Original Research Article

Growth performance and physiological responses of helmeted guinea fowl (*Numida meleagris*) to different stocking densities in humid tropical environment

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Abstract

Commercialisation of helmeted guinea fowl production in Africa is still in its rudimentary stage and there is a dearth of information on the optimal stocking density of the birds. This study was conducted to evaluate the effect of stocking density on the physiological responses and performance of guinea fowl. A total of 240 indigenous guinea fowl were used for this study. The birds were weighed and assigned to different stocking densities of 14, 16, 18 and 20 birds/m² (SD1, SD2, SD3 and SD4, respectively) at four weeks of age. Feed and water were provided *ad libitum*. Data were collected on growth performance, haematochemical profile and plasma triiodothyronine of the birds. The results showed that from the 7th to the 13th week, birds stocked at 14 and 16 birds/m² were significantly (P < 0.05) heavier than birds stocked at 18 and 20 birds/m², while at weeks 14 and 15, body weight of the birds was in the order 14 birds/m² > 16 birds/m² > 18 birds/m² > 20 birds/m². Feed conversion ratios of the birds increased with stocking density. It was concluded that stocking density of helmeted guinea fowl higher than 16 birds/m² adversely affected growth performance and welfare of the birds.

Keywords: Production; physiology; welfare; guinea fowl; haematology; tropics

INTRODUCTION

Guinea fowl production in Nigeria is still in its vestigial stage despite the fact that these birds originated from Africa (Moreki and Radikara, 2013) and the ready market for guinea fowl in Africa (Nahashon et al., 2006). These birds are still raised with minimal input by subsistent farmer with low productivity (Oke et al., 2015). Whereas guinea fowl production as a meat bird is a potentially profitable enterprise in different parts of the world (Embury, 2001; Nahashon et al., 2006) including developing countries like Nigeria. It has been reported that guinea fowl has a high socio-economic value in the rural society where they are mainly kept to supply meat and eggs and as a source of income for the rural farmers (Schwanz, 1987). Most of the guinea fowl in Nigeria are reared extensively by peasant farmers at the back yard and few farmers are interested in rearing guinea fowl intensively. There is a lack of information on the optimum stocking density to be used for the birds and some farmers therefore adopt the stocking density of chickens for guinea fowl. Our earlier study (Oke et al., 2015) indicated that guinea fowl reared on deep litter performed better than those in cages. Optimized floor density would minimize overhead costs associated with maintenance of poultry houses and improve efficiency of feed utilization (Nahashon et al., 2011). The ultimate goal of poultry producers worldwide is to maximize kg of poultry produced per square metre of space while preventing production losses due to overcrowdings to achieve a satisfactory economic return (Abudabos et al., 2013).

Excessive stocking density may have adverse effect on the bird's performance (including body weight gain, feed intake, and feed conversion ratio), health, welfare and often a greater incidence of footpad dermatitis, scratches, bruising, poor feathering, and

Ingredient	0–4 weeks	5–8 weeks	9–16 weeks
Maize	540.00	500.00	473.00
Soybean meal	163.00	140.00	60.00
Fishmeal	60.00	20.00	10.00
Wheat offal	35.00	150.00	300.00
Groundnut cake	160.00	125.00	65.00
Bone meal	20.00	40.00	40.00
Limestone	15.00	18.00	45.00
Salt (NaCl)	2.50	2.50	2.50
*Premix	2.50	2.50	2.50
Methionine	1.00	1.00	1.00
Lysine	1.00	1.00	1.00
TOTAL	1000.00	1000.00	1000.00
Calculated Nutrient			
Metabolizable Energy(MJ/kg)	12.15	11.54	10.97
Crude Protein (%)	24.09	20.23	15.59
Crude Fat (%)	4.92	4.36	3.85
Crude Fibre (%)	3.48	4.03	4.47
Calcium (%)	1.24	1.79	2.70
Phosphorus (%)	0.70	0.87	0.77

*I kg contains: Vit A: 10,000,000 IU, Vit D3: 2,000,000 IU, Vit. E: 12,000 IU, Vit. K3: 2,000 mg, Vit. B1: 1,500 mg, Vit. B2: 5,000 mg, Vit. B6: 1500 mg, Vit. B12: 10,000 mg, Biotin: 20 mg, Niacin: 15,000 mg, Panthotenic Acid: 5,000 mg, Manganese: 75,000 mg, Zinc: 50,000 mg, Iron: 25,000 mg, Copper: 5,000 mg, Iodine: 1,000 mg, Selenium: 100 mg, Cobalt: 300 mg, Choline: 150,000 mg, Antioxidant: 125,000 mg

condemnations (Nahashon et al., 2009) and also on the immunity of the birds (Heckert et al., 2002).

Experimental site

It has been reported that higher stocking densities affect the welfare of birds through reduction of feeder space allocation, which may induce aggression, chronic stress, and even mortality (Thogerson et al., 2009). Adaptive changes in response to stressful conditions can be reflected by the plasma corticosterone, glucose concentration, cholesterol concentration in plasma, leukocyte number, etc. (Puvadolpirod and Thaxton, 2000). There is, however, a scarcity of data on the physiological response of guinea fowl to different stocking densities in the tropical environment. We hypothesised that stocking density would influence the growth and physiological responses of guinea fowl. The aim of the present study therefore was to investigate the effect of stocking density on physiological responses and performance of guinea fowl in a tropical environment.

MATERIALS AND METHODS

Ethical Approval

The research was conducted in accordance with the Institutional Animal Ethics Committee guidelines of the Federal University of Agriculture, Abeokuta, Nigeria. All birds used in the experiment were provided proper care and management without unnecessary discomfort. The experiment was carried out at the Poultry unit of the Teaching and Research Farms and the laboratory of the Department of Animal Physiology, Federal University of Agriculture, Abeokuta, Nigeria lying within latitude 7°10' N, longitude 3°2' E and altitude 76 m, located in the derived savannah zone of South-Western Nigeria having a prevailing tropical climate with a mean annual rainfall of 1,037 mm, annual mean temperature and relative humidity of 34 °C and 82%, respectively (Amujoyegbe et al., 2008).

Experimental Birds and General Procedure

A total of 240 indigenous guinea fowl keet were purchased from a reputable hatchery in Abeokuta, Nigeria and were raised using standard breeding and rearing techniques (Bell and Weaver, 2002). Wood shavings were used as the litter materials at depth of 10 cm. Feeder and drinker space was provided as described by Nahashon et al. (2011). The birds were wing-tagged, weighed and randomly assigned to four treatments (60 birds each) of stocking density at four weeks. The experiment was laid out as a Completely Randomized Design (CRD) consisting of four (4) stocking densities (14 birds/m² (SD1), 16 birds/m² (SD2), 18 birds/m² (SD3) and 20 birds/m² (SD4) having four replicates each of 15 birds. Feed and water were provided ad libitum. Composition of the experimental diets is shown in Table 1.

Week	SD 1	SD2	SD3	SD4	SEM	P value
5	184.0	189.0	171.0	178.0	4.34	0.5250
6	231.0	225.0	195.0	200.0	5.43	0.0568
7	292.0ª	278.0^{a}	244.0 ^b	246.0 ^b	5.54	0.0001
8	370.0ª	353.0ª	302.0 ^b	286.0 ^b	8.69	0.0001
9	435.0ª	424.0ª	354.0 ^b	324.0 ^b	11.81	0.0001
10	520.0 ^a	505.0ª	424.0 ^b	393.0 ^b	13.20	0.0001
11	628.0ª	608.0ª	496.0 ^b	440.0°	18.51	0.0001
12	734.0ª	720.0ª	572.0 ^b	518.0°	21.50	0.0001
13	844.0ª	838.0ª	680.0 ^b	630.0°	21.88	0.0001
14	992.0ª	972.0 ^b	804.0°	742.0^{d}	24.64	0.0001
15	1132.0ª	1072.0^{b}	882.0°	832.0 ^d	29.33	0.0001
16	1216ª	1168ª	968.0 ^b	920.0 ^b	29.68	0.0001

Table 2. Effect of stocking density on the body weights (g) of guinea fowl

^{abcd}: Means within a row with different superscript differ significantly at P < 0.05

Sample Collection and Analytical Determination

Body weights of the guinea fowl were taken weekly using a digital scale. Feed intake (FI) of the birds was taken as the difference between the feed supplied and the left over at the end of each period. Feed conversion ratio (FCR) (g/g) of the birds was determined by dividing the feed intake per replicate weekly by the weight gain of the birds.

At 8th and 14th week of age, two birds per replicate were randomly selected for blood sampling, 2 ml of blood samples were collected from the wing veins into heparinized bottles for haematological indicators including packed cell volume (PCV), red blood cell count (RBC) and white blood cell count (WBC). Haemoglobin was determined using the cyanmethaemoglobin method and PCV using the microhematocrit method as described by Bernard et al. (2000). RBC and WBC were determined using Neubaur's hemocytometer and Toluidine blue (0.015%) saline as diluent following the description of Brar et al. (2002). The blood films stained with Wright's stain (Benjamin, 1985) were used for the differential leucocyte counts. Mean corpuscular haemoglobin (MCH), mean cell volume (MCV), and mean corpuscular haemoglobin concentration (MCHC) were calculated following the method of Stockham et al. (2002). Blood samples used for serum biochemical determination were collected in unheparinized bottles for the determination of total protein, globulin, creatinine, triglyceride, glucose and albumin and were measured with the use of standard commercial kits produced by Roche Diagnostics GmbH, Sandhofer Strasse, Germany.

Plasma triiodothyronine

Plasma was obtained from the blood samples by centrifuging the heparinized blood collected from the birds for 15 min (1372 \times *g*) and plasma triiodothyronine concentrations were analysed using immunoenzymatic ELISA kit as described by Tachibana et al. (2007).

Carcass Composition

At week 16, 2 birds per replicate in each treatment were fasted for 12 hours, weighed and then slaughtered, exsanguinated and eviscerated. Each bird was dissected and carcass composition was determined.

Statistical Analysis

Data were analyzed by ANOVA with stocking density as the independent variable in a completely randomized design using SAS (2008). When stocking densities were found to be significant (P < 0.05), theTukey's HSD test was used to separate means.

RESULTS

Growth Performance

Table 2 shows the effect of stocking density on the body weights of guinea fowl at different weeks. There was no difference in the weight gains of the birds at weeks 5 and 6 of age. At week 7, 8, 9, 10 and 16, the weight gains of the birds in SD1 and SD2 were similar but significantly higher than those of the birds in SD3 and SD4, while the weights of SD3 and SD4 were similar. At weeks 11, 12 and 13, the weight gain of the birds in SD1 and SD2 were similar the other treatment groups. Moreover, the weight gain in the birds in SD3 was higher than that of SD4. At weeks 14 and 15, the weight gain of the birds decreased as the stocking density increased progressively.

Table 3 shows the effect of stocking density on the feed conversion ratio of guinea fowl. The feed conversion ratio of the birds in SD3 and SD4 was comparable but higher than those of SD1 and SD2 whose values were similar at week 6. The feed conversion ratio increased progressively as the stocking density increased at weeks 7, 8 and 10. At week 9, there was a similarity in the FCR of the birds in SD1 and SD2 but significantly lower than those of SD4 and SD3. At

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Week	SD1	SD2	SD3	SD4	SEM	P value
WCCK	301	302	303	504	JLIVI	r value
6	2.26 ^b	2.30 ^b	2.38 ^a	2.39ª	0.015	0.0001
7	2.56^{d}	2.66 ^c	2.76^{b}	2.84^{a}	0.025	0.0001
8	2.83°	2.92^{b}	2.98^{ab}	3.02 ^a	0.019	0.0001
9	3.55 ^b	3.54^{b}	3.67 ^a	3.74 ^a	0.020	0.0001
10	3.82 ^d	3.93°	4.12 ^b	4.23ª	0.037	0.0001
11	4.45 ^b	4.61 ^{ab}	4.72ª	4.75 ^a	0.033	0.0005
12	4.74 ^b	4. 83 ^a	4.87ª	4.87ª	0.015	0.0001
13	4.86 ^b	5.13ª	5.17 ^a	5.16 ^a	0.032	0.0001
14	3.89 ^b	4.77 ^{ab}	4.84 ^{ab}	5.66 ^a	0.204	0.0099
15	3.79°	4.18 ^{bc}	4.79 ^{ab}	5.11 ^a	0.144	0.0004
16	4.17°	4.36°	5.09 ^b	6.33 ^a	0.208	0.0001

Table 3. Effect of stocking density on the feed conversion ratio (g/g) of guinea fowl

 $^{\rm abcd}$: Means within a row with different superscript differ significantly at P < 0.05

Table 4.	Effect of the stocking	density on the haematochen	nical profile of guinea	fowl at 8 weeks of age
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	SD 1	SD2	SD3	SD4	SEM	P-value
Indicators						
Packed cell volume (%)	38.25	38.50	38.00	35.00	1.90	0.6455
Haemoglobin (Hb/g)	12.30	12.53	12.60	11.48	0.60	0.8487
Red blood cell (× $10^{12}/L$)	3.05	3.20	3.10	2.90	0.22	0.7659
White blood cell (× 10º/L)	11.85	12.00	11.60	11.28	1.01	0.4476
Heterophil(%)	27.00^{b}	28.25 ^b	35.25ª	37.25ª	2.02	0.0001
Lymphocyte (%)	69.00 ^{ab}	70.25 ^a	63.00 ^{bc}	61.00 ^c	1.96	0.0003
Eosinophil (%)	1.00	0.50	0.50	0.25	0.26	0.4262
Basophil (%)	1.00	0.25	0.75	0.50	0.20	0.3829
Monocyte (%)	2.00ª	0.75^{b}	0.50^{b}	1.00 ^{ab}	0.34	0.0132
Heterophil/Lymphocyte	0.39 ^b	0.41 ^b	0.57 ^a	0.61ª	0.05	0.0449
Total Protein (g/L)	38.5	45.5	40.8	38.8	4.60	0.4697
Albumin (g/L)	10.0	10.5	11.5	10.3	1.80	0.9065
Globulin (g/L)	28.5	34.8	29.3	28.5	3.5	0.2168
Triglyceride (g/L)	0.84 ^{ab}	0.90 ^a	0.61 ^b	0.79 ^{ab}	0.068	0.0436
Creatinine(g/L)	0.08	0.08	0.09	0.13	0.019	0.5006
Glucose (mg/L)	1.26	1.31	1.35	1.29	0.046	0.5001

abc: Means within a row with different superscript differ significantly at P < 0.05

SEM: Standard Error of the Mean

week 11, the FCR of the birds in SD3 and SD4 were similar but higher than that of SD1 but comparable with the SD2. The FCR of the birds in SD1 was lower than those of the other treatment groups at week 13. The FCR of the birds in SD4 was similar to that of SD3 but higher than those of the other treatment groups. The least FCR was in SD1. At week 16, the FCR was similar in SD1 and SD2 but lower than that of SD3 while that of SD3 was lower than that of SD4.

Blood indicators

There were no significant (P > 0.05) differences in the packed cell volume, haemoglobin concentration, red blood cell counts with the stocking density at 8 weeks of age (Table 4). Birds stocked at higher densities (SD3 and SD4) had significantly (P < 0.05) lower heterophil percentage than birds stocked at lower rates (SD1 and SD2). Lymphocyte percentage of the birds in SD4 was lower than of those in SD1 and SD2 but similar to that of SD3. Eosonophil and basophil percentage were not affected (P > 0.05) by the stocking density. Monocyte percentage decreased with increasing stocking rate from birds stocked at SD1 to SD3. Birds stocked at higher densities (SD3 and SD4) had higher (P < 0.05) heterophil/lymphocyte percentage than birds stocked at lower rates (SD1 and SD2). Total protein, albumin and globulin were not affected (P > 0.05) by the stocking densities, while the lowest triglycerides was found in the birds of SD3.

There were no significant (P > 0.05) differences in the packed cell volume, haemoglobin concentration, red blood cell and white blood cell counts with

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Table 5. Effect of the	stocking density of	on the haematochemical	profile of guinea fowl at 14 weeks
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Indicatorss	SD 1	SD2	SD3	SD4	SEM	P-value
Packed cell volume (%)	51.25	48.00	50.00	47.25	2.33	0.6048
Haemoglobin (Hb/g)	16.48	15.70	16.28	15.50	0.84	0.6133
Red blood cell (× 10 ¹² /L)	4.03	3.78	3.95	3.75	0.21	0.8627
White blood cell (×10 ⁹ /L)	15.25	11.28	14.13	14.55	1.56	0.9635
Heterophil(%)	24.25c	29.25b	31.25b	35.00a	1.08	0.0112
Lymphocyte (%)	73.75a	68.50b	67.00b	62.00c	1.23	0.0177
Eosinophil (%)	0.25	0.50	0.50	1.00	0.26	0.4262
Basophil (%)	0.50	0.75	1.00	0.25	0.30	0.1678
Monocyte (%)	1.25ab	1.00ab	0.25b	1.75a	0.39	0.0458
Heterophil/Lymphocyte	0.33c	0.43b	0.47b	0.57a	0.02	0.0087
Total Protein (g/L)	55.00	53.30	52.00	48.0	2.80	0.9577
Albumin (g/L)	31.80	30.80	30.80	27.0	2.20	0.7868
Globulin (g/L)	23.30	22.50	21.30	21.0	2.30	0.1543
Triglyceride (g/L)	0.91	0.81	0.83	0.96	0.054	0.4095
Creatinine(g/L)	0.09	0.09	0.08	0.08	0.008	0.8701
Glucose (g/L)	1.30	1.25	1.25	1.19	0.05	0.0173

^{abc}: Means within a row with different superscript differ significantly at P < 0.05

Table 6. Effect of stocking density on plasma triiodothyronine level (ng/ml) of guinea fowl

Week	SD 1	SD2	SD3	SD4	S.E.M	P Value
6	2.01	2.04	1.99	1.93	0.098	0.9893
9	2.05	2.01	1.88	1.70	0.112	0.7207
12	2.24	2.22	2.13	1.54	0.140	0.2522
14	1.77	1.89	1.74	1.56	0.114	0.7935

Stocking density (per m²)	Breast muscle (g)	Thigh (g)	Drumstick (g)	GIT weight (g)	Liver weight (g)	Heart weight (g)	Lung (g)	Gizzard (g)
SD1	128.20ª	37.60ª	48.40ª	37.8ª	23.20ª	3.4ª	5.00ª	33.80ª
SD2	120.40 ^{ab}	33.20 ^{ab}	42.20 ^b	36.0 ^{ab}	20.40 ^{ab}	3.2ª	4.60 ^{ab}	30.40 ^b
SD3	115.80 ^{bc}	30.80 ^b	41.60 ^b	33.4 ^b	17.60 ^{bc}	2.8ª	3.60 ^b	28.80^{bc}
SD4	108.80°	26.00 ^c	36.60°	29.2°	13.00 ^c	1.4^{b}	2.20 ^c	25.20°
SEM	2.226	1.201	1.222	0.917	1.120	0.219	0.302	0.905
P value	0.0062	0.0008	0.0012	0.0006	0.0019	0.0004	0.0003	0.0016

 Table 7. Effect of stocking density on organ weights of guinea fowl

^{abcd}: Means within a row with different superscript differ significantly at P < 0.05

the stocking density at 14 weeks of age (Table 5). Birds in SD4 had the highest (P < 0.05) heterophil percentage (35.00 %), while lymphocyte percentage decreased as the stocking density increased. Eosinophil and basophil percentage were not significantly (P > 0.05) affected by the stocking density, while percentage monocyte ranged from 0.25 % in birds in SD3 to 1.75 % in birds in SD4. The heterophil/lymphocyte ratio increased with increasing stocking density. Other evaluated variables were not significantly (P > 0.05) affected by the stocking density.

Effect of stocking density on plasma triiodothyronine level (ng/ml) of guinea fowl is presented in Table 6. Stocking density did not have the significant effect on the plasma triiodothyronine levels of the birds across the treatment groups throughout the experimental period.

Table 7 shows the effect of stocking density on organ weights of guinea fowl. The gastrointestinal tract (GIT) length of the birds decreased as the stocking density increased. A similar trend was observed in the weight of the GIT. There was a similarity in the heart weights of the birds except for the lower values obtained in the birds in SD4. The liver weight of the birds in SD1 was similar to those of SD2 but higher than those of the other treatment groups. SD2 was also similar to those SD3 but significantly higher than those of SD4. A similar trend was observed in the lungs, thigh, and the breast muscle weight of the birds. The gizzard of

the birds in SD1 was higher than those of the other treatment groups. Those of SD2 were comparable to the birds in SD3 but significantly higher than those SD4. Drumstick of the birds in SD1 was higher than the birds in the other treatment groups while the birds in SD2 and SD3 were similar but higher than that of SD4.

DISCUSSION

Increased stocking density has been reported to adversely influence poultry performance (Estevez, 2007). Generally, the present study showed that the weight gain of the birds decreased as the stocking increased beyond 16 birds per m². This finding partially agrees with the report of Nahashon et al. (2012) who reported that the body weight gain of French guinea fowl broiler birds reared at stocking density of 10.7, 12, and 13.6 birds/m² was higher than that of birds raised at floor density of 15.6 birds/m². Moreover, consistent with our findings in the present study, earlier study has also indicated that the weight gain of guinea fowl reared at floor density of 18 and 15.6 birds/m² was poorer than those in 12 birds/m² for most part of the rearing period (Nahashon et al., 2011). The similarity in the weight gain of the birds up to week 6 of age in the present study indicates the birds were not encumbered by the space available to them. Even though there is a lack of scientific data on the effects of stocking density on guinea fowl, our finding in the present study is in agreement with the report of Keeling et al. (2003) who indicated that stocking density reduced the body weight gain of Leghorn layers. Previous studies have also shown that stocking density affected the weight gain of broiler chickens adversely (Dozier et al., 2006; Ravindra et al., 2006; Mtileni et al., 2007; Škrbic et al., 2009; Beloor et al., 2010; Adeyemi et al. (2015)

The lower FCR observed in the guinea fowl reared at floor density of 14 birds/m² than those in 18birds/m² at week 6 in this study contradicts the report of Nahashon et al. (2011) who showed that guinea fowl reared at floor density of 18 birds/m² was significantly lower than that of birds reared at stocking density of 15.6, 13.6, and 12 birds/ m^2 . The better FCR obtained in this study at this stage suggests that the floor space was not excessive enough to bring about reduction in feed efficiency as a result of increased physical activity (Hill, 1986). Wang et al. (2009) also reported that birds reared in expansive floor space incline to have susceptibility to exhibit a higher metabolism resulting consumption of more energy. The discrepancy in the observations of the two studies may also be due to the variation in environmental factors, including relative humidity, temperature, and stable gases like NH₃, CO₂, CO, and H₂S, etc.

Beyond week 7 of age, Nahashon et al. (2011) reported that there was a shift in the trend of feed conversion ratios as the birds in the higher floor density had higher feed conversion ratios. This is in accordance with the findings of the present study. The generally higher FCR from 18 birds/m² suggests that decreasing floor space or increasing floor density is usually associated with poor feed efficiency or higher FCR because of competition for feeder space (Thogerson et al., 2009). The higher FCR as the floor density increased at later age are consistent with the observation of Nahashon et al. (2011). Other studies (Estevez, 2007; Dozier et al., 2006) on chickens have also indicated that stocking densities adversely affected feed conversion ratio.

It has been shown that environmental factors affect haematological indicators in birds (Maxwell, 1990). The fluctuation in the blood system could be a part of the thermoregulatory responses acquired by broilers exposed to stress (Arieli et al., 1979) while the determination of serum enzymes panel often reflects the degree of hepatocellular damage and leakage (Jaensch, 2000). The similarity observed in the total protein across the treatment groups at different phases in the present study is at variance with the observation of Tong et al. (2012) who reported that stocking density of local chickens was found to significantly affect the levels of blood total protein. The heterophil to lymphocyte ratio (H:L) is a reliable indicator of avian stress (Heckert et al., 2002; Zulkifli et al., 2003). Increase in the heterophil as the stocking density increased in the present study resulted in higher H:L in the birds at higher stocking density. This indicates that the birds reared at 20 birds/m² were more stressed than those of the other stocking densities. A similar observation was made by Feddes et al. (2002) who reported that H:L ratio increased with stocking density broilers. On the contrary, some authors (Spinu et al., 2003; Dozier et al., 2006; Sekeroglu et al., 2011) observed a similar H:L ratio in broilers reared under different stocking densities. There is, however, a scarcity of data on the effect of stocking density on guinea fowl. The similarity in the blood glucose level in the present study is in accordance with the report of Thaxton et al. (2006).

Plasma triiodothyronine (T_3) has been used as a biomarker of stress as Stratakis and Chrousos (1995) reported that there is a suppressed secretion of thyroid-stimulating hormone and decreased conversion of the relatively inactive T_4 to more biologically active T_3 during stress. There was, however, no significant difference in the plasma T_3 of the birds across the treatment groups at the different weeks of observation. This similarity suggests that the stocking density was not high enough to depress the hormonal levels. In agreement with our study, Tong et al. (2012) observed that stocking did not have a recognizable effect on the plasma triiodothyronine of chickens.

The organ weights of the birds decreased progressively as the stocking density increased in the present study. Literature is replete with reports on the adverse effect of stocking density on growth and external carcass quality of chickens (Stanley et al., 1989; Puron et al., 1995; Feddes et al., 2002). There is, however, a dearth of information on guinea fowl. Our observation is consistent with the findings of Dozier et al. (2006), which indicated that some organ weights were negatively affected by high stocking density in broiler chickens. On the contrary, Nahashon et al. (2009) reported that there was no significant difference in most of the weights of the parts of guinea reared on different floor density.

CONCLUSION

In conclusion, helmeted guinea fowl can be raised in the tropical environments using a floor density of 16 birds/m² without compromising the performance and welfare of the birds. This stocking density is therefore recommended to the farmers.

Conflicts of Interest Statement

The authors declare they did not have conflict of interest.

REFERENCES

- Abudabos A. M., Samara E. M., Hussein E. O. S., Al-Ghadi M. Q., Al-Atiyat R. M. (2013): Impacts of stocking density on thee performance and welfare of broiler chickens. Italian Journal of Animal Science12: 66–71.
- Adeyemi O. A., Adedoyin E. O., Olaleye O. O., Njoku P. C., Sanwo K. A. (2015): Effects of ascorbic acid supplementation on broiler chickens stocked at two different densities in a humid tropical environment. Malaysian Journal of Animal Science 18: 89–101.
- Arieli A., Meltzer A., Berman A. (1979): Seasonal acclimatization in the hen. British Poultry Science 20: 505–513.
- Bell D. E., Weaver Jr W. D. (2002): Feeding commercial egg type layers. Pp. 287–328 in Commercial Chicken Meat and Egg Production, D. E. Bell, W. D. Weaver, Eds, Kluwer Academic Publishers, Dordrecht, the Netherlands.
- Beloor J., Kang H. K., Kim Y. J., Subramani V. K., Jang I. S., Sohn S. H., Moon Y. S. (2010): The effect of stocking density on stress related genes and telomeric length in broiler chickens. Asian Australian Journal of Animal Science 23: 437–443.
- Benjamin M. M. (1985): Outline of veterinary clinical pathology. 3rd Ed. The Iowa State University Press, Ames.

- Bernard F. F., Joseph G. Z., Jain N. C. (2000): Schalm's Veterinary Hematology. 5th Ed. Lippincott Williams and Wilkins, Philadelphia.
- Brar R. S., Sandhu H. S. Singh A. (2002): Veterinary clinical diagnosis by laboratory methods. 1st Ed. Kalyani Publishers, New Delhi.
- Dozier W. A., Thaxton J. P., Purswell J. L., Olanrewaju H. A., Branton S. L., Roush W. B. (2006): Stocking density effects on male broilers grown to 1.8 kilograms of body weight. Poultry Science 85: 344–351.
- Embury I. (2001): Raising guinea fowl. Agfact A5.0.8. New South Wales Agriculture Publications, New South Wales, Australia.
- Estevez I. (2007): Density allowances for broilers: Where to set the line. Poultry Science 86: 1265–1272.
- Feddes J. J. R., Emmanuel E. J., Zuidhof M. J. (2002): Broiler performance, bodyweight variance, feed and water intake, and carcass quality at different stocking densities. Poultry Science 81: 774–779.
- Heckert R. A., Estevez I., Russek-Cohen E., Pettit-Riley R. (2002): Effect of density and perch availability on the immune status of broilers. Poultry Science 81: 451–457.
- Hill J. A. (1986): Egg production in alternative systems–A review of recent research in the UK. Research Development in Agriculture 3: 13–18.
- Moreki J. C. , Radikara M. V. (2013): Challenges to Commercialization of Guinea Fowl in Africa. International Journal of Science and Research 2: 436–440
- Keeling L. J., Estevez I., Newberry R. C., Correia M. G. (2003): Production-related traits of layers in different sized flock: The concept of problematic intermediate group sizes 1. Poultry Science 82: 1393–1396.
- Maxwell M. H. (1990): Haematological and histopathological findings in young broilers reared in poorly and well ventilated environments. Research in Veterinary Science 48: 374–376.
- Mtileni B. J., Nephawe K. A., Nasamvuni A. E., Benyi K. (2007): The influence of stocking density on body weight, egg weight, and feed intake of adult broiler breeder hens. Poultry Science 86:1615–1619.
- Nahashon S. N., Adefope 1 N., Amenyenu A., Tyus II J., Wright D. (2009): The effect of floor density on growth performance and carcass characteristics of French guinea broilers. Poultry Science 88: 2461–2467.
- Nahashon S. N., Adefope N., Wright D. (2011): Effect of floor density on growth performance of Pearl Grey guinea fowl replacement pullets. Poultry Science 90: 1371–1378.
- Nahashon S. N., Adefope N. A., Amenyenu A., Wright D. (2006): Laying Performance of Pearl Gray Guinea Fowl Hens as Affected by Caging Density. Poultry Science 85: 1682–1689.
- Oke O. E., Adejuyigbe A. E., Idowu O. P., Sogunle O. M., Ladokun A. O., Oso A. O., Abioja M. O., Abiona J. A., Daramola J. O., Whetto M., Jacobs E. B., Williams

T. J., Njoku C. P. (2015): Effects of housing systems on reproductive and physiological response of guinea fowl (*Numida meleagris*). Journal of Applied Animal Science 8: 47–55.

- Puron D., R. Santamaria J. C., Segura Alamilla J. L. (1995): Broiler performance at different stocking densities. Journal of Applied Poultry Research 4: 55–60.
- Puvadolpirod S., Thaxton J. P. (2000): Model of physiological stress in chickens. 1. Response parameters. Poultry Science 79: 363–369.
- SAS User's Guide (2008): Version 9.02 ed., SAS Institute Inc., Cary, NC.
- Schwanz L. (1987): The Family Poultry Flock. Library of Congress, London, UK.
- Sekeroglu A., Sarica M., Gulay M. S., Duman M. (2011): Effect of stocking density on chick performance, internal organ weights and blood parameters in broilers. Journal of Animal and Veterinary Advances 10: 246–250.
- Škrbic Z., Pavlovski Z., Lukic M. (2009): Stocking density–factor of production performance, quality and broiler welfare. Biotechnology in Animal Husbandry 25: 359–372.
- Spinu M., Benveneste S., Degen A. A. (2003): Effect of density and season on stress and behaviour in broiler breeder hens. British Poultry Science 44: 170–174.
- Stanley V. G., Bailey J. E., Krueger W. F. (1989): Effect of iodine-treated water on the performance of broiler chickens reared under various stocking densities. Poultry Science 68: 435–437.
- Stockham S. L., ScottM. A. (2002): Fundamentals of veterinary clinical pathology. Iowa State University Press, Blackwell Publishing Company.

- Stratakis C. A., Chrousos G. P. (1995): Neuroendocrinology and pathophysiology of the stress system. Annals of New York Academy of Science 771: 1–18.
- Tachibana T., Oikawa D., Takahashi H., Boswell T., Furuse M. (2007): The anorexic effect of alpha-melanocyte stimulating hormone is mediated by corticotrophin-releasing factor in chicks. Compartive Biochemistry and Physiology A, 147: 173–178.
- Thaxton J. P., Dozier III W. A.,Branton S. L., Morgan G. W., Miles D. W., Roush W. B., Lott B. D., Vizzier-Thaxton Y. (2006): Stocking density and physiological adaptive responses of broilers. Poultry Science 85: 819–824.
- Thogerson C. M., Hester P. Y., Mench J. A., Newberry R. C., Okura C. M., Pajor E. A. (2009): The effect of feeder space allocation on productivity and physiology of Hy-Line W-36 hens housed in conventional cages. Poultry Science 88: 1793–1799.
- Tong H. B., Lu J., Zou J. M., Wang Q., Shi S. R. (2012): Effects of stocking density on growth performance, carcass yield, and immune status of a local chicken breed. Poultry Science 91: 667–673.
- Wang X. L., Zheng J. X., Ning Z. H., Qu L. J., Xu G. Y., Yang N. (2009): Laying performance and egg quality of blueshelled layers as affected by different housing systems. Poultry Science 88: 1485–1492.
- Zulkifli I., Liew P.K., Israf D.A., Omar A. R., Hair-Bejo M. (2003): Effect 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. Journal of Thermal Biology 28: 217–222.

Received: November 28, 2019 Accepted after revisions: April 5, 2020