groups compared to control ones.

Similar findings were obtained by Puron et al. (1995); de Buisonje (1999) and Onbasilar et al. (2008) and could be related to the decrease in both the feed consumption and the true assimilation of amino acids and protein (Onbasilar et al., 2008).

Regarding to the findings of the feed consumption and the feed conversion ratio, it could be elucidated as high stocking density leads to an increase in the environmental temperature around birds that stimulate the peripheral thermal receptors to transfer suppressive nerve impulse to the appetite center in the hypothalamus causing a reduction in the body weight, feed intake and feed conversion (Kusnadi et al., 2008).

Concerning the findings of carcass characteristics (body weight at slaughtering time, eviscerated body weight) and edible giblets, these results may be related to a decrease in the feed intake and reduction in the digestibility and assimilation of nutrients (Al-Homidan and Robertson, 2007).

Reduction in the dressing percentage in the stocking density groups could be related to increased glucocorticoids that have catabolic effects, results in a decrease in the average of synthesis of amino acids and in turn muscle wasting and retardation of growth (Al-Homidan and Robertson, 2007).

Bone mineralization has a necessary function in preserving bone health and prohibiting leg problems (Mohammed et al., 2021). In the current study, serum calcium and phosphorus levels were reduced in stocking density groups compared to controls. Similar results were obtained by The World Conservation Union (IUCN) (2007). These results may be related to the increase in the calcium and phosphorus in urine and the elevation in osmolar excretion.

The current results indicated that the total proteins were reduced in the stocking density groups but the total albumin was decreased in the high stocking density group only in comparison with control group. These findings were in consistent with (Jewel et al., 2023) who reported that total protein and albumin contents were the high in the highest stocking density of Gulsa tengra (Mystus *cavasius*) catfish. These results may be returned to the reduction in the protein intake and consequently the deficiency of the essential amino acids because of reduced feed intake.

Thyroid hormones (T3 and T4) are crucial in controlling nutrient metabolism and thermoregulation, these hormones are influenced by internal and external stimuli, including managerial stressors i.e. stocking density (Garasto et al., 2017). In the present experiment, the serum concentration of T4 was reduced in the stocking density groups but the T3 was reduced in the high stocking density group only in comparison with the control groups.

These findings were like those of Decuypere and Buyse (2005); Shibata et al. (2007) and European Commission. (2013). These results may be attributed to lowering the concentration of thyroid releasing hormone (TRH) secretion from hypothalamus in high stocking density group, this decreases thyroid gland production to thyroid hormone (Shafaq et al., 2018). It was approved that; crowded ducks show a difficulty in loosing body heat to the environment, drink much and consume less feed and consequently growth rate as well as weight of body and lymphoid organs decrease, so synthesis and secretion of the active calorigenic hormone T3 and T4 are also reduced (Decuypere and Buyse, 2005).

In the present study, serum corticosterone concentration was increased in the stocking density groups in comparison with controls. A corresponding result were obtained by Puvadolpirod and Thaxton (2000); Heckert et al. (2002); Kusnadi et al. (2008) and Onbasilar et al. (2008).

Rearing ducks under stress activates the secretion of hypothalamus corticotropin-releasing hormone (CRH) and so adrenal adrenocorticotrophic hormone (ACTH) increased from pituitary gland that then stimulates the adrenal glands to synthesize and releases norepinephrine and glucocorticoids, primarily cortisol (Mohammed et al., 2021 and Federica et al., 2021).

When birds are reared under stress, such as high stocking density, excessive release of reactive oxygen species (ROS) happens, causing free radical deterioration and lipid peroxidation to proteins and consequently oxidative injury (Slimen et al., 2014). In the current study, serum malondialdehyde was increased; however, the total antioxidant was decreased in the stocking density groups compared to controls. These findings were in accordance with Ozturk and Gumuslu (2004); Seven et al. (2009) and McMillan and Beeching (2010).

These results could be attributed to the increase in the erythrocyte osmotic fragility and elevation in the level of thiobarbituric acid and other reacting substances which are products of lipid peroxidation in the erythrocyte membrane (Ozturk and Gumuslu, 2004).

Conclusion

In the current study, the findings indicated that, increasing the stocking density to 9 or 12 birds per m^2 reduced the feeding, crouching, standing, walking and feather ruffling but increased drinking, huddling, sitting, feather pecking, wall pecking and preening behaviors. It also reduced the performance parameters including feed intake, body weight gain, eviscerated weight, and dressing %. Furthermore, it lowered serum calcium, phosphorus, total protein, T4 and total antioxidant; however, it increased serum malondialdehyde and corticosterone. Overall, our findings elucidate that increasing the stocking density to 9 or 12 Muscovy duckling per m^2 leads to

deleterious effects on bird welfare and health.

Conflict of Interest

The authors do not have conflicts of interest to detect.

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