



Research Article

Effect of Stocking Density and Dietary Antimicrobial Inclusion of Male Broilers Grown to 35 Days of Age¹ Part 1: Biochemical and Enzymatical Variables

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Abstract

Objective: This study investigated the effects of recommended stocking densities and dietary antimicrobial inclusion of male broilers grown to 35 days of age on selected hemato biochemical and enzymatical variables. **Materials and Methods:** In each study, a total of 1024 1-day-old Ross × Ross 708 male chicks were randomly distributed into 32 pens based on 4 assigned stocking density treatments. The treatments consisted of 4 densities (27, 29, 33, 39 kg m⁻²) and 2 diets (AGP+, ABF) arranged in a 4 × 2 factorial with eight replicates. Conventional (antimicrobial-growth-promoters, AGP+) and antibiotic free (ABF) diets were equally assigned to each pen with feed and water provided *ad libitum*. Blood samples were collected from the brachial wing vein of 3 birds per pen on d 15, 28 and 35. The collected blood samples were centrifuged to separate the plasma and used to determine the concentration of biochemical parameters and enzyme activities using an ACE-AXCEL automatic analyzer. **Results:** Results show that only uric acid (UA) was affected by stocking density. However, in comparison to broilers fed the ABF diet, broilers with AGP+ had higher significant (p<0.05) effects on albumin (ALB), total protein (TP), alkaline phosphatase (ALP), Gamma-glutamyl transferase (GGT), Ca²⁺ and K⁺ along with lower total bilirubin (TBILI). The results are in broad agreement with those reported in the literature and contribute to our knowledge of blood metabolites and homeostatic variation in developing male broilers. **Conclusion:** The results indicating that stocking densities up to 39 kg m⁻² with appropriate environmental management regardless of antimicrobial addition in the diets may be suitable for both poultry integrators and contract growers to enhance broilers production efficiency without compromising the welfare of broilers grown to 35 days of age.

Key words: Stocking-density, antimicrobial, biochem-enzyme, broilers, welfare

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Data Availability: All relevant data are within the paper and its supporting information files.

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INTRODUCTION

The poultry industry is making rapid progress in improving the efficiency of broiler growth. Advances include improved genetics and nutrition along with changes in environmental management, resulting in more rapid broiler growth. Consumer demand for breast meat has driven a shift in market composition towards increased market weights. Over the past five decades, consumption of poultry meat has increased dramatically, which is expected to continue in the future due to the relative price-competitiveness compared to other meat products¹⁻³. In addition, poultry meat production globally will further grow by 2.4% per year over the next 20 years and the total production in 2030 will be around 160 million tons that will have a share of 39% in total meat production⁴. However, as the demand for animal protein products increases, increasing animal production and production efficiencies will be critical to the continued viability of the U.S. poultry and livestock industries.

Significant expansion in conventional (antimicrobial-growth-promoters, AGP+) and antibiotic-free (ABF) broiler production is expected to continue as demand for poultry products continues to increase and feed prices remain stable. However, there is worldwide concern over the present state of antimicrobial resistance (AMR) among zoonotic bacteria that potentially circulate among food-producing animals including poultry⁵⁻⁸. This has resulted in the general public's perception that antibiotic use by human beings and in food animals selects for the development of AMR among food-borne bacteria that could complicate public health therapies⁹⁻¹³. There is also the public perception that increased production efficiency takes a toll on the well-being and welfare of the animal including poultry. With increasing movement away from antibiotic growth promoters (AGPs) coupled with increasing market weights, modification of traditional methods of housing management and environmental control is necessary to maintain or enhance production efficiency. In addition, much concern nowadays is expressed about stocking density as it relates to the well-being and welfare of broilers. However, as the demand for food animal products increases, increasing production and production efficiencies will be critical to the continued viability of the U.S. livestock industries. A larger part of this concern centers on the question of whether stocking density causes adaptive responses that are characteristic of physiological stress. Moreover, stress imposed by AGP-free production may be mitigated through adjusting stocking density, yet optimum stocking density and associated feeder and drinker space is undefined for broilers reared under AGP and AGP-free

systems. Exposure of poultry to inadequate environmental conditions including stocking density during the course of poultry production has an adverse impact on biochemical variables, electrolytes and metabolites, which can lead to production inefficiencies, compromised animal well-being and increased animal morbidity and mortality¹⁴⁻¹⁶. It is important to recognize that many poultry production managements including stocking density and dietary antimicrobial inclusion can potentially influence blood homeostasis reference ranges and welfare of birds.

The American public concern regarding animal well-being in food production systems continues to grow and increased production efficiency is perceived to negatively impact the well-being of the animal. Environmental management strategies must be developed that increase efficiency without detrimental effects on the welfare of poultry and livestock. Techniques must be developed that increase efficiency without detrimental effects on the welfare of livestock. For these reasons, there is a need for research to quantify the relationship between animal well-being, production and economic factors including genetics, behavior, housing, health, nutrition, management, level of performance, profitability, production efficiencies and food safety. As antibiotic use is reduced or eliminated in animal feed, proper husbandry and environmental management become critical to ensure production efficiency, animal well-being and efficient use of inputs. As use of AGPs are reduced, modification of housing management is necessary to improve sustainability and food safety. In addition, ABF production imposes stressors which may interact to diminish well-being and welfare; however, physiological and immune stress responses to ABF production in commercial broiler chickens are undocumented in the literature.

Changes in the levels of various blood plasma biochemistry and enzyme activity biomarkers are indicators of internal organs' (heart, liver, kidney) health and overall systemic homeostasis. Blood analyses along with other biochemical evaluations have been used to assess the health status of animals¹⁷. Changes in major selected blood variables are routinely used to determine various influences of environmental, nutritional and pathological factors¹⁸. For instance, alterations in the levels of blood biochemistry parameters have been observed for alkaline phosphatase (ALP) and total cholesterol^{19,20}. It has been documented that green muscle disease in broilers is associated with large increase in plasma CK and AST activities²¹. Hence, this study examined the main effects of stocking density, dietary antimicrobial inclusion and their interaction if any on selected

blood plasma biochemical, enzymatical variables and electrolytes levels of male broilers grown to 35 days of age to ensure the health and welfare of broilers.

MATERIALS AND METHODS

Bird husbandry: All procedures relating to the use of live birds in this study were approved by the USDA-ARS Animal Care and Use Committee at the Mississippi State location. In addition, unnecessary discomfort to the birds was also avoided by using proper housing and handling techniques²². This experiment was repeated two times and in each 1024 1-day-old male chicks were obtained from a commercial hatchery. Chicks were equally and randomly allocated to 32 groups inside 32 pens based on stocking density treatment assigned at 50% RH. Conventional (antimicrobial-growth-promoters, AGP+) and antibiotic free (ABF) diets were equally assigned to each pen to give 4 by 2 treatments with 4 replicates. Each pen was equipped with one tube feeder and nipple drinkers. Lighting was provided with 5000K LED bulbs. Lighting intensity, photoperiod and room temperature were adjusted according to experimental design, while minimum ventilation rates were adjusted weekly according to NPTC guidelines. Chicks were vaccinated for Marek's, Newcastle and infectious bronchitis diseases at the hatchery. At 12 day of age, birds received a Gumboro vaccination via water administration. The chicks remained in their respective rooms from 1-day-old throughout the experimental period (1-56 day of age). All birds were fed the same diet throughout the study. Birds were provided a 3-phase feeding program (starter: 1-14 day; grower: 15-28 day and finisher: 29-35 day of age). Diets were formulated to meet or exceed NRC²³ nutrient recommendations for each feeding phase. Starter feed was provided as crumbles and subsequent feeds were provided as whole pellets. Feed and water were offered *ad libitum*. Temperature and relative humidity (RH) on d 1 were maintained at $32 \pm 1.1^\circ\text{C}$ and $50 \pm 5\%$, respectively and RH was held constant across all treatments. Temperature was decreased as the birds progressed in age until 15.6°C was reached at 49 day of age. Bedding material was sourced from a commercial cooperator to mimic commercial conditions and litter microflora. To prevent cross-contamination between AGP+ and ABF pens, shoe covers were used any time any personnel entered pens and shoe covers were changed when going between AGP+ and AGP- treatment pens.

Experimental treatments: The experimental design was a 4×2 factorial consisting of 4 Densities (kg m^{-2}) [27, 29, 33, 39] and 2 Diets [antibiotic free (ABF), conventional (antimicrobial-

growth-promoters, AGP+)]. The AGP diet contained 2 additives of *salinomycin* and *bacitracin* based on manufacture's recommended levels, while the ABF diet did not contain any antimicrobial. Play sand was used in place of the medicated additives. In addition, used litter was obtained from AGP and ABF commercial farms to simulate commercial conditions for both conventional and ABF groups and then placed in respective diet treatment pen. The AGP and ABF treatments pens were partitioned to avoid cross contamination of litters. The LED light source was adjusted to the spectral sensitivity of broiler as indicated in our previous study²⁴. Light intensity settings were verified from the center and four corners of each room at bird level (30 cm) using a photometric sensor with National Institute of Standards and Technology-Traceable calibration (403125, Exttech Instruments, Waltham, MA) for each intensity adjustment. The light bulbs were cleaned weekly to minimize dust build-up, which would otherwise reduce their intensity.

Measurements

Blood collections and chemical analyses: On day 15, 28 and 35 within 45 sec after birds were caught, blood samples were collected between 0800 and 0900 h from a brachial vein of 3 randomly selected birds from each pen. The birds were then returned to the appropriate pens without unnecessary discomfort using proper housing and handling techniques²². Blood samples were put on ice and transferred to the laboratory. Blood samples were then centrifuged at 4000 g for 20 min at 4°C to obtain plasma samples and 2 mL of each of the plasma samples were stored in 2.5 mL graduated tubes at -20°C for later chemical analyses. Plasma samples were removed from the freezer, thawed and analyzed for the plasma concentrations and activities of albumin (ALB), total bilirubin (TBILI), blood urea nitrogen (BUN), creatinine (CREAT), total protein (TP), uric acid (UA), cholesterol (CHOL), low density lipoprotein (LDL-C), high density lipoprotein (HDL), triglycerides (TRIG), alanine aminotransferase (ALT), alkaline phosphatase (ALP), amylase (AMYL), aspartate aminotransferase (AST), creatine kinase (CK), Gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) and lipase (LIP) using ACE-AXCEL (Alfa Wassermann Diagnostic Tech, West Caldwell, NJ) through biochemical and enzymatic rate reactions. Some of these blood parameters are indicators of internal organ health and systemic homeostasis.

Statistical analysis: The experimental design was a randomized complete block design. Treatment structure was a 4×2 factorial arrangement with the main factors consisting of 4 stocking densities (kg m^{-2}) [27, 29, 33, 39] and 2 Diets

[antibiotic free (ABF), conventional (AGP+)]. Individual sample data within each of the replicate units were averaged before analysis and data from the two trials were pooled and analyzed together. A mixed model ANOVA employing PROC MIXED procedure of SAS software²⁵ was used to analyze the data. Trial was a random effect, whereas the stocking density and diets were the fixed effect. The main effects of stocking density and diets and the interaction of these 2 factors on biochemical and enzymatical variables were tested¹³. Means comparisons on d 15, 28 and 35 were assessed by least significant differences and statements of significance were based on $p \leq 0.05$ unless otherwise stated. Analyses of variance combined across days were performed to obtain treatment comparisons averaged across days and to test for treatment interactions with equal variances between days. In addition, ANOVAs for each of the blood sampling days interval was performed. The repeated measures were modeled using a compound symmetry error structure.

RESULTS

The influence of stocking density and dietary antimicrobial inclusion on plasma biochemistry variables are presented in Table 1. As shown in Table 1, there was no effect of stocking density noted on selected blood plasma biochemistry variables but only on uric acid. However in comparison with broilers fed antibiotic free diet (ABF), broilers fed antimicrobial growth promoter (AGP) up to 35 days of age had higher levels of ALB ($p < 0.001$) and TP ($p < 0.035$) along with reduced levels of plasma TBILI ($p < 0.005$). The influence of stocking density and dietary antimicrobial inclusion on plasma enzymes activities are presented in Table 2. There was no effect of stocking density noted on selected plasma enzymes activities but in comparison with broilers fed ABF, broilers fed AGP had higher enzyme activity levels of ALP ($p < 0.001$) and GGT ($p < 0.003$) at 35 days of age. The influence of stocking density

Table 1: Combined main effects of stocking density and dietary antimicrobial inclusion on selected blood plasma biochemistry of male broilers grown to 35 days of age¹

Treatments/variables	Stocking density (kg m ⁻²)					p-value	Diets ²			
	27	29	33	39	SEM ³		AGP+	ABF	SEM ³	p-value
BW (kg)	2.015	1.984	1.995	1.976	0.015	0.547	2.038	1.947	0.011	0.483
Chemistry assays										
Albumin (ALB), g dL ⁻¹	1.054	1.036	1.057	1.056	0.023	0.778	1.077 ^a	1.024 ^b	0.012	0.001
Bilirubin, OR total (TBILI), mg dL ⁻¹	0.526	0.588	0.523	0.511	0.043	0.354	0.294 ^b	0.580 ^a	0.031	0.005
Blood Urea Nitrogen (BUN), mg dL ⁻¹	0.931	0.889	0.896	0.875	0.056	0.398	0.0931	0.856	0.039	0.364
Creatinine (CREAT), mg dL ⁻¹	0.268	0.259	0.259	0.255	0.006	0.686	0.263	0.257	0.004	0.398
Total protein (TP), g dL ⁻¹	2.334	2.244	2.308	2.357	0.056	0.348	2.416 ^a	2.205 ^b	0.039	0.035
Uric acid (UA), mg dL ⁻¹	6.713 ^a	6.558 ^a	6.642 ^a	6.235 ^b	0.197	0.364	6.576	6.348	0.139	0.285
Cholesterol (CHOL), mg dL ⁻¹	104.980	103.320	106.490	106.600	2.625	0.658	105.750	104.440	1.856	0.569
Low density lipoprotein (LDL-C), mg dL ⁻¹	11.000	10.750	11.424	11.764	0.458	0.365	11.639	11.630	0.324	0.065
High density lipoprotein (HDL), mg dL ⁻¹	77.222	76.389	77.958	77.320	1.881	0.356	76.618	77.826	1.330	0.463
Triglycerides (TRIG), mg dL ⁻¹	58.111	54.187	54.771	53.389	2.746	0.265	54.649	55.580	1.941	0.364
Glucose (GLU), mg dL ⁻¹	250.380	249.220	244.290	244.700	2.340	0.176	240.770	243.520	1.667	0.074

^{a,b}Means within a row not sharing a common superscript are significantly different at $p \leq 0.05$. ¹Table values are least squares means of eight replicate pens for each treatment. ²Diets: 1 = conventional (AGP+), 2 = antibiotic free (ABF). ³Pooled SEM for main effects ($n = 8$)

Table 2: Combined main effects of stocking density and dietary antimicrobial inclusion on selected blood plasma enzymes activity levels of male broilers grown to 35 days of age¹

Treatments/variables	Stocking density (kg m ⁻²)					p-value	Diets ²			
	27	29	33	39	SEM ³		AGP+	ABF	SEM ³	p-value
BW (kg)	2.015	1.984	1.995	1.976	0.015	0.547	2.038	1.947	0.011	0.483
Enzyme assays										
Alanine Aminotransferase (ALT), U L ⁻¹	0.042	0.035	0.014	0.014	0.024	0.463	0.017	0.035	0.017	0.356
Alkaline Phosphatase (ALP), U L ⁻¹	6277.700	5751.400	6166.200	5249.600	487.650	0.658	7375.600 ^a	4346.800 ^b	344.820	0.001
Amylase (AMYL), U/L	675.140	595.470	640.900	602.880	34.541	0.597	652.360	604.830	24.424	0.653
Aspartate Aminotransferase (AST), U L ⁻¹	133.910	130.260	129.190	132.840	4.061	0.298	132.130	130.980	2.872	0.697
Creatine Kinase (CK), U L ⁻¹	3546.300	3469.800	3476.400	3595.400	424.920	0.654	3913.100	3930.900	300.070	0.002
Gamma-Glutamyl Transferase (GGT), U L ⁻¹	15.104	15.326	15.139	14.910	0.380	0.365	15.640 ^a	14.600 ^b	0.268	0.003
Lactate Dehydrogenase (LDH), U L ⁻¹	387.320	385.210	371.710	396.360	20.182	0.654	373.480	396.480	14.271	0.569
Lipase (LIP), U L ⁻¹	25.701	20.722	27.188	24.514	3.939	0.543	22.413	26.649	2.785	0.351

^{a,b}Means within a row not sharing a common superscript are significantly different at $p \leq 0.05$. ¹Table values are least squares means of eight replicate pens for each treatment. ²Diets: 1 = conventional (AGP+), 2 = antibiotic free (ABF). ³Pooled SEM for main effects ($n = 8$)

Table 3: Combined main effects of stocking density and dietary antimicrobial inclusion on selected blood plasma electrolytes and Angap levels of male broilers grown to 35 days of age¹

Treatments/variables	Stocking density (kg m ⁻²)				SEM ³	p-value	Diets ²		SEM ³	p-value
	27	29	33	39			AGP+	ABF		
BW (kg)	2.015	1.984	1.995	1.976	0.015	0.547	2.038	1.947	0.011	0.483
Electrolytes										
Calcium (Ca ²⁺), mg dL ⁻¹	2.990	2.980	2.970	2.970	0.011	0.581	2.970 ^a	2.590 ^b	0.008	0.042
Magnesium (Mg) mg dL ⁻¹	2.240	2.370	2.190	2.220	0.170	0.534	2.210	2.260	0.14	0.486
Sodium (Na ⁺), mmol L ⁻¹	148.510	148.530	148.270	148.220	0.491	0.953	148.270	148.490	0.347	0.659
Chloride (Cl ⁻), mmol L ⁻¹	104.800	104.840	104.850	104.780	0.283	0.967	104.830	104.700	0.200	0.636
Potassium (K ⁺), mmol L ⁻¹	4.310	4.270	4.250	4.250	0.044	0.733	4.460 ^a	4.080 ^b	0.031	0.001
Anion Gap (Angap), mEq L ⁻¹	22.650	22.870	22.540	22.210	1.061	0.978	22.590	22.570	0.750	0.983

^{a,b}Means within a row not sharing a common superscript are significantly different at $p \leq 0.05$. ¹Table values are least squares means of eight replicate pens for each treatment. ²Diets: 1 = conventional (AGP+), 2 = antibiotic free ABF) ³Pooled SEM for main effects (n = 8)

and dietary antimicrobial inclusion on plasma electrolytes and angap levels are presented in Table 3. There was no effect of stocking density noted on any of the plasma electrolytes and angap levels. However, in comparison with broilers fed ABF, broilers fed AGP had higher levels of Ca²⁺ ($p < 0.042$) and K⁺ ($p < 0.001$) at 35 days of age.

DISCUSSION

Global Animal Partnership (GAP) stated that SD could not exceed 29 kg/m⁻² for Step 1 in the welfare rating program²⁶. High SD may restrict bird movement, increase litter moisture, microbial growth and impede air flow among others, thereby affecting production performance and welfare. Currently, consumers perceive stocking density to be one of the most important factors that influence animal welfare and the application of optimal welfare standards where low stocking density is believed to result in a higher product quality that relates to the welfare and well-being of the broilers²⁶⁻²⁸. Furthermore, antimicrobials have been added in animal feed to improve growth, feed conversion efficiency and to prevent diseases²⁹. However, there are concerns that the use of antibiotics leads to development of antimicrobials resistance that posing a potential threat to human health³⁰. These mixed opinions still exist on the transfer of antimicrobials resistance genes from animal to human pathogens. In view of the increasing concerns over antibiotic use, the quest for alternate replacements of antibiotic use in animal agriculture has grown over the past two decades.

As it can be seen from Table 1-3 in the present study, final BW of broilers, did not differ among the treatment groups. In addition, as shown in Table 1, there was no significant effect of stocking density on the selected blood plasma biochemistry, enzymes activity, electrolytes variables and angap levels except uric acid. However, in comparison with broilers fed antibiotic free diet (ABF), broilers fed

conventional (antimicrobial growth promoters [AGPs]) diet, had significant ($p < 0.05$) higher levels of albumin (ALB) and total protein (TP), along with significantly ($p < 0.05$) lower levels of total bilirubin (TBILL). Albumin constitutes a large part of the protein fraction of plasma and changes in ALB values in the present study were consistent with those of TP, since ALB will increase when protein intake increases and both are directly related to BW. This specifically occurs after the protein requirement for maintenance and growth are met. Presented results of the biochemical blood parameters on stocking density are without statistically significant differences between treatments groups that indicate the conclusion that no difference in broiler welfare in investigates stocking densities. Concentrations of certain plasma metabolites such as glucose among others have been suggested to be sensitive indicators of stress levels in broiler chickens^{31,32}. The nonsignificant effect of treatments on blood glucose observed in the current study, suggest that these treatments did not present as stressors to broilers under this condition. Similar results are reported by others^{33,34} and they concluded that stocking densities between 30 and 45 kg BW/m² to 49 days of age do not represent causes of stress. Furthermore, they reported that the established concentrations of glucose and total cholesterol in blood of broilers from their studies were within limits of reference values. The lack of treatments effects on HDL and LDL in this study reflected the nonsignificant results that have merged in the cholesterol values, which revealed that values of the lipid biochemical parameters studied were not differed significantly within broilers under different stocking densities. These values reported here were like the results found by others who found no significance on serum cholesterol under different bird densities^{34,35}.

The activity of most enzymes in the blood circulatory system are sensitive indicators of disease processes since they appear in the blood even when damage to the cell membrane is minor. The most enzymes activities in poultry can be

affected by many factors, including sex, age, physiological state, species, breed, nutrition and environmental conditions including stocking density^{36,37}. Most of these enzymes are intracellular enzymes that enter the blood due to cell damage, organs disturbances or diseases, stress or death among others. As indicated in Table 2, stocking density had no significant effect on plasma enzymes activity, indicating that the stocking density used in this study does not compromise broilers welfare and wellbeing. In addition, broilers fed AGP diet had significant higher levels of alkaline phosphatase (ALP) ($p < 0.001$) and gamma-glutamyl transferase (GGT) ($p < 0.003$) in comparison with those fed ABF diet, which may be associated with broilers higher BW (2.038 vs 1.947 lbs.).

As shown in Table 3 in this study, broilers fed AGP diet had significant higher levels of Ca^{2+} ($p < 0.042$) and K^+ ($p < 0.001$) in comparison with those fed ABF diet may be associated with broilers higher in BW (2.038 vs 1.947 lbs.). Calcium is the most abundant electrolyte in the body of poultry that consisting of 1.5% of its body weight, serve as a transmitter of an intracellular signal and improved growth performance and tibia characteristics in broilers³⁸⁻⁴⁰. Physiologically, calcium provides the structural strength of the poultry skeleton by the formation of calcium salts. Moreover, it plays vital roles in many of the biochemical reactions within the body via its concentration in the extracellular fluid. In addition, calcium is also critical for muscle contraction, nerve signaling, blood clotting and maintaining normal heart function. Potassium ion has been shown to be more involved in many metabolic processes, amino acid absorption and transport, including protein synthesis⁴¹.

As shown in Table 3 in this study, birds fed AGP diet in comparison to those fed ABF diet had higher K^+ that may be associated with a higher BW, since K^+ ion concentration have been shown to be involved in many metabolic processes, including amino acid absorption and transport including protein synthesis⁴². Furthermore, it has been shown that potassium is required for the formation of a complex of amino acyl-s-RNA, ribosome(s), messenger RNA and amino acid transport⁴³. It has been documented that K^+ also regulates protein synthesis in the cell and that relatively small decreases in intracellular K^+ result in a reduction in protein synthesis⁴⁴. In addition, Bell⁴⁵ indicated that 50% of the calcium present in blood is bound to ALB, which may explain the higher concurrent TP, ALB and Ca^{2+} levels in broilers fed AGP diet in this study. The activities of ALT and AST, LDH, ALP, GGT in the broilers are indicators of protein metabolism and hepatic damage or injury, while Ck is an indicator of muscle and cardiac damage and stress⁴⁶. Hence nonsignificant treatments effect on these enzymes in the present study, indicates that the welfare of these broilers is not compromise.

CONCLUSION

The nonsignificant final BW and those of selected blood plasma biochemistry and enzyme activities observed are in broad agreement with those reported in the literature and contribute by enhancing our knowledge of homeostasis variation and the range of various blood metabolites in developing male broilers. They would be very useful in detecting not only broiler chicken's welfare conditions but also metabolic nutritional disorders. Therefore, stocking densities up to 39 kg m⁻² with appropriate environmental management regardless of antimicrobial addition in the diets may be suitable for both poultry integrators and contract growers to enhance broilers production efficiency without compromising the welfare of broilers grown to 35 days of age.

SIGNIFICANCE STATEMENT

This study revealed that stocking densities up to 39 kg m⁻² with appropriate environmental management regardless of antimicrobial do not affect broiler welfare and growth performance. This study will help researchers and commercial poultry industry uncover critical broiler management information that will lead to profitable broiler production. Our study adds to the accumulating evidence that stocking density and antimicrobial we used in the study do not trigger organs and muscle damage and stress indicators in broiler chickens.

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