Research Article

The Effect of Different Cage Floors and Feed Nutrient Content on Cage Microclimate, Energy and Protein Retention in Joper Village Chickens

I Made Nuriyasa[1]*, Yonni Koesmaryono[2], I Ketut Puja[3]

[1]Animal Nutrition. Faculty of Animal Husbandry. Udayana University. Bali Indonesia; [2]Department of Geophysics and Meteorology. FMIPA. Bogor Agricultural Institute.Bogor. Indonesia; [3]Veterinary Genetics and Reproduction Technology Laboratory. Faculty of Veterinary Medicine. Udayana University. Bali. Indonesia.

Abstract | This study aimed to determine the retention of metabolizable energy and crude protein in Joper native chickens aged 0–8 weeks, raised on different cage floor types and fed diets with varying nutrient levels. A completely randomized design with a 2 × 4 split-plot arrangement and four replications was employed. The main plot was the cage floor type, consisting of a litter floor (L1) and a battery floor (L2). The subplot was the feed composition, which included four treatments: 2800 kcal/kg ME and 18% CP (P1), 2900 kcal/kg ME and 19% CP (P2), 3000 kcal/kg ME and 20% CP (P3), and 3100 kcal/kg ME and 21% CP (P4). Observed variables included cage microclimate and energy and protein retention in Joper chickens. No significant interaction ($P > 0.05$) was found between cage type and feed across all variables. However, cages with battery floors provided a more comfortable microclimate ($P < 0.05$) than those with litter floors. Feed nutrient level (P1–P4) did not significantly affect cage comfort. Energy and protein retention in chickens reared in battery cages (L2) were 22.98 kcal/bird/day and 4.04 g/bird/day, respectively, compared to 21.18 kcal/bird/day and 3.21 g/bird/day in litter cages (L1). It can be concluded that battery cages create a more favorable microclimate and enhance energy and protein retention in Joper native chickens compared to litter-floor cages.

Keywords | Joper chicken, Different cage floors, Microclimate, Energy retention, Protein retention

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***Correspondence** | I Made Nuriyasa, Animal Nutrition. Faculty of Animal Husbandry. Udayana University. Bali Indonesia; **Email:** madenuriyasa@unud.ac.id
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INTRODUCTION

The continuous growth of the global population has led to an increasing demand for food, electricity, and transportation. These activities contribute to higher greenhouse gas emissions, even when environmentally friendly technologies are adopted. Efforts to reduce methane (CH_4) emissions from the livestock sector and carbon dioxide (CO_2) emissions from human activities are therefore essential (Susan *et al*., 2021). Key factors that require attention to enhance livestock productivity include improving the genetic potential of local breeds, optimizing husbandry management, providing nutritionally balanced feed according to animal requirements, and ensuring comfortable housing environments (Park *et al*., 2018). Poultry housing floor systems markedly affect greenhouse gas emissions. Litter floors promote higher CH_4 and N_2O emissions due to moisture retention and anaerobic zones that enhance microbial activity. In contrast, battery or slatted floors allow rapid manure drying, reducing CH_4 and N_2O formation, although NH_3 volatilization may increase with poor ventilation. Thus, litter systems generally yield higher overall GHG emissions than battery systems.

The Joper native chicken is a crossbreed between a commercial layer hen and a Bangkok native rooster (Samadi *et al*., 2021). Through strict selection, this crossbreeding has produced a superior local chicken line. Joper chickens can be mass-produced with relatively uniform body weights, grow faster than traditional native chickens, and retain the preferred taste of native chicken meat. The production of superior native chickens such as Joper is not limited to commercial enterprises but is also widely practiced by smallholder farmers and backyard producers, particularly in developing countries (Rahayu *et al*., 2023). Although Joper chickens have superior growth genetics, their performance remains strongly dependent on microclimate conditions. Improving temperature, humidity, and ventilation allows birds to express their growth potential more efficiently, reducing maintenance energy costs and improving feed intake. Economically, microclimate improvements usually offer a higher return on investment than installing battery cages because they require lower capital outlay and yield immediate performance benefits. By comparison, battery cages are expensive and improve manure management but contribute less directly to growth. Thus, microclimate optimization is still essential and economically justified even for genetically superior Joper chickens

Previous studies have reported differences in growth rates among native chicken breeds. Saifullah *et al*. (2024) found that the daily weight gain of KUB native chickens was 11.32 g/bird/day, Merawang native chickens 9.07 g/bird/day, and Joper chickens 12.13 g/bird/day (Nuriyasa *et al*., 2024). According to Nuriyasa *et al*. (2023), higher growth performance in chickens is associated with increased feed intake, which consequently raises the demand for metabolizable energy and crude protein. Nutritional requirements in poultry are influenced not only by growth rate but also by the microclimate of the rearing environment. Chickens exposed to heat stress conditions require higher maintenance energy to regulate body temperature, reducing the amount of energy available for growth. Therefore, achieving optimal poultry production requires feed formulations that are adjusted to the environmental conditions of the housing system. Wirapartha *et al*. (2024) demonstrated that properly formulated diets tailored to the needs of poultry under specific environmental conditions can enhance their overall performance.

The present study aimed to evaluate the effects of different cage floor types (litter and battery) and dietary metabolizable energy and protein levels on the cage microclimate and on metabolizable energy and crude protein retention in Joper chickens aged 0–8 weeks. The findings are expected to assist poultry farmers in selecting suitable cage floor systems and adjusting dietary formulations based on local farm conditions, thereby optimizing the growth performance of Joper chickens according to their genetic potential.

MATERIALS AND METHODS

Research location and housing

The research was conducted in a tropical lowland area (150 m above sea level) in Dajan Peken village, using well-controlled experimental cages. In general, tropical lowland regions situated at elevations of 0–500 meters above sea level (m asl) are classified as areas with distinct microclimatic conditions. The battery cages were equipped with wire floors positioned 75 cm above the ground, and the litter-floor cages consisted of a soil base covered with rice husks. Both cage types had identical dimensions: 70 cm in length, 50 cm in width, and 50 cm in height.

Research design

The study employed a split-plot design arranged within a completely randomized structure. The main-plot factor was the cage-floor type consisting of (1) a litter floor (L1) and (2) a battery floor (L2). Each floor type was assigned randomly to the main-plot units, and each main plot was replicated four times. Within each main plot, the subplot factor consisted of four dietary treatments differing in metabolizable energy (ME) and crude protein (CP) levels: P1 (2800 kcal/kg ME, 18% CP), P2 (2900 kcal/kg ME, 19% CP), P3 (3000 kcal/kg ME, 20% CP), and P4 (3100 kcal/kg ME, 21% CP). Subplot treatments were randomly assigned within each main plot, resulting in a total of 2 × 4 treatment combinations with 4 replications, for a total of 32 experimental units.

Each experimental unit consisted of a cage containing 5 Joper native chickens, yielding a total population of 160 birds. Each cage containing five Joper chickens was considered a single experimental unit. All birds within a cage received the same treatment combination and were group-housed; therefore, measurements from individual birds were averaged to obtain cage-level means to preserve statistical independence. Accordingly, all analyses were performed using these cage means, with four replicated cages per treatment. The split-plot structure created two levels of randomization: (1) main plots were randomized first to floor treatments, and (2) subplots were randomized independently within each main plot to feed treatments. This study was conducted following the ethical guidelines for the use and care of experimental animals, and the research protocol was reviewed and approved by the Animal Ethics Committee of Faculty of Veterinary Medicine, Udayana University. No.EC:B/168/UN14.2.9/PT.01.04/2025.

Experimental diets

The research feed used yellow corn flour, rice bran, fish meal, coconut oil, bread waste flour. mineral mix, and NaCl. The composition of the feed ingredients and nutrient content are presented in Tables 1 and 2.

Table 1: Composition of ingredients in treatment feed.

Feed ingredients (%)	Treatment			
	P1	P2	P3	P4
Yellow Corn Flour	45	45	46	49.6
Rice bran	16.5	12.6	12.1	7.5
Fish meal	18.9	21.6	24.6	27.5
Coconut oil	1	2.4	4.6	5.7
Bread waste flour	17.9	17.7	12	9
Mineral mix	0.45	0.45	0.45	0.45
NaCl	0.25	0.25	0.25	0.25
Total	100	100	100	100

Table 2: Nutrient content of treated feed.

Feed nutrient content	Treatment			
	P1	P2	P3	P4
Metabolic Energy (Kcal/kg)	2808.15	2910.57	3000.68	3100.46
Crude Protein (%)	18.03	19.03	20.11	21.12
Crude fat (%)	6.02	5.78	5.98	5.77
Crude fiber (%)	3.39	2.95	2.83	2.33
Ca (%)	0.58	0.63	0.67	0.71
P (%)	0.44	0.47	0.51	0.54

Research variables and measurements

Microclimate variables including air temperature, relative humidity, temperature-humidity index (THI), solar radiation, and wind speed were measured at 07:30, 13:30, and 17:30 daily. The average microclimate conditions were then estimated using the following formula: (07:30 data × 2) + 13:30 data + 17:30 data / 4. This approach provides values that closely approximate those obtained from hourly measurements (24 observations). Air temperature was measured using an EBRO thermometer, air humidity was measured using a digital thermometer-hygrometer model GMK-930HT. Solar radiation intensity was measured using a digital net solar meter and wind speed was measured using a digital anemometer type LM-81AM.

Energy retention

The total energy content of the treatment feed (GE) and the metabolizable energy content of the feces were calculated using a bomb caloriemeter. Crude protein in the treatment feed and feces was analyzed by Kjeldhal method. The amount of gross energy consumed was calculated by multiplying feed consumption by the gross energy content of the feed. Metabolizable energy (ME) was calculated as GE intake minus energy in feces. Energy retention was obtained by subtracting the amount of energy in the body of the Joper village chicken at the end of the study from the amount of body energy of the Joper village chicken at the beginning of the study. Heat Production (PP) was calculated by subtracting ME from RE. Maintenance energy requirements were estimated as the energy requirement when RE = 0 (Nuriyasa *et al*., 2018).

Data analysis

All data obtained were analyzed using a general linear model (GLM) procedure and conducted with IBM SPSS Statistics 25 software. The Least Significant Difference (LSD) test was used to compare mean differences.

RESULTS AND DISCUSSION

The statistical analysis revealed that there was no significant interaction ($P > 0.05$) between the cage floor type and the feed treatment on cage microclimate parameters, metabolizable energy retention, or crude protein retention in Joper village chickens aged 0–8 weeks. This indicates that the effects of floor type and feed treatment were independent, meaning that variations in one factor did not significantly influence the response to the other.

The results of air temperature measurements showed that the cage with a battery base (L2) had an average air temperature of 26.21°C, while the cage with a litter base (L1) showed a 3.63% higher temperature ($P < 0.05$) compared to L2 (Table 3). The significantly lower temperature observed in the battery cage (L2) can be attributed to the better ventilation system and air circulation. In battery-type cages, airflow enters not only from the sides but also from the bottom part of the structure, which facilitates more effective heat dissipation and reduces the accumulation of warm air inside the cage. In contrast, the litter-based cage (L1) exhibited higher air temperatures, likely due to heat generated from microbial activity within the litter material. The fermentation and decomposition processes of organic matter in the litter floor produce metabolic heat, contributing to the overall increase in ambient temperature. This finding is consistent with the report of Nuriyasa *et al*. (2014), who stated that litter fermentation could elevate cage temperatures due to microbial respiration and biochemical reactions occurring in the bedding material. The attribution of higher temperatures in litter cages solely to microbial fermentation remains inconclusive because litter moisture was not measured. The insulating properties of rice husk litter may also contribute to reduced heat dissipation. Moreover, the discussion should acknowledge broader literature showing that although battery cages improve thermal conditions, they may compromise animal welfare compared with litter systems. These factors warrant further investigation in future studie.

Table 3: Microclimate in the Joper village chicken coop maintained with litter and battery coop floors.

Variable	Cage floor treatment[1)]		
	L1	L2	SEM
Air Temperature in the Cage (°C)	27.17[b]	26.21[a]	0.07
Air Humidity in the Cage (%)	76.75[b]	75.82[a]	0.05
Temperature Humidity Index (THI)	28.09[b]	27.13[a]	0.06
Solar radiation intensity (fc)	5.48[a]	5.85[a]	0.93
Wind Speed (m/s)	0.47b	0.89a	0.01

1) L1: Cage with litter floor, L2: Cage with battery floor, 2) The same superscripts in the same row indicate a non-significant difference (P>0.05), and different superscripts in the same row indicate a significant difference (P<0.05).

Table 4: Energy balance in the body of native Joper chickens raised in litter and battery floor cages.

Variable	Treatment[1)]		
	L1	L2	SEM[3)]
Gross Energy Consumption (K.cal/day)	96.63[a2)]	99.18[a]	4.25
Fecal Energy (K.cal/day)	6.12[a]	6.15[a]	0.81
Metabolizable Energy (Kcal/day)	90.51[a]	93.03[a]	2.74
Energi Retensi (K.cal/day)	21.18[a]	22.98[b]	0.23
Heat Production (K.cal/day)	69.33[a]	70.05[a]	2.95
Energy for Growth (Kcal/day)	30.25[b]	32.83[a]	0.89
Maintenance energy (Kcal/day)	60.25 [a]	60.20 [a]	3.05
Maintenance Energy/W0.75(Kcal/day)	72.85[a]	68.04[b]	3.26
Total Energy Requirements (Kcal/day)	90.51[a]	93.03[a]	1.82
ME/growth requirement (K.cal/1 g growth)	10.27[a]	9.61[b]	0.22
Heat Production (K.cal$W^{0.75}$/day)	83.82[a]	79.18[b]	2.60
Growth Energy (Kcal/1 g growth)	0.041[a]	0.40a	0.03
Energy Efficiency (%)	23.40 [a]	24.70 [a]	0.61

1) L1: Cage with litter floor, L2: Cage with battery floor, 2) The same superscripts in the same row indicate a non-significant difference (P>0.05) and different superscripts in the same row indicate a significant difference (P<0.05), 3) SEM: Standard Error of the Treatment Means.

Furthermore, Dharmawan *et al.* (2023) found similar results, where a battery cage system with three tiers produced a lower temperature (26°C) compared to a single-tier cage system (27.5°C). This supports the notion that air movement and cage design, including vertical stratification, play an important role in regulating thermal conditions within the housing environment.

Overall, the results indicate that battery cage systems provide a cooler and more thermally stable microclimate compared to litter-based systems. The reduced temperature in battery cages could potentially enhance animal comfort and reduce heat stress, especially under tropical conditions. Conversely, while litter cages may offer other management or welfare benefits, their tendency to retain heat should be considered in designing ventilation systems or choosing suitable bedding management practices. The average air humidity was 75.82% in L2 cages, which was significantly lower (P<0.05) than the 76.75% measured in L1 cages. The air humidity in the litter cage was higher than the battery cage because the litter cage could store air so it was more humid than the battery cage (L2). The temperature in the litter cage (L1) was higher than the battery cage (L2). which would accelerate the evaporation of air from the litter so that the measured humidity was higher. in accordance with the opinion of Nuriyasa *et al.* (2024). The Temperature Humidity Index (THI) is calculated using the empirical formula according to Marai (2002) as follows:

$$THI = T - [(0.31 - 0.31 \times RH)(T - 14.4)]$$

Description: THI: Temperature Humidity Index, T: Average temperature in the enclosure (°C), RH: Relative humidity.

Table 5: Protein balance in the body of native Joper chickens raised in litter and battery floor cages.

Variable	Treatment		
	L1	L2	SEM
Protein Consumption (g/bird/day)	4.82a	4.82[a]	0.48
Protein in Feces (g/bird/day)	0.41[a]	0.43[a]	0.10
Digestible Protein (g/bird/day)	4.41	4.39 [a]	0.38
Protein Retention (g/bird/day)	3.27 [b]	4.04[a]	0.21
Growth Protein (g/bird/day)	0.073[a]	0.067[b]	0.02
Protein Maintenance (g/bird/day)	4.75[a]	4.76[a]	0.67
Total Protein Requirement (g/bird/day)	4.82a	4.83[a]	0.69

1) L1: Cage with litter floor, L2: Cage with battery floor, 2) The same superscripts in the same row indicate a non-significant difference (P>0.05) and different superscripts in the same row indicate a significant difference (P<0.05), 3) SEM: Standard Error of the Treatment Means.

Furthermore: a THI value below 27.8 indicates that the livestock are comfortable, THI value in the range of 27.8 - 28.9 indicates that the livestock in the enclosure are experiencing moderate heat stress, THI in the range of 28.9 - 30 indicates that the livestock are experiencing high levels of heat stress, and a THI value above 30 indicates very high levels of heat stress. The THI calculation for a battery-bottomed enclosure yielded a value of 27.13, indicating that the enclosure is comfortable. The cage with litter base is 28.09 which indicates that the livestock is in a mild heat stress condition.

The L1 cage floor recorded solar radiation entering the cage, namely 5.48 fc, while the L2 cage floor caused solar radiation 6.75% higher (P>0.05) compared to L1. The difference in different cage floors did not affect the intensity of solar radiation, because the cage plot with the litter cage floor and the battery cage floor were in one cage, so the intensity of solar radiation did not differ between different cage floors.

The wind speed in the L2 cage was 0.89 m/s. while the wind speed in the L1 cage was 47.19 lower (P<0.05) compared to L2. The wind speed in the L2 cage was higher than the L1 cage because the L2 cage floor was higher than the L1 floor. The L1 cage floor had higher frictional resistance to the wind than the L2 floor so that the wind speed reaching the L1 cage was lower, in accordance with the opinion of Nuriyasa *et al*. (2014). The difference in different cage floors did not affect the solar radiation intensity. because the cage plot with the litter cage floor and the battery cage floor were in one cage. so the solar radiation intensity did not differ between different cage floors.

Energy retention in the body of Joper village chickens

Gross energy consumption of Joper native chickens raised in litter floor cages (L1) was 96.63 Kcal/day while energy consumption in battery floor cages (L2) was 2.64% higher (P>0.05) compared to L1. This was because the feed consumption of Joper chickens raised in L2 cages (33.74 g/bird/day) was not significantly different compared to the feed consumption of chickens in L1 cages (33.35 g/bird/day). The energy content in the feces of chickens raised in L1 cages was 6.12 Kcal/kg while the feces content of chickens in L2 cages was 6.15 Kcal/kg which was not statistically significantly different (P>0.05). Joper chickens raised in L1 cages produced 90.51 Kcal/kg/day of metabolizable energy while chickens in L2 cages were 2.78% higher (P>0.05) compared to L1 cages.

The gross energy consumption of Joper native chickens did not differ significantly between treatments, and the gross energy content of Joper native chicken feces did not differ significantly between treatments. Indigenous chickens exhibit strong environmental adaptability, including tolerance to a wide range of dietary energy and protein levels. The broad ranges of energy and protein contents selected in this study are intended to provide sufficient flexibility for Joper native chickens to regulate their feed intake in accordance with their physiological energy and protein requirements. This resulted in no significant difference in metabolizable energy consumption of chickens in cage L1 compared to cage L2. Joper native chickens raised in cage L1 produced an energy retention of 21.18 kcal/day, while those raised in cage L2 produced an energy retention 8.50% higher (P<0.05) than those raised in cage L1. Energy retention in Joper village chickens kept in L1 cages is lower than in L2 cages due to the use of energy to overcome environmental conditions (maintenance) in Joper village chickens kept in L1 cages being higher than in L2 (72.85 K.cal/hr vs 68.04 K.cal/hr). This increase in energy for maintenance is due to an increase in heart rate and respiration rate which is the livestock's response to supply more oxygen to the body. in accordance with the opinion of (Nuriyasa *et al*., 2014).

Heat production of chickens in cage L2 was 70.05 Kcal/day while chickens in cage L1 were 1.03% lower (P>0.05) (Table 4). Energy for heat production is the energy used for the needs of maintaining body temperature. metabolic processes and carrying out physical activities (McDonald *et al*., 2010). In terms of quantity. heat production of chickens in cage L2 was slightly higher than L1 because the gross energy consumption of chickens in cage L2 was higher so that the metabolic process was slightly higher and heat production was also slightly higher. The energy requirement for maintenance per metabolic body weight (W0.75) in joper chickens kept in L2 cages is 68.04 Kcal/kg/day while in L1 cages it is 7.07% higher than L2. Joper village chickens kept in L1 cages require higher maintenance energy than L2 cage chickens because the microclimate conditions of L1 cages are more uncomfortable compared to L2 cages, which is indicated by the THI value of L1 cages being higher than L2 cages (Table 1). Uncomfortable cage conditions cause the energy used for maintenance to be higher in accordance with the opinion of (Nuriyasa *et al*., 2014, 2020).

The total energy requirement of Joper chickens raised in L2 cages is 93.03 Kcal/day, while the total energy requirement of chickens in L1 cages is 2.71% (P>0.05) compared to chickens in L2 cages. Joper chickens require an average of 91.77 Kcal/head/day of metabolizable energy in accordance with the opinion of (Sheikh *et al*., 2010). According to calculations. the metabolizable energy requirement per 1 g of body weight gain. chickens in L2 cages require 9.61 Kcal/day of metabolizable energy while chickens in L1 cages require 6.87% lower energy (P<0.05) than L2 cages. The energy for growth of Joper chickens raised in L2 cages is 0.40 Kcal/1 g of growth while those in L1 cages require 2.50% lower energy than those in L1 cages. In terms of calculations. the efficiency of energy use in the L2 cage was 24.70% while the energy efficiency in the L1 cage was 23.40% which was not statistically significantly different (P>0.05).

The total energy requirement of Joper village chickens housed in L2 cages was quantitatively higher than those in L1 cages due to their faster growth rate (14.21 g/bird/day in L2 vs. 13.22 g/bird/day in L1), as reflected in their greater weight gain (Wirapartha *et al*., 2024). However, the energy needed to produce each unit of body weight gain was actually lower in chickens kept in L2 cages, indicating that their energy utilization efficiency was higher than that of chickens in L1 cages.

Protein retention in the body of Joper village chickens

The protein consumption of Joper native chickens in L1 and L2 cages was the same, namely 4.82g/day (Table 5). The protein content in the feces of Joper native chickens in L1 cages was 0.41g/day, while in L2 cages it was 4.88% lower (P>0.05) than L1. Digestible protein in Joper native chickens raised in L1 cages was 4.41g/day. while in L2 it was 2.0% lower (P>0.05) compared to L1.

The L1 and L2 cage treatments did not affect the amount of protein consumption of Joper chickens because feed consumption in different cage treatments did not have a significant effect. The protein content in the feces of chickens raised in litter floor cages (L1) and battery (L2) did not differ significantly because the feed ingredients were the same. This condition caused the digestible protein of Joper chickens in cages with different cage floors to also not differ significantly. The results of this study are in accordance with the results of research (Nuriyasa *et al*., 2014).

Protein retention in the body of Joper village chickens in L1 cages was 3.27 g/bird/day while protein retention in the body of Joper village chickens in L2 cages (14.21 g/ bird/day) was 23.55% higher (P<0.05) compared to L1 cages (13.22 g/ bird/day). Protein retention in the body of Joper village chickens in L2 cages was higher compared to L1 cages because the growth rate of chickens in L2 cages was higher than L1, in accordance with research (Saifullah *et al*., 2024).

Protein for growth in Joper native chickens kept in L1 cages is 0.07g/bird/day while in L2 cages it is 8.96% lower (P<0.05) compared to L1 cages. Protein for maintenance needs of Joper native chickens in L1 cages is 4.75g/bird/day while in L2 cages it is 0.21% higher (P>0.05) compared to L1. This is related to the less comfortable conditions of L1 cages, which is indicated by a higher THI value compared to L2 cages (Table 1). Uncomfortable conditions in L1 cages cause chickens to have to expend extra energy to maintain body temperature (panting), increase blood circulation, and metabolic activity for cooling. Energy that should be used for growth is instead diverted to the process of maintaining body temperature according to (Qaid *et al*., 2021).

The total protein requirement for Joper native chickens raised in L1 cages is 4.82 g/bird/day, while in L2 cages it is 0.21% higher compared to L1. This is because the protein requirement for growth of Joper native chickens in L1 cages is higher and the protein requirement for maintenance is lower, so the total protein requirement is not significantly different. Chickens in L1 cages require 6.87% lower energy (P<0.05) than L1 cages. This is a clear typo that confuses the interpretation. Which cage is it? This must be corrected.

CONCLUSIONS

This study demonstrates that, under the specific conditions tested, the battery cage system provided a more stable microclimate and was associated with more favorable physiological indicators compared to the deep-litter system. These findings reflect microclimate-related physiological comfort only, and do not imply superior overall welfare, as important behavioral dimensions such as opportunities for exploration, dust-bathing, and foraging were not assessed. Future research integrating behavioral welfare indicators, long-term performance metrics, and economic assessments is needed to form comprehensive recommendations for poultry housing systems.

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NOVELTY STATEMENT

This study is the first to link cage floor-induced microclimate differences with metabolizable energy and crude protein retention in Joper native chickens under tropical conditions. By quantifying maintenance energy requirements and nutrient retention, this research moves beyond conventional performance indicators and provides physiological evidence of how housing systems influence nutrient utilization efficiency.

AUTHOR'S CONTRIBUTION

IMN and IKP designed the research and conducted laboratory analyses. IMN and YK were responsible for statistical data analysis. IMN, YK and IKP prepared the publication article. All authors contributed equally to the final version of the paper.

Generative AI and AI-assisted technology statement

The authors used generative AI and AI-assisted technologies only for language polishing. All analyses, interpretations, and conclusions remain the sole responsibility of the authors.

Conflict of interest

The authors have declared no conflict of interest.

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