Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

e-ISSN: 1806-9061 2026 / v.28 / n.2 / 001-011

http://dx.doi.org/10.1590/1806-9061-2026-2264

Original Article

Effects of Stocking Density on Growth Performance, Hematobiochemical Responses, Carcass Traits, and Economic Efficiency of Broilers Raised in Open-Sided Housing During Seasonal Heat Stress

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■Keywords

Broiler, carcass, FCR, heat stress, stocking density, serum biochemistry.

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Section Editor: Irenilza de Alencar Nääs

Submitted: 16/May/2026
Approved: 24/June/2026

ABSTRACT

This study assessed the impact of four stocking densities (14.35, 10.76, 8.61, and 7.18 birds/m²) on the growth performance, carcass characteristics, hematological and serum biochemical indices, and economic efficiency of broilers raised in open-sided housing under natural heat stress (32–38 °C; relative humidity 65–80%). A total of 360 male chicks (Ross-308) were randomly allocated to four treatments, with six replicates of 15 birds each, in a completely randomized design over 35 days. Broilers raised at 8.61 and 7.18 birds/m² showed higher (p<0.05) feed intake and body weight gain than those raised at 14.35 and 10.76 birds/m². Feed conversion ratio was improved in birds provided 8.61 and 7.18 birds/m². Carcass characteristics and cut-up part yields were not significantly affected by stocking density. Hematological analysis revealed higher red blood cell count, hemoglobin, packed cell volume, lymphocyte percentage, and serum protein levels in birds reared at lower densities (8.61 and 7.18 birds/m²). Broilers kept at 14.35 birds/m² had higher levels of heterophils, heterophil-to-lymphocyte ratio, glucose, triglycerides, cholesterol, corticosterone, ALT, AST, and ALP. In terms of economics, 8.61 birds/m² produced the highest net profit and cost-benefit ratio, even though 7.18 birds/m² generated higher overall income. According to these results, under the environmental and management conditions of the present study, 8.61 birds/m² appeared to provide the most favorable balance between growth performance and economic return in broilers raised in open-sided housing during a hot season.

INTRODUCTION

The welfare and productivity of broilers are affected by various management and environmental factors, including diet, ventilation, genetics, stress exposure, and, notably, stocking density (Yakubu *et al.*, 2009). In contemporary intensive poultry farming, optimizing stocking density is essential for reconciling economic efficiency with avian welfare. The Council Directive (2007) defines flock density as the number of birds or total live body weight per square meter of usable floor space. Although higher stocking density enhances space efficiency and reduces housing costs, excessive overcrowding can adversely affect growth performance, health, and welfare.

Environmental stresses, including elevated ambient temperature, intensify the adverse impacts of high stocking density (SD). Heat stress alters metabolic and endocrine responses, reduces feed intake (FI), and compromises immune function, thereby impairing growth performance (Bilal *et al.*, 2021). Inadequate space restricts physical activity, influences behavior, and modifies physiological responses, encompassing endocrine and hematological markers (López-López

et al., 2022; Janicka *et al.*, 2025; Sakr *et al.*, 2025). Prior research has yielded inconsistent results concerning the influence of stocking density on broiler production. Sakr *et al.* (2025) reported reduced body weight gain (BWG), inferior carcass characteristics, and heightened stress indicators at higher stocking densities, whereas Houshmand *et al.* (2012) found negligible effects on performance and stress markers. These inconsistencies indicate that environmental factors, especially temperature, may influence the response to stocking density.

Overcrowding has been linked to diminished locomotion, heightened leg problems, and impaired welfare (Estevez, 2007; Ventura *et al.*, 2010). While certain studies indicate that broilers may be raised at densities of up to 20 birds/m² without considerable negative impacts (Farhadi & Hosseini, 2016), other research has shown declines in feed intake, body weight gain, and immunological function at elevated densities (Uzum & Toplu, 2013; Singh *et al.*, 2018). Moreover, an increased heterophil-to-lymphocyte (H:L) ratio, elevated corticosterone levels, and serum biochemical changes have been observed in broilers subjected to high density under heat-stress conditions (Najafi *et al.*, 2015; Kim *et al.*, 2021).

Heat stress remains a significant limitation in poultry production, especially in tropical and subtropical regions. The activation of the hypothalamic–pituitary–adrenal (HPA) axis in response to temperature stress increases corticosterone levels, inhibits immunological responses, and diminishes feed intake and growth (Quinteiro-Filho *et al.*, 2010). Despite the proposal of various mitigation measures, such as nutritional modifications and management adjustments (Kumari & Nath, 2018), optimizing stocking density remains a pragmatic and cost-effective approach to alleviate stress levels.

Although extensive research has examined stocking density in broiler production, three important knowledge gaps remain. First, most studies have been carried out under controlled environmental conditions, whereas little information is available on the effects of various stocking densities under naturally occurring heat stress in open-sided housing systems commonly used in developing countries. Second, past studies have tended to examine productive performance or physiological responses, but few have assessed growth performance, carcass characteristics, and hematobiochemical responses simultaneously. Third, although the interface between biological responses and economic outcomes is critical in practical farm decision-making, it remains underexplored. In light of this, the present study was conducted to assess the growth performance, carcass characteristics, blood biochemical constituents, and economic benefit of broilers under natural seasonal heat stress in an open-sided housing system with different stocking densities. Heat stress was not experimentally induced or manipulated; rather, birds were exposed to prevailing seasonal environmental conditions to assess practical field-level responses relevant to commercial poultry production.

MATERIALS AND METHODS

Ethical Consideration

Approval for this study was obtained from the Ethical Committee of the Faculty of Animal Husbandry and Veterinary Sciences at the University of Agriculture Peshawar, Pakistan (No: 428/PS/UAP, dated October 04, 2020). The research activities took place on a poultry farm at the University of Agriculture, Peshawar, and the laboratory work was conducted at the National Veterinary Laboratories (NVL) in Islamabad.

Experimental Location, Bird Management, and Housing

A total of 400 one-day-old male Ross-308 broiler chicks of average body weight (42 ± 0.15 g) were initially procured from the local commercial market. During the 7-day brooding/acclimatization period, chicks were managed under uniform conditions. After the brooding period, 360 chicks were allocated to four groups: D1, D2, D3, and D4. The remaining birds were excluded to maintain uniformity among replicates. Each group had six replicates, each with 15 chicks. The experimental replicates consisted of separate floor pens. The full experimental stocking densities were applied after the 7-day brooding period. The pen dimensions were adjusted according to the assigned stocking density treatments to maintain the required floor area per bird. The total pen areas were 1.05, 1.39, 1.74, and 2.09 m² for groups D1 (14.35 birds/m²), D2 (10.76 birds/m²), D3 (8.61 birds/m²), and D4 (7.18 birds/m²), respectively. Each pen replicate was considered the experimental unit.

During the brooding stage, birds were initially confined to one-fourth of the pen area, and the available space was gradually increased as birds aged and grew throughout the experiment. There was no thermoneutral control treatment. The feed provided

to all groups for raising day-old chicks consisted of a starter diet until the second week, followed by a finisher diet thereafter (Table 1). To minimize competition among birds for feeders and drinkers, feeder and drinker access were standardized across all pens. Each pen was equipped with identical feeders and drinkers, and sufficient feeder and drinker space was maintained to ensure unrestricted access to feed and water for all birds throughout the experimental period. Feed and water were provided *ad libitum* under uniform management conditions. Feeder and drinker number, feeder frontage, drinker access, ventilation exposure, and location within the house were equivalent across pens.

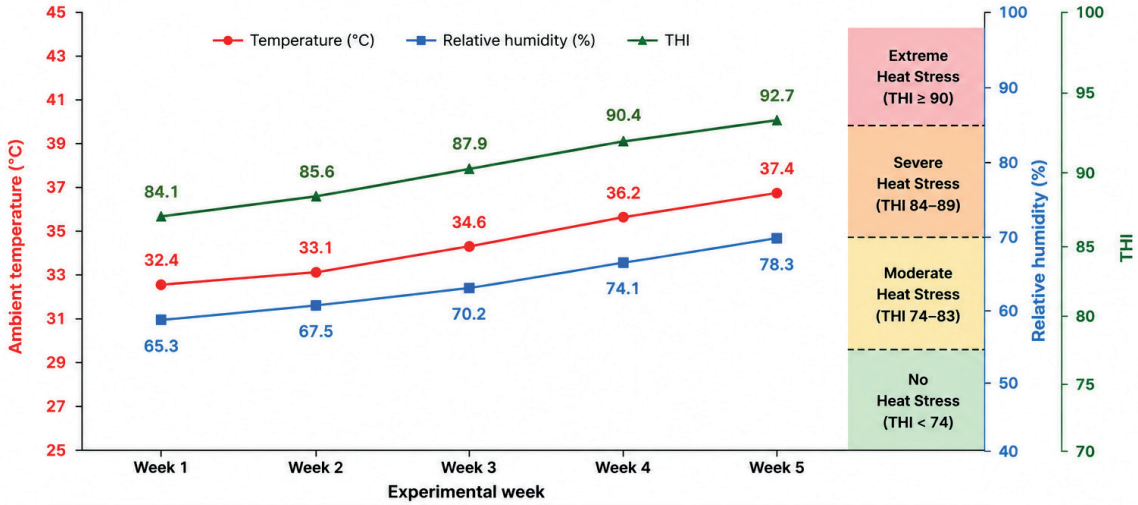
For the first three days, the birds were exposed to continuous lighting; thereafter, the light period was adjusted to 23 hours per day for the remainder of the study. The house temperature was 35 °C during the first week of the brooding phase. Sawdust was used as the bedding material.

The research trial was conducted under natural heat-stress conditions, with ambient temperatures ranging from 32 °C to 38 °C and relative humidity between 65% and 80% after the brooding period (Figure 1). Temperature measurements were recorded daily at six-hour intervals at the birds' level. This study was designed to evaluate broiler responses to varying stocking densities under naturally occurring seasonal heat-stress conditions rather than to compare thermoneutral and heat-stress environments. Therefore, all treatment groups were exposed to the same environmental conditions, and stocking density was considered the sole experimental factor. The following formula was used to measure the temperature-humidity index (Buffington *et al.*, 1981).

Table 1 – Commercial starter and finisher diet compositions

Ingredients	Starter phase (%)	Finisher phase (%)
Corn	58.10	63.38
Soybean meal (CP=44%)	34.55	29.18
Animal fat	3.54	3.58
Dicalcium phosphate	1.53	1.26
Limestone	1.10	1.01
DL-methionine	0.25	0.19
L-lysine HCL	0.07	0.54
L-threonine	0.024	0.01
Sodium chloride	0.40	0.40
Vitamin mineral premix[1]	0.44	0.45
Calculated nutrients %		
Dry matter	87.41	87.49
ME (kcal/kg)	2900	3100
Crude protein	21.60	18.80
Ether extract	4.66	6.08
Crude fiber	2.16	2.06
Calcium	0.88	0.78
Total P	0.60	0.63
Avail. P	0.52	0.39
Sodium	0.23	0.19
Chlorine	0.26	0.28
Choline	1.84	1.75
Folate	0.85	0.83

[1]1 kg of premix provided vitamin E 80 mg, vitamin D3 3,000 IU, A 15,000 IU, vitamin B1 3 mg, vitamin K_3 3 mg, vitamin B_2 8 mg, vitamin B_3 60 mg, vitamin B_5 15 mg, vitamin B12 0.02 mg, Choline chloride (60%) 700 mg, vitamin B_6 4 mg, vitamin B9 2 mg, vitamin H 0.2 mg/kg, Zn (as ZnSO4.H2O) 80 mg, Mn (MnSO4.H2O) 80 mg, Cu (CuSO4.H2O) 10 mg, Se 0.2 mg, Fe (FeSO4.H2O) 60 mg, I (KI) 1 mg. Ca = Calcium; Total P = Total phosphorus; Avail.P = Available phosphorus, Na = Sodium; Cl = Chlorine.



Week	Week 1	Week 2	Week 3	Week 4	Week 5
Ambient temperature (°C)	32.4 ± 1.2	33.1 ± 1.5	34.6 ± 1.4	36.2 ± 1.6	37.4 ± 1.8
Relative humidity (%)	65.3 ± 2.8	67.5 ± 3.1	70.2 ± 2.9	74.1 ± 3.5	78.3 ± 3.8
THI	84.1 ± 1.3	85.6 ± 1.4	87.9 ± 1.3	90.4 ± 1.5	92.7 ± 1.6

Figure 1 – Weekly environmental conditions during the broiler trial. Weekly ambient temperature, relative humidity, and THI during the experimental period. Values are weekly mean ± SD. THI= temperature-humidity index (THI calculated using the formula of Buffington *et al.*, 1981). Environmental temperature and relative humidity were recorded daily at six-hour intervals (06:00, 12:00, 18:00 and 24:00 h).

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)];$$

where T = ambient temperature (°C); RH = relative humidity (%)

The biosecurity program and strategy were implemented on the poultry farm to reduce the risk of disease transmission. To safeguard the broilers against viral infections, an appropriate vaccination regimen (Marek's disease, Newcastle disease, and Gumboro disease) was followed on designated days during the study. The trial lasted five weeks (35 days).

Production Performance Indicators

Each week, production performance metrics were evaluated. Feed intake (FI) was calculated by deducting the daily feed leftovers from the total feed given. Body weight gain (WG) was calculated as the difference between the initial and final body weight. The feed conversion ratio (FCR) was calculated as the birds' weekly feed consumption divided by their weekly BWG. The method outlined by Shuaib *et al.* (2021) was followed. Mortality was recorded daily throughout the experiment and used to adjust the feed conversion ratio and economic calculations when applicable.

$$\text{Feed Intake} = \text{Feed offered} - \text{Feed refused}$$
$$\text{Weight gain} = \text{Final weight} - \text{Initial weight}$$
$$\text{Feed conversion ratio} = \text{Feed intake} \div \text{Weight gain}$$

Week 1 data in the results section (Tables 2, 3, and 4) represent the common brooding/acclimatization period during which birds were managed under uniform conditions, before the full stocking-density treatments were implemented. Consequently, no significant treatment differences were expected during this period, and biological interpretation was focused primarily on responses observed from day 8 onward. While weekly performance responses are presented for completeness, interpretation primarily focuses on the starter, finisher, and overall production phases, which provide the most biologically and commercially relevant assessment of stocking-density effects.

Carcass Characteristics and Cut-Up Parts

On day 35 of the experiment, four broiler chickens were randomly selected from each replicate pen based on the average pen body weight to represent the respective treatment group. Before slaughter, birds were fasted for 8 hours with free access to water. The birds were individually weighed, humanely slaughtered by severing the cervical vessels following standard halal slaughter procedures, and then defeathered and eviscerated manually. Dressing percentage was calculated by dividing the hot carcass weight (the carcass after bleeding, defeathering, and removal of the head, shanks, and viscera) by the live body weight, then multiplying by 100.

$$\text{Dressing percentage} = (\text{Hot carcass weight} / \text{live body weight}) \times 100$$

Eviscerated weight was determined after the removal of visceral organs, being calculated as:

$$\text{Eviscerated weight (\%)} = \text{Eviscerated carcass weight} / \text{live body weight} \times 100$$

Giblet weight is the combined weight of the liver, heart, and gizzard were expressed in grams. Abdominal fat percentage was calculated relative to live body weight. Abdominal fat (%) was calculated as:

$$\text{Abdominal fat (\%)} = \text{Abdominal fat weight} / \text{live body weight} \times 100$$

The relative weights of the cut-up parts (breast, legs, thighs, and wings) were determined as percentages of dressed carcass weight, as follows:

$$\text{Cut up part (\%)} = \text{Cut up part weight} / \text{dressed carcass weight} \times 100$$

Meat color was measured on the surface of the pectoralis major (breast) muscle approximately 30 min postmortem using a portable colorimeter. The instrument was calibrated against a standard white calibration tile according to the manufacturer's instructions before measurements were taken. Because only a composite color index was recorded during data collection, individual L* (lightness), a* (redness), and b* (yellowness) coordinates were not available for analysis.

Carcass pH was measured with a digital pH meter. For statistical analysis, carcass measurements obtained from birds within the same replicate pen were averaged, and pen means were considered the experimental unit.

Blood Biochemical Profile

Blood samples were collected from four randomly selected broilers in each replicate using sterile 22-gauge syringes and needles via the brachial vein. For hematological examinations, blood was drawn into

EDTA tubes; for serum biochemical analysis, into gel-clot tubes. Each sample was then immediately placed in an ice box and stored at 4 °C until analysis.

Biochemical parameters such as total protein (g/dL), glucose (mg/dL), triglycerides (mg/dL), cholesterol (mg/dL), alanine aminotransferase (ALT; U/L), aspartate aminotransferase (AST; U/L), alkaline phosphatase (ALP; U/L) and corticosterone (ng/mL) were determined by commercial diagnostic kits (Merck®, Germany; Greiner Diagnostic Kits, Germany), following the manufacturer's instructions. Biochemical analysis was performed on Semi-Automatic Biochemistry Analyzers (Merck®, Germany; MicroLab-300 and Cobas Hitachi Analyzer, Japan).

An automated veterinary hematology analyzer (Urit 3000 Plus Vet Hematology Analyzer, Germany) was used to determine hematological parameters such as red blood cell count (RBCs; M/µL), hemoglobin concentration (Hb; g/dL), packed cell volume (PCV; %), heterophil percentage (%), and lymphocyte percentage (%). A differential leukocyte count was also confirmed by Field stain A and B blood smear examination. The ratio of heterophil to lymphocyte (H:L) was determined by dividing the heterophil count by the lymphocyte count. Biochemical and hematological analyses were performed in duplicate to ensure analytical accuracy and consistency.

Economics

Economic analysis was conducted on a per-bird basis using prevailing local market prices during the experimental period (Pakistani Rupees; PKR, 2024). The economic parameters included total production cost (TPC), total revenue (TR), net profit, and cost-benefit ratio (CBR). Feed cost was calculated using cumulative feed intake and the market prices of starter and finisher diets during the study period. The average purchase cost per day-old chick, vaccination expenses, litter costs, electricity charges, and routine management expenses were also included in the total production cost calculations. However, depreciation costs for fixed infrastructure and housing were not included because all treatment groups were housed in the same facility.

Total revenue per bird was estimated using the final live body weight and the prevailing market price of live broilers at the end of the experiment. Net profit per bird was calculated by subtracting total production cost from total revenue. The cost-benefit ratio (CBR) was determined by dividing total revenue by total production cost. The following equations were used to calculate the economic parameters (Shuaib *et al.*, 2024):

$$\textit{Total production cost (TPC)} = \textit{Chick cost} + \textit{Feed cost} + \textit{Vaccination and management expenses}$$

$$\textit{Total revenue} = \textit{Total meat kg} \times \textit{Market Price per kg live broiler}$$

$$\textit{Net profit} = \textit{Total revenue} - \textit{Total production cost}$$

$$\textit{Cost-benefit ratio} = \textit{Total revenue} \div \textit{Total production cost}$$

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) under a Completely Randomized Design (CRD), following the procedures described by Steel *et al.* (1997). Pen replicate was considered the experimental unit for feed intake, body weight gain, feed conversion ratio, and economic parameters. For carcass and blood variables, measurements obtained from sampled birds within each pen were averaged, and the pen mean was used as the experimental unit.

Performance variables were recorded weekly and analyzed separately for each week to evaluate treatment responses at specific growth stages under real production conditions. Although repeated measurements were collected from the same pens over time, weekly ANOVA was used to facilitate stage-specific treatment comparisons. This approach does not account for within-pen correlations across weeks and may increase the risk of multiple testing; therefore, results should be interpreted with this limitation in mind.

Before analysis, data were assessed for normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively. Statistical analyses were performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Treatment means were compared using Tukey's multiple comparison test. Differences among treatments were considered significant at $p<0.05$.

The statistical model applied was:

$$Yij = \mu + Ti + \varepsilon ij;$$

where Yij = observed response variable; μ = overall mean; Ti = fixed effect of the i^{th} stocking density treatment (i = 1–4), εij = random error associated with each observation, assumed to be independent

and normally distributed with mean zero and constant variance (N(0,σ2)).

RESULTS

Production Performance Indicators

The feed intake (FI) results are shown in Table 2. The FI during the second and third weeks, as well as during the starter phase, was higher (p<0.05) in groups D4 and D3 than in groups D2 and D1. The same trend was observed for the fourth and fifth weeks, the finisher phase, and the overall experiment period. Over the entire 35-day trial, broilers reared in the D3 and D4 groups exhibited approximately 7.0% and 7.3% greater overall feed intake, respectively, compared with birds reared in D1. Similarly, D3 and D4 showed approximately 3.8% and 4.1% higher overall feed intake than D2.

Table 2 – Stocking density impact on feed intake (FI) in seasonal heat-stressed broilers.

Weeks	Groups (g/bird)[1]				SEM	*p*-value
	D1	D2	D3	D4		
1	132	136	135	136	0.71	0.140
2	300^{c}	312^{b}	332^{a}	333^{a}	4.29	0.026
3	535^{c}	556^{b}	584^{a}	585^{a}	6.33	0.017
Starter phase (1-21d)	967^{c}	1004^{b}	1050^{a}	1054^{a}	10.9	0.043
4	693^{c}	712^{b}	734^{a}	734^{a}	5.20	0.011
5	873^{c}	896^{b}	926^{a}	931^{a}	7.14	0.026
Finisher phase (22-35d)	1566^{c}	1608^{b}	1659^{a}	1665^{a}	12.2	0.012
Overall period (1-35d)	2533^{c}	2612^{b}	2710^{a}	2719^{a}	23.0	0.033

Data are reported as the mean and the pooled standard error of the mean (SEM). Means within the same row followed by different superscripts differ significantly (p<0.05)
[1]D1 = 14.35 birds/m²; D2 = 10.76 birds/m²; D3 = 8.61 birds/m²; and D4 = 7.18 birds/m²
No mortality was recorded in any treatment during the experimental period; therefore, feed intake calculations were not adjusted for mortality.

Table 3 presents the findings for the weight gain (WG) of broilers in this study. During the second, third, fourth, and fifth weeks, as well as in the starter, finisher, and overall phases, groups D3 and D4 exhibited significantly higher WG (p<0.05) compared with groups D2 and D1. Over the entire 35-day trial, broilers reared at D3 and D4 exhibited approximately 15.6% and 16.1% greater overall weight gain, respectively, compared with birds reared at D1. Similarly, D3 and D4 showed approximately 6.2% and 6.7% higher overall weight gain than D2.

The results for the broilers' feed conversion ratio (FCR) are presented in Table 4. The FCR during the second week and starter phase was improved (p=0.034) in group D3 compared with group D1, but in the third week (p=0.019), it was improved in group D3 compared with groups D1 and D2. At the fourth week and finisher phase, FCR was significantly improved (p<0.05) in group D4 compared with groups D1 and D2, and at the fifth week (p=0.027), in group D4 compared with group D1. The overall period showed a better FCR (p=0.042) in groups D3 and D4 than in groups D1 and D2. Overall, in the 1-35-day trial, broilers reared in D3 and D4 exhibited approximately 7.5% lower FCR compared with birds reared in D1, indicating improved fineed utilization efficiency. Similarly, D3 and D4 showed approximately 2.5% lower overall FCR than D2.

Table 3 – Stocking density impact on weight gain (WG) in seasonal heat-stressed broilers.

Weeks	Groups (g/bird)[1]				SEM	*p*-value
	D1	D2	D3	D4		
1	116	117	118	118	0.68	0.820
2	202^{c}	218^{b}	234^{a}	233^{a}	3.93	0.014
3	230^{c}	259^{b}	282^{a}	281^{a}	6.46	0.031
Starter phase (1-21d)	548^{c}	594^{b}	634^{a}	632^{a}	10.6	0.022
4	311^{c}	338^{b}	358^{a}	361^{a}	6.21	0.011
5	337^{c}	370^{b}	390^{a}	395^{a}	7.08	0.026
Finisher phase (22-35d)	647^{c}	707^{b}	748^{a}	756^{a}	13.1	0.038
Overall period (1-35d)	1196^{c}	1301^{b}	1382^{a}	1388^{a}	23.5	0.012

Data are reported as the mean and the pooled standard error of the mean (SEM). Means within the same row followed by different superscripts differ significantly (p<0.05)
[1]D1 = 14.35 birds/m²; D2 = 10.76 birds/m²; D3 = 8.61 birds/m²; and D4 = 7.18 birds/m²
No mortality was recorded in any treatment during the experimental period; therefore, feed intake calculations were not adjusted for mortality.

Table 4 – Stocking density impact on feed conversion ratio (FCR) in seasonal heat-stressed broilers.

Weeks	Group[1]				SEM	*p*-value
	D1	D2	D3	D4		
1	1.13	1.16	1.14	1.16	0.01	0.420
2	1.48^{a}	1.43^{ab}	1.42^{b}	1.43^{ab}	0.01	0.034
3	2.33^{a}	2.15^{b}	2.07^{c}	2.08^{c}	0.03	0.019
Starter phase (1-21d)	1.76^{a}	1.69^{b}	1.66^{b}	1.67^{b}	0.01	0.034
4	2.23^{a}	2.11^{b}	2.05^{bc}	2.03^{c}	0.02	0.011
5	2.59^{a}	2.42^{b}	2.37^{b}	2.36^{b}	0.03	0.027
Finisher phase (22-35d)	2.42^{a}	2.27^{b}	2.22^{c}	2.20^{c}	0.03	0.014
Overall period (1-35d)	2.12^{a}	2.01^{b}	1.96^{c}	1.96^{c}	0.02	0.042

Data are reported as the mean and the pooled standard error of the mean (SEM). Means within the same row followed by different superscripts differ significantly (p<0.05)
[1]D1 = 14.35 birds/m²; D2 = 10.76 birds/m²; D3 = 8.61 birds/m²; and D4 = 7.18 birds/m²
No mortality was recorded in any treatment during the experimental period; therefore, feed intake calculations were not adjusted for mortality.

Carcass Characteristics and Cut-Up Parts

The influence of stocking density on carcass characteristics and cut-up parts is described in Table 5. The results revealed no effect of stocking density on carcass characteristics, including dressing percentage, eviscerated weight, giblet weight, abdominal fat, meat

color, and pH. Dressing percentage showed a numerical increase at lower stocking density, but the difference did not reach statistical significance. Similarly, stocking density had no significant effect on the percentages of breast, leg, thigh, and wing on the 35th day of the trial.

Table 5 – Overall impact of stocking density on carcass characteristics and cut-up parts in heat-stressed broilers.

Carcass characteristics	Groups[1]				SEM	*p*-value
	D1	D2	D3	D4		
Dressing (%)	61.8	65.3	65.9	66.2	2.11	0.062
Eviscerated weight (%)	76.9	76.7	78.2	78.3	1.93	0.181
Giblet weight (gm)	76.0	77.2	80.2	86.0	6.02	0.264
Abdominal fat weight (%)	1.80	1.70	1.70	1.60	0.25	0.431
Meat color index	1.20	1.20	1.30	1.30	0.45	0.860
Meat pH	6.00	6.00	6.00	6.00	0.08	0.542
Cut up parts						
Breast (%)	16.2	16.5	16.1	16.5	1.02	0.142
Legs (%)	14.2	14.4	15.1	14.3	0.93	0.221
Thighs (%)	13.5	13.2	13.2	13.2	0.30	0.152
Wings (%)	3.30	3.30	3.30	3.50	0.18	0.174

Data are reported as the mean and the pooled standard error of the mean (SEM).
[1]D1 = 14.35 birds/m²; D2 = 10.76 birds/m²; D3 = 8.61 birds/m²; and D4 = 7.18 birds/m².

Blood Biochemical Profile

Table 6 shows the current research findings on blood profile and serum biochemical values. The number of RBCs and lymphocytes was higher (p<0.05) for group D4 compared with groups D1 and D2. The Hb and PCV levels were higher (p<0.05). in group D4 compared with groups D1, D2, and D3. The heterophil value was higher (p=0.024) in group D1 than in groups D3 and D4. The heterophil-to-lymphocyte ratio (H:L) was higher (p=0.038) in group D1 than in groups D2, D3, and D4. Broilers raised at D3 and D4 exhibited approximately 68.1% and 76.3% lower H:L ratios, respectively, compared with birds reared at D1. Similarly, the H:L ratio in D3 and D4 was reduced by approximately 35.3% and 52.0%, respectively, compared with D2, indicating a markedly reduced stress response at lower stocking densities.

Serum biochemical parameters such as ALT, triglycerides, glucose, corticosterone, AST, and ALP were higher (p<0.05) for group D1 compared with groups D2, D3, and D4. Broilers reared at D3 and D4 exhibited approximately 63.6% and 66.1% lower serum corticosterone concentrations, respectively, compared with birds reared at D1. Similarly, corticosterone levels in D3 and D4 were reduced by approximately 53.2% and 56.4%, respectively, compared with D2, indicating a markedly lower physiological stress response at lower stocking densities. The protein level was higher (p=0.012) in groups D3 and D4 than in groups D1 and D2, while the cholesterol level was higher (p=0.026) in groups D1 and D2 compared with groups D3 and D4. Values were interpreted as stress-related physiological responses rather than diagnostic evidence of clinical disease.

Table 6 – Overall impact of stocking density and seasonal heat stress on blood biochemical parameters in broilers.

Parameters[2]	Groups[1]				SEM	*p*-value
	D1	D2	D3	D4		
RBCs (M/µL)	2.33^{b}	2.38^{b}	2.83^{a}	3.24^{a}	0.13	0.034
Hb (g/dL)	8.50^{b}	9.03^{b}	9.53^{b}	10.8^{a}	0.28	0.013
PCV (%)	30.4^{c}	33.3^{b}	33.8^{b}	39.4^{a}	1.08	0.027
Heterophil (%)	61.7^{a}	48.0^{ab}	39.3^{b}	32.0^{c}	3.62	0.024
Lymphocyte (%)	30.3^{c}	47.0^{b}	57.7^{a}	64.0^{a}	4.02	0.012
H:L	2.07^{a}	1.02^{b}	0.66^{b}	0.49^{c}	0.19	0.038
ALT (U/L)	60.7^{a}	43.0^{b}	33.7^{bc}	24.7^{c}	4.31	0.045
Triglyceride (mg/dL)	126^{a}	97.3^{b}	42.3^{c}	41.7^{c}	11.1	0.033
Protein (g/dL)	2.96^{c}	3.46^{b}	4.44^{a}	4.58^{a}	0.21	0.012
Glucose (mg/dL)	270^{a}	214^{b}	183^{b}	182^{b}	11.4	0.022
Corticosterone (ng/mL)	1.21^{a}	0.94^{b}	0.44^{c}	0.41^{c}	0.10	0.015
Cholesterol (mg/dL)	186^{a}	182^{a}	146^{b}	143^{b}	6.24	0.026
AST (U/L)	181^{a}	103^{b}	55.0^{c}	50.3^{c}	16.1	0.020
ALP (U/L)	209^{a}	176^{b}	106^{c}	97^{c}	14.5	0.019

Data are reported as the mean and the pooled standard error of the mean (SEM). Means within the same row followed by different superscripts differ significantly (p<0.05).
[1]D1 = 14.35 birds/m²; D2 = 10.76 birds/m²; D3 = 8.61 birds/m²; and D4 = 7.18 birds/m².
[2]RBCs = red blood cells; Hb = hemoglobin; PCV = packed cell volume; H:L = heterophil-to-lymphocyte ratio; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase.

Economics

The economic performance of broilers under different stocking densities during the starter, finisher, and overall phases is summarized in Table 7. During the starter phase, total production cost per bird (TPC bird^{-1}) was highest (p=0.025) in group D4 as compared with D1, D2, and D3. Total revenue per bird (TR bird^{-1}) was significantly higher (p=0.011) in groups D3 and D4 than in D1 and D2. Net profit bird^{-1} was lowest (p=0.026) in D4, while the cost-benefit ratio (CBR) was greater (p=0.033) in D1 and D2 than in D3 and D4. During the finisher phase, group D4 again had the highest TPC bird^{-1} (p=0.016). TR bird^{-1} was greater (p=0.022) in D3 and D4 compared with D1 and D2. Net profit bird^{-1} was highest (p=0.040) in D2, and the CBR was significantly greater (p=0.018) in D1, D2, and D3 than in D4. For the overall period, the TPC bird^{-1} was highest (p=0.031) in D4, while the TR bird^{-1} was higher (p=0.023) in D3 and D4 relative to D1 and D2. Net profit bird^{-1} was greatest (p=0.037) in

D3, and the CBR was higher (p=0.014) in D2 and D3 compared with D1 and D4.

For the overall period, broilers reared at D3 showed approximately 22.2% more net profit bird^{-1} compared with birds reared at D1, while D4 showed a 12.7% increase relative to D1. Similarly, net profit bird^{-1} in D3 was approximately 5.5% higher than in D2 and 8.5% higher than in D4, showing that the stocking density of 8.61 birds/m² provided the greatest economic return on a per-bird basis.

Table 7 – Impact of stocking density on economics in heat-stressed broilers.

Economics parameters (Rs)[2]	Groups[1]				SEM	*p*-value
	D1	D2	D3	D4		
Starter phase (1-21d)						
TPC bird^{-1}	73.0^{d}	81.0^{c}	90.0^{b}	96.0^{a}	2.61	0.025
TR bird^{-1}	104^{c}	113^{b}	120^{a}	120^{a}	1.98	0.011
Net profit bird^{-1}	31.0^{a}	32.0^{a}	31.0^{a}	24.0^{b}	0.92	0.026
CBR	1.42^{a}	1.39^{a}	1.34^{b}	1.25^{c}	0.02	0.033
Finisher phase (22-35d)						
TPC bird^{-1}	108^{d}	117^{c}	125^{b}	131^{a}	2.62	0.016
TR bird^{-1}	123^{c}	135^{b}	142^{a}	144^{a}	2.47	0.022
Net profit bird^{-1}	15.0^{ab}	18.0^{a}	17.0^{a}	13.0^{b}	0.70	0.040
CBR	1.14^{a}	1.15^{a}	1.14^{a}	1.10^{b}	0.01	0.018
Overall period (1-35d)						
TPC bird^{-1}	164^{d}	175^{c}	186^{b}	192^{a}	3.23	0.031
TR bird^{-1}	227^{c}	247^{b}	262^{a}	264^{a}	4.47	0.023
Net profit bird^{-1}	63.0^{c}	73.0^{b}	77.0^{a}	71.0^{b}	1.53	0.037
CBR	1.38^{b}	1.42^{a}	1.41^{a}	1.37^{b}	0.01	0.014

Data are reported as the mean and the pooled standard error of the mean (SEM). Means within the same row followed by different superscripts differ significantly (p<0.05).
[1]D1 = 14.35 birds/m²; D2 = 10.76 birds/m²; D3 = 8.61 birds/m²; and D4 = 7.18 birds/m².
[2]Rs = Rupees; TPC = Total production cost; TR = Total revenue; Net profit = Total revenue – Total production cost (expressed on a per-bird basis); CBR = Cost benefit ratio.
Total production cost (TPC) included feed cost, chick cost, and routine management expenses, and calculations were based on prevailing local market prices during the experimental period (in Pakistani Rupees, PKR, 2024). Profitability was expressed on a per-bird basis.
No mortality was recorded in any treatment during the experimental period; therefore, economic calculations were not adjusted for mortality.

Table 8 compares profit per bird and estimated profit per unit floor area (m²) to illustrate the economic efficiency of broiler production under various stocking densities. According to the results, increasing the stocking density increased the number of birds accommodated per square meter, thereby increasing total profit per unit area. Despite having a lower individual profit per bird (Rs. 63), birds raised at 14.35 birds/m² (D1) achieved the highest estimated profit per m² (Rs. 904). The main cause was the higher stocking density (14.35 birds/m^2), which enabled the production of more birds in a comparable floor area.

On the other hand, birds with 8.61 birds/m² (D3) yielded the highest profit per bird (Rs. 77), indicating improved individual growth performance and production efficiency at a moderate stocking density. However, as fewer birds were housed per unit space (8.61 birds/m^2), the estimated profit per m^2 was reduced to Rs. 663. Similarly, the lowest estimated profit per m² was recorded in D4 (Rs. 510), where the lowest stocking density (7.18 birds/m²) limited stocking capacity substantially, although profit per bird remained adequate. Overall, the optimal stocking density depends on whether the priority is individual bird performance/welfare or profit per floor area.

Table 8 – Economic efficiency per unit floor area under different stocking densities.

Treatment[1]	Space allowance (cm²/bird)	Birds/m²	Live weight density (kg/m²)	Profit/bird (Rs)	Estimated profit/m² (Rs)
D1	697	14.3	17.8	63.0	904
D2	929	10.7	14.5	73.0	785
D3	1161	8.61	12.3	77.0	663
D4	1394	7.18	10.3	71.0	510

[1]D1 = 14.35 birds/m²; D2 = 10.76 birds/m²; D3 = 8.61 birds/m²; and D4 = 7.18 birds/m².
cm^2 = square centimeter; m^2 = square meter; Rs = rupees.
Note: This estimated calculation does not include potential welfare penalties, mortality risk, litter deterioration, ventilation costs, or market restrictions related to stocking density.

DISCUSSION

Stocking density is a significant environmental factor that influences broiler productivity and well-being, especially under heat stress. The current research revealed that reducing the stocking density from 14.35 to 8.61 or 7.18 birds/m² markedly enhanced feed intake (FI) and body weight gain (BWG) and improved the feed conversion ratio (FCR) in broilers subjected to seasonal heat stress. The findings indicate that increased stocking density intensifies the adverse impact of increased ambient temperature on growth performance.

Decreased feed intake and body weight gain at increased stocking densities may be ascribed to limited access to feeders, heightened competition, and compromised thermoregulation. In crowded situations, birds struggle to dissipate metabolic heat, leading to decreased voluntary feed intake as a compensatory strategy to reduce heat production. Comparable declines in growth performance at elevated stocking densities have been shown by Feddes *et al.* (2002), Dozier *et al.* (2006), and Uzum & Toplu (2013), who noted diminished weight gain and reduced feed efficiency in congested environments. Furthermore, heat stress is known to reduce appetite and alter feed metabolism, thereby exacerbating performance declines when birds are raised in high-density conditions.

The improved feed conversion ratio observed in broilers housed at 8.61 and 7.18 birds/m² suggests enhanced nutrient utilization efficiency, likely because of reduced competition and lower stress levels in more spacious housing conditions. Ample space likely enhanced feeder accessibility, improved air circulation at bird level, and reduced behavioral stress, thereby optimizing metabolic efficiency. These findings align with other studies demonstrating that a mild reduction in stocking density enhances growth performance under high-temperature conditions.

In contrast, carcass attributes and yields of cut-up portions were not significantly influenced by stocking density. This indicates that while the growth rate varied across treatments, the carcass composition remained largely consistent. Liu *et al.* (2021) and López-López *et al.* (2022) have reported analogous findings, noting a negligible effect of stocking density on carcass yield metrics. The absence of carcass variation may suggest that density predominantly influences overall growth rather than tissue distribution.

Significant changes were observed in hematological and serum biochemical markers, indicating physiological responses to increased stocking density during heat stress. Birds raised at a higher density (14.35 birds/m²) demonstrated an increased percentage of heterophils, an enhanced heterophil-to-lymphocyte (H:L) ratio, and higher corticosterone levels, all of which are recognized markers of stress activation through the hypothalamic–pituitary–adrenal (HPA) axis. Increased corticosterone enhances gluconeogenesis and lipid mobilization, explaining the elevated levels of glucose, triglycerides, and cholesterol observed in densely stocked birds. Elevated ALT, AST, and ALP activities indicate hepatic stress and potential cellular leakage under combined space-and-heat stress conditions.

In contrast, broilers allotted 8.61 and 7.18 birds/m² exhibited elevated red blood cell counts, hemoglobin concentration, packed cell volume, lymphocyte percentage, and serum protein levels, indicating improved physiological condition and immune function. The diminished H:L ratio in these groups indicates a reduced burden of chronic stress. The results align with studies by Najafi *et al.* (2015), Singh *et al.* (2018), and Kim *et al.* (2021), which indicated that increased stocking density during heat stress elevates stress biomarkers and diminishes immune indicators.

The physiological stress response and metabolic disturbance in broilers may have been caused by overcrowding at elevated ambient temperature. Previous studies have indicated that heat stress can exacerbate oxidative stress and alter metabolic homeostasis, but the oxidative stress markers and molecular pathways were not directly investigated in the present study.

Economically, birds raised at 7.18 birds/m² yielded the highest overall revenue because of higher body weight and increased feed consumption. Despite higher biological performance, the increased production cost at 7.18 birds/m² reduced overall economic efficiency compared with 8.61 birds/m². The optimal balance between biological performance and production cost was achieved at 8.61 birds/m², yielding the highest profit and a favorable cost-benefit ratio. Furthermore, higher stocking densities allowed a greater number of birds to be housed per square meter, resulting in higher estimated profit per unit floor area. However, broilers reared at 8.61 birds/m² exhibited superior individual profitability and physiological performance, indicating a more favorable balance between economic return and bird welfare. Thus, the most economical stocking density depends on whether the production goal is output per bird or output per unit floor area.

These findings emphasize that maximum biological performance does not necessarily correspond to maximum economic efficiency. Instead, a moderate stocking density appears to provide the most sustainable production strategy under seasonal heat stress in open-sided housing systems. Overall, the results indicate that stocking density significantly modulates growth performance, physiological stress responses, and economic returns in broilers exposed to natural heat stress. Providing 8.61 birds/m² appears to represent an optimal compromise between welfare, productivity, and profitability. Providing 8.61 birds/m² maximized per-bird performance and profitability, whereas higher stocking density increased estimated profit per floor area at the expense of physiological and performance indicators.

Moreover, the present study was conducted in an open-sided housing system at a single geographic location and at a single time of year, which may limit the generalizability of the results to other environmental and management contexts. Only male Ross-308 broilers were used in the experiment, and, therefore, sex, genotype, or strain differences were not examined. Furthermore, it was not possible to directly assess the specific effects of heat stress without a thermoneutral control group. In addition, detailed welfare-related assessments, such as behavioral observations, gait scoring, foot pad dermatitis, and litter quality, were not performed, although growth performance and

economic outcomes were assessed. In addition, the economic analysis is a simplified production analysis and assumes no variation in markets, input costs, or long-term factors of production. Physiological measures of stress and health status, including measures of oxidative stress biomarkers, immune-function parameters, and other metabolic measures that may give additional insight into the mechanisms of the observed performance differences, were not studied. Moreover, meat color was recorded only as a composite color index. Consequently, the individual CIE color coordinates (L*, a*, and b*), which provide a more comprehensive characterization of meat appearance, could not be evaluated. Future studies should include objective color measurements using the complete CIE Lab* system to enable a more detailed assessment of meat quality attributes.

An additional limitation of this study relates to the statistical analysis of weekly performance data. Although the pen was considered the experimental unit, weekly measurements were analyzed separately using ANOVA rather than a repeated-measures mixed model. Consequently, correlations among repeated observations within the same pen were not explicitly accounted for, and multiple week-by-week comparisons may have increased the risk of Type I error. Therefore, the phase-wise and overall performance responses should be considered more robust indicators of treatment effects than individual weekly comparisons. Future studies should employ mixed-model methodologies incorporating treatment, time, and treatment × time interactions to better characterize longitudinal growth responses.

It is therefore important to note that the results of this study should be interpreted in light of its specific environmental and management conditions, and that future research should include thermoneutral controls, different broiler strains and sexes, full welfare assessments, and physiological measures to validate and extend these findings.

CONCLUSION

Broilers subjected to seasonal heat stress demonstrated enhanced growth performance and feed efficiency when stocked at 8.61 or 7.18 birds/m², compared with higher stocking densities. Blood biochemical indicators confirmed reduced physiological stress at lower density, while carcass traits remained unaffected. Although 7.18 birds/m² generated higher revenue, 8.61 birds/m² provided the most favorable profit and cost-benefit ratio. Under the conditions of this study, 8.61 birds/m² provided the best compromise among individual bird performance, physiological status, and per-bird profitability, whereas a higher stocking density increased estimated profit per floor area but was associated with poorer performance and stress-related indicators.

ACKNOWLEDGEMENTS

The authors sincerely thank the staff of the Department of Poultry Science, University of Agriculture Peshawar, for providing technical and laboratory facilities. The authors also appreciate the Deanship of Graduate Studies and Scientific Research, Taif University, for supporting the APC of the manuscript.

AUTHOR CONTRIBUTIONS

AH: Conceptualization, methodology, formal analysis, supervision, writing-review & editing; MHN: Conceptualization, methodology, interpreted the study findings, writing, review, and editing; MS: Formal analysis, software, writing original draft preparation, visualization; OU, MM, SHW, IM, KSA, MSU, WFL, ABI: Validation, data curation, writing-review & editing; AAS: Validation, data curation, writing-review & editing and funding acquisition. All authors read and approved the final manuscript.

FUNDING

The authors would like to acknowledge Deanship of Graduate Studies and Scientific Research, Taif University, for funding this work.

DATA AVAILABILITY STATEMENT

Data from the corresponding author is available upon reasonable request.

CONFLICT OF INTEREST

No conflicts of interest were disclosed by the authors.

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