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Effects of housing system and dietary mannan oligosaccharides on broiler performance, corticosterone response to transportation, and selected caecal bacterial populations under humid tropical conditions

ZULAIKHA ZAINOOL ABIDIN
Universiti Putra Malaysia
ZULKIFLI IDRUS
Universiti Putra Malaysia https://orcid.org/0000-0002-2082-7430
ELMUTAZ ATTA AWAD
Universiti Putra Malaysia
SURIYA KUMARI RAMIAH
International Medical University

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Abstract

This study examined the effects of housing system and dietary mannan oligosaccharides (MOS) on growth performance, serum corticosterone (CORT), and selected caecal bacterial populations in broiler chickens exposed to road transportation under humid tropical conditions. A total of 440 one-day-old male Cobb 500 chicks were assigned to a 2 × 2 factorial arrangement involving naturally ventilated (NVH) or mechanically ventilated housing (MVH) and a control diet or the same diet supplemented with MOS at 2.0 g/kg. On day 42, separate groups of birds were sampled before transport or after 4 h of road transportation. Broilers reared in MVH had greater final body weight and better feed conversion than those reared in NVH. MOS supplementation also improved final body weight and feed conversion ratio. Transportation increased serum CORT concentrations, while birds reared in MVH and those fed MOS had lower overall CORT concentrations. No significant interactions were detected among housing system, diet, and sampling time for CORT. Housing system also affected selected caecal bacterial populations, with lower *Clostridium* spp. and higher *Lactobacillus* spp. counts in MVH birds. MOS supplementation and transportation did not significantly affect the measured bacterial populations. These findings indicate that housing environment and MOS supplementation mainly influenced broiler performance and physiological status independently under humid tropical conditions, while short-term transportation increased CORT without measurable changes in the selected caecal bacterial populations.

Introduction

Poultry production has increased rapidly in tropical and subtropical regions of Southeast Asia because of population growth and increasing demand for affordable animal protein (Godfray et al. 2010; Scanes 2018). Broiler production in these regions, however, is often challenged by high environmental temperature and humidity. Prolonged exposure to these conditions can reduce growth and affect physiological stability and bird welfare (Renaudeau et al. 2012; Lara and Rostagno 2013). These problems may become more important with climate change, particularly where broilers are commonly reared under naturally ventilated conditions (Tawfeek et al. 2014).

Housing conditions shape much of the thermal environment broilers experience. Mechanically ventilated housing (MVH) provides better control of temperature, humidity, and air movement, which can help reduce heat load under tropical conditions. Naturally ventilated housing (NVH), on the other hand, depends more on the prevailing environment and birds may experience greater fluctuations in temperature and humidity (Zulkifli et al. 2009; Lara and Rostagno 2013). High temperature and humidity can impair nutrient digestion and absorption, increase oxidative stress, and affect intestinal integrity. These effects are commonly associated with lower feed intake, reduced weight gain, and poorer feed efficiency (Lara and Rostagno 2013; Sohail et al. 2010; Zhao et al. 2014; Wasti et al. 2020). Despite MVH's advantages, its higher installation and operating costs may limit its use in some production systems.

The rearing environment may also influence how birds respond to stress later in life. Transportation is a major stressor for broilers before slaughter. During transport, birds are exposed to handling, confinement, vibration, noise, and changes in environmental conditions. These challenges can activate the hypothalamic–pituitary–adrenal axis and increase circulating corticosterone concentrations (Nijdam et al. 2005; Al-Aqil and Zulkifli 2009). Most transportation studies have focused on journey duration and environmental conditions during transport. The possible influence of the housing environment before transport has received less attention.

Transportation stress may also affect the intestinal environment. Changes in gut motility, mucosal integrity, and immune regulation can influence gastrointestinal bacterial populations (Bailey et al. 2011; Tannock 2016). Changes in selected caecal bacterial populations may therefore provide additional information on intestinal responses to transportation. However, these changes should not be taken as direct evidence of food-safety risk because the study did not measure bacterial shedding or carcass contamination. Little is known about whether birds reared under different housing conditions show different intestinal bacterial responses when transported under humid tropical conditions.

Dietary intervention may offer another way to support birds exposed to environmental and preslaughter stress. Mannan oligosaccharides (MOS) have been used in poultry diets because of their reported effects on growth performance and gut health (Spring et al. 2000; Hooge 2004). Prebiotics may influence intestinal morphology, epithelial integrity, immune responses, and host–microbe interactions, although changes in bacterial populations are inconsistent (Chacher et al. 2017). The effect of MOS on broilers' response to road transportation has received relatively little attention, particularly when birds are reared under different housing systems in a humid tropical environment.

Few studies have considered housing system, MOS supplementation, and transportation together under tropical conditions. Examining these factors in the same experiment may help determine whether the effects of housing and MOS on physiological responses and selected intestinal bacterial populations are independent or whether they change the birds’ response to transportation. The present study therefore evaluated the effects of housing system and dietary MOS supplementation on growth performance, serum corticosterone response to road transportation, and selected caecal bacterial populations in broiler chickens reared under humid tropical conditions.

Materials and methods

Ethics statement

All procedures were approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (Approval number: UPM/IACUC/AUP-R056/2020)

Experimental design, animals, and housing

A total of 440 day-old male Cobb 500 chicks were obtained from a local commercial hatchery. Chicks were randomly allocated to 20 floor pens (22 birds per pen), with 10 pens located in a conventional naturally ventilated house (NVH) and 10 pens in a mechanically ventilated house (MVH). Pens were constructed on concrete floors and bedded with wood shavings to a depth of 8 cm. The floor space allowance was 0.10 m² per bird in both housing systems.

In the NVH, birds were exposed to cyclic ambient temperatures ranging from 24°C (minimum) to 34°C (maximum) and relative humidity (RH) between 80% and 90%. In the MVH, ambient temperature was set at 32°C on day 1 and gradually reduced to 23°C by day 21, while RH was maintained between 65% and 75%. Lighting was provided continuously during the first two days, after which an 18-h light schedule was applied from day 3 onwards in both housing systems.

Birds were fed commercial broiler starter diets (crumble form; 12.56 MJ/kg metabolizable energy and 21.0% crude protein) from days 1 to 21 and finisher diets (pellet form; 12.98 MJ/kg metabolizable energy and 19.0% crude protein) from days 22 to 42. Chicks were vaccinated against Newcastle disease via the intraocular route at 7 and 21 days of age.

Within each housing system, birds were assigned to one of two dietary treatments from days 1 to 42: a basal diet (control) or the basal diet supplemented with a commercial mannan oligosaccharide product (Bio-Mos®; Alltech Inc., Nicholasville, KY, USA) at 2.0 g/kg diet. The experimental diets were formulated to be iso-caloric and iso-nitrogenous. Each housing system × diet combination included five pens with 22 birds per pen.

Road transportation

On day 42, 20 birds from each housing system × diet subgroup (four birds per pen) were randomly selected from their home pens and placed into plastic transport crates (0.80 × 0.60 × 0.31 m) at a density of 10 birds per crate. At 0900 h, the crates were loaded onto an open truck and transported for 4 h at an average speed of approximately 80 km/h. The transport route included highways, high-traffic roads, and traffic-light intersections. Ambient temperature and relative humidity during transportation were approximately 27–34°C and 70–85%, respectively. Feed and water were not provided to the crated birds during transportation.

Blood and caecal content sampling

Separate groups of birds were sampled before transportation (0 h) and after 4 h of road transportation. At 0 h, 20 birds from each housing system × diet subgroup (four birds per pen) that had not been crated or transported were randomly selected from their home pens. A separate group of 20 birds from each subgroup (four birds per pen) underwent 4-h road transportation and was sampled immediately after transport. Thus, we used different birds at the two sampling times.

All sampled birds were slaughtered in accordance with halal procedures (Farouk et al. 2014), during which blood samples were collected by exsanguination. Following blood collection, caecal contents were collected from each bird, rapidly frozen in liquid nitrogen, and stored at − 80°C until analysis.

Serum corticosterone determination

Serum corticosterone concentrations were measured using a commercial high-sensitivity enzyme immunoassay (EIA) kit (AC-15F1, IDS, Boldon, UK). The intra- and inter-assay coefficients of variation were < 6.7% and < 7.8%, respectively, and the assay range was 0.17–16 ng/mL. We performed the assay according to the manufacturer's instructions, as previously described by Zulkifli et al. (2014) and Najafi et al. (2015).

Caecal bacterial quantification

DNA was extracted from the caecal contents using QIAamp® DNA Stool Mini Kits according to the manufacturer's instructions. Selected caecal bacterial populations (*Lactobacillus* spp., *Escherichia coli*, *Clostridium* spp., and *Salmonella* spp.) were quantified by quantitative PCR (qPCR) following the procedures described by Mitin et al. (2026) and Navidshad et al. (2012). PCR products were purified using the MEGA quick-spin™ system (Intron Biotechnology, Inc.), and DNA purity and concentration were determined using a NanoDrop ND-1000 spectrophotometer. Standard curves were generated using serial dilutions of PCR products obtained from pure cultures. Quantitative PCR was performed using a Bio-Rad CFX96 Touch system (Bio-Rad, USA) with optical-grade plates. Melting-curve analysis was conducted to confirm amplification specificity. Amplification efficiency and correlation coefficients (R^2) of the standard curves were verified. Bacterial populations were expressed as $\log_{10}$ CFU/g of caecal contents. The primer sequences used for bacterial quantification are presented in Table 1.

Table 1
Primers used for the quantitative PCR determination of selected caecal bacterial populations

Bacterial group	Primer	Sequence (5'–3')
Salmonella spp.	Forward	TCGTCATTCCATTACCTACC
	Reverse	AAACGTTGAAAAACTGAGGA
Lactobacillus spp.	Forward	CAT CCA GTG CAA ACC TAA GAG
	Reverse	GAT CCG CTT GCC TTC GCA
Escherichia coli	Forward	GTG TGA TAT CTA CCC GCT TCG C
	Reverse	AGA ACG CTT TGT GGT TAA TCA GGA
Clostridium spp.	Forward	GAG TTT GAT CMT GGC TCA G
	Reverse	CCC TTT ACA CCC AGT AA
Primer sequences were as reported by Mitin et al. (2026), following Navidshad et al. (2012).		

Growth performance

Bird body weights were recorded on a pen basis on days 1 and 42. Feed intake was recorded, and feed conversion ratio (FCR) was calculated as feed intake divided by body weight gain. Mortality was recorded daily and used to adjust performance calculations where appropriate. Growth performance data were summarised and analysed by pen.

Statistical analysis

Data were analysed using the General Linear Model (GLM) procedure of SAS software (SAS Institute Inc., Cary, NC, USA). Growth performance variables (body weight, feed intake, and feed conversion ratio) were analysed as a 2 × 2 factorial arrangement with housing system (naturally ventilated vs. mechanically ventilated) and diet (basal diet [control] vs. basal diet supplemented with mannan oligosaccharides [MOS]) as fixed effects, including the housing system × diet interaction. The pen was considered the experimental unit for growth performance measurements. Cumulative mortality data were analysed using the chi-square test to compare the frequency of mortality among the four housing system × diet treatment groups.

Serum corticosterone concentrations and caecal bacterial population data (*Escherichia coli*, *Clostridium* spp., and *Lactobacillus* spp.) were analysed using a 2 × 2 × 2 factorial model with housing system, diet, and sampling time relative to transportation (0 h vs 4 h) as fixed effects. The model included all two-way interactions and the three-way housing system × diet × sampling time interaction. Because different birds were sampled before and after transportation, we treated sampling time as a between-subjects

factor. The individual bird was considered the experimental unit for serum CORT and caecal bacterial measurements.

Normality of the data was first assessed using the Shapiro–Wilk test. Homogeneity of variance was also assessed before analysis. Serum CORT concentrations were square-root-transformed where necessary to meet model assumptions, whereas bacterial counts were analysed following $\log_{10}$ transformation. *Salmonella* spp. were not included in the statistical analysis because they were not detected in the samples. When significant main effects or interactions were detected, means were compared using Tukey's test. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Growth performance and mortality

There were no significant housing system × diet interactions for total feed intake, final body weight, or overall feed conversion ratio (FCR) ($p > 0.05$; Table 2). Housing system significantly affected all growth performance variables. Broilers reared in NVH had lower total feed intake ($p < 0.0001$) and final body weight ($p < 0.0001$), and a higher overall FCR ($p < 0.0001$) than broilers reared in MVH. Dietary supplementation with mannan oligosaccharides (MOS) significantly increased total feed intake ($p = 0.0062$) and final body weight ($p < 0.0001$), and improved overall FCR ($p < 0.0001$) compared with the control diet. Mortality did not differ significantly among the four housing system × diet treatment groups ($\chi^2 = 3.70$, df = 3, p = 0.296). Mortality was 10.9%, 9.1%, 6.4%, and 4.5% in the NVH-control, NVH-MOS, MVH-control, and MVH-MOS groups, respectively.

Table 2
Effects of housing system and dietary MOS supplementation on total feed intake, final body weight, and overall feed conversion ratio (FCR) in broiler chickens

Variable	Total feed intake days 1–42 (g/bird)	Final body weight day 42 (g/bird)	Overall FCR days 1–42 (feed/gain)
Housing system (HS)			
NVH	4372 ± 26.26	2606 ± 21.08	1.68 ± 0.004
MVH	4812 ± 37.32	2893 ± 25.86	1.66 ± 0.002
Diet (D)			
Control	4553 ± 59.13	2712 ± 39.14	1.68 ± 0.003
MOS	4631 ± 59.40	2787 ± 40.20	1.66 ± 0.003
p-value			
HS	< 0.0001	< 0.0001	< 0.0001
D	0.0062	< 0.0001	< 0.0001
HS × D	0.8866	0.8693	0.3168
Values are presented as mean ± SEM.			
NVH, naturally ventilated house; MVH, mechanically ventilated house; Control, basal diet; MOS, basal diet supplemented with 2.0 g/kg Bio-Mos.			
, Means within a column and within the same main effect with no common superscripts differ significantly at $p < 0.05$.			

Serum corticosterone concentrations

No significant two- or three-way interactions among housing system, diet, and sampling time were observed for serum corticosterone (CORT) concentrations ($p > 0.05$; Table 3). Sampling time relative to transportation significantly affected serum CORT concentrations, with birds sampled after 4 h of road transportation having higher CORT concentrations than birds sampled before transportation ($p < 0.0001$). Housing system also significantly affected serum CORT concentrations, with broilers reared in NVH having higher concentrations than those reared in MVH ($p = 0.0057$). Dietary MOS supplementation resulted in lower serum CORT concentrations than the control diet ($p = 0.0262$), irrespective of housing system or sampling time.

Table 3
Effects of housing system, dietary MOS supplementation, and sampling time relative to transportation on serum corticosterone concentrations (CORT; ng/mL) in broiler chickens

Variable	CORT (ng/mL)
Housing system (HS)	
MVH	1.27 ± 0.11
NVH	1.50 ± 0.08
Diet (D)	
Control	1.50 ± 0.11
MOS	1.30 ± 0.08
Sampling time (T)	
Before transport (0 h)	1.22 ± 0.09
After transport (4 h)	1.77 ± 0.17
p-value	
T	< 0.0001
HS	0.0057
D	0.0262
T × HS	0.1321
T × D	0.3392
HS × D	0.1507
HS × D × T	0.9777
Values are presented as mean ± SEM.	
MVH, mechanically ventilated house; NVH, naturally ventilated house; Control, basal diet; MOS, basal diet supplemented with 2.0 g/kg Bio-Mos; T, sampling time relative to transportation.	
, Means within the same main effect with no common superscripts differ significantly at $p < 0.05$.	

Caecal bacterial populations

No significant two- or three-way interactions among housing system, diet, and sampling time were detected for any of the caecal bacterial populations examined ($p > 0.05$; Table 4). Housing system significantly affected caecal *Clostridium* spp. and *Lactobacillus* spp. populations. Broilers reared in MVH had lower *Clostridium* spp. counts than those reared in NVH ($p = 0.0207$), whereas *Lactobacillus* spp. counts were higher in MVH birds ($p = 0.0001$). Caecal *Escherichia coli* populations were not affected by

housing system (p = 0.7618), diet (p = 0.4933), sampling time (p = 0.9891), or their interactions. Dietary MOS supplementation and sampling time did not significantly affect caecal *Clostridium* spp. or *Lactobacillus* spp. populations (p > 0.05). *Salmonella* spp. were not detected in any samples and were therefore not included in the statistical analysis.

Table 4
Effects of housing system, dietary MOS supplementation, and sampling time relative to transportation on caecal bacterial counts (log_{10} CFU/g) in broiler chickens

Variable	***Clostridium* spp.**	***E. coli***	***Lactobacillus* spp.**
Housing system (HS)			
MVH	6.53^{b} ± 0.17	3.751 ± 0.092	6.11^{a} ± 0.08
NVH	6.95^{a} ± 0.06	3.696 ± 0.065	5.59^{b} ± 0.09
Diet (D)			
Control	6.84 ± 0.06	3.703 ± 0.074	5.81 ± 0.10
MOS	6.64 ± 0.18	3.810 ± 0.077	5.83 ± 0.09
Time (T)			
Before transport (0 h)	6.68 ± 0.17	3.94 ± 0.11	5.78 ± 0.09
After transport (4 h)	6.81 ± 0.08	3.93 ± 0.09	5.91 ± 0.10
p-value			
HS	0.0207	0.7618	0.0001
D	0.2563	0.4933	0.8152
T	0.5192	0.9891	0.3274
HS x D	0.3135	0.7873	0.8740
HS x T	0.5343	0.3578	0.8100
D x T	0.1396	0.3820	0.6046
HS x D x T	0.2051	0.1570	0.4930
Values are presented as mean ± SEM.			
MVH, mechanically ventilated house; NVH, naturally ventilated house; Control, basal diet; MOS, basal diet supplemented with 2.0 g/kg Bio-Mos; T, sampling time relative to transportation.			
□, □ Means within the same main effect with no common superscripts differ significantly at p < 0.05.			

DISCUSSION

The broilers housed in the mechanically ventilated house (MVH) performed better than those housed in the naturally ventilated house (NVH). This difference likely resulted from more stable temperature and relative humidity, and better air movement, in the MVH. Birds under hot, humid conditions likely eat less and expend more energy on thermoregulation, which may reduce growth and feed efficiency (Lara and Rostagno 2013; Sohail et al. 2010; Zhao et al. 2014; Wasti et al. 2020). Growth performance was also improved by MOS supplementation. Previous studies have reported beneficial effects of MOS on the performance of broilers exposed to heat stress (Sohail et al. 2012). In the present study, however, MOS improved performance similarly under both housing conditions, as there was no housing system × MOS interaction. This suggests that the response to MOS was not dependent on the housing environment under the conditions of this study. Mortality did not differ significantly among treatment groups, although it was numerically lower in MVH and MOS-supplemented birds.

Serum corticosterone increased after 4 h of transport. This was expected, as transport involves handling, crating, vibration, noise and changes in environmental conditions, which can stimulate the hypothalamic–pituitary–adrenal axis (Al-Aqil and Zulkifli 2009; Nijdam et al. 2005). CORT concentrations were higher overall in birds from NVH than from MVH. This difference may reflect the rearing environment. However, CORT increased after transportation in both housing systems, and the housing system × sampling time interaction was not significant.

MOS also showed a similar trend. Birds that received MOS had lower overall CORT concentrations than control birds. This is consistent with reports that prebiotics may decrease corticosterone concentrations in broilers subjected to heat stress (Sohail et al. 2010, 2012). There was also no housing system × MOS interaction for CORT, indicating that MOS had a similar effect in NVH and MVH birds. However, MOS did not influence the response to transportation, as the diet × sampling time interaction was not significant. Thus, the lower CORT in MOS-fed birds seems to be a general effect rather than a specific protection against transport stress. This study did not elucidate the mechanism, but improved intestinal integrity and immune regulation may have reduced physiological stress signalling and subsequent activation of the hypothalamic–pituitary–adrenal axis (Sohail et al. 2012).

The housing system also influenced some of the caecal bacterial populations. Birds reared in MVH had lower counts of *Clostridium* spp. and higher counts of *Lactobacillus* spp. These differences may reflect better environmental conditions at MVH, since heat stress may affect intestinal integrity and immune function. However, this finding should not be extrapolated beyond the bacterial groups measured. Only certain populations were studied, and the results do not represent the entire gut microbiome (Song et al. 2014).

MOS did not significantly affect the bacterial populations measured in this study. This is not surprising, as the effects of prebiotics on intestinal bacterial numbers in chickens have not always been consistent (Pourabedin and Zhao 2015). In this study, the improved performance and lower CORT concentration in birds fed MOS did not coincide with any measurable change in *Clostridium*, *Lactobacillus* or *E. coli*.

Therefore, MOS may exert its effects through mechanisms other than changing the numbers of these bacteria.

Despite the clear increase in CORT, transportation did not strongly affect bacterial counts. One explanation is that the endocrine response to stress occurs much faster than changes in caecal bacterial populations. Four hours of transport was enough to increase CORT, but perhaps not enough to change bacterial numbers. The caecal microbial population is also relatively well established and may be less sensitive to short-term stressors.

Another point is that qPCR gives data on bacterial abundance but not necessarily bacterial activity. Thus, microbial metabolism or host-microbe interactions can change even without changes in bacterial numbers. This might explain why the selected bacterial populations were not affected, but the physiological response to transport was evident. Whyte et al. (2001) observed increased bacterial shedding in transported broilers, but their birds faced longer and more variable transport and holding conditions, along with prolonged feed and water withdrawal. These conditions involved greater cumulative preslaughter stress than the transport protocol used here and may partly explain the different findings.

A few limitations remain. The study assessed selected caecal bacterial populations but did not measure bacterial shedding or carcass contamination; therefore, the findings should not be interpreted as evidence of changes in food-safety risk. Another limitation is that the study used only one NVH and one MVH. Although each house contained several replicate pens, the housing system was not replicated at the house level, so the housing effect cannot be fully separated from other house-specific differences. Future studies should include more than one independent house for each housing system and, when possible, combine microbiome analysis with carcass microbiology.

Overall, broilers reared in MVH had better growth performance and lower serum CORT concentrations than those reared in NVH, while MOS supplementation improved growth performance and lowered overall CORT. Transportation increased CORT but did not alter the selected caecal bacterial populations. The absence of significant interactions suggests that housing, MOS supplementation, and transportation acted mainly independently under the conditions of this study. These findings provide useful information on housing and nutritional management of broilers exposed to humid tropical conditions and subsequent transportation stress.

Declarations

Authors' Contributions

Zulkifli Idrus and Zulaikha Zainool Abidin conceptualised and designed the study, curated the data, and performed the formal analysis. Zulaikha Zainool Abidin, Zulkifli Idrus, Elmutaz Atta Awad, and Suriya Kumari Ramiah developed the methodology and conducted the investigation. Zulaikha Zainool Abidin managed the software and validation processes. Zulkifli Idrus and Zulaikha Zainool Abidin drafted the

manuscript, while Zulkifli Idrus, Elmutaz Atta Awad, and Suriya Kumari Ramiah reviewed and edited the manuscript.

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Data availability

The corresponding author can provide the datasets upon reasonable request.

Conflicts of interest

The authors declare no conflict of interest.

Ethical approval

All experimental procedures were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) of Research Policy at Universiti Putra Malaysia (UPM) (Approval number: UPM/IACUC/AUP-R056/2020).

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References

1. Al-Aqil A, Zulkifli I (2009) Changes in heat shock protein 70 expression and blood characteristics in transported broiler chickens as affected by housing and early age feed restriction. Poult Sci 88:1358–1364.
2. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M (2011) Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain Behav Immun 25:397–407.
3. Chacher MFA, Kamran Z, Ahsan U, Ahmad S, Koutoulis KC, Qutab Ud Din HG, Cengiz Ö (2017) Use of mannan oligosaccharide in broiler diets: an overview of underlying mechanisms. Worlds Poult Sci J 73:831–844.
4. Farouk MM, Al-Mazeedi HM, Sabow AB, Bekhit AED, Adeyemi KD, Sazili AQ, Ghani A (2014) Halal and kosher slaughter methods and meat quality: a review. Meat Sci 98:505–519.
5. Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818.

6. Hooge DM (2004) Meta-analysis of broiler chicken pen trials evaluating dietary mannan oligosaccharide, 1993–2003. Int J Poult Sci 3:163–174.
7. Lara LJ, Rostagno MH (2013) Impact of heat stress on poultry production. Animals 3:356–369.
8. Mitin H, Idrus Z, Hussein AN, Goh YM, Sazili AQ (2026) Effects of multimodal early-life stress on transport-induced responses in Pekin and Muscovy ducks. Trop Anim Health Prod 58:520.
9. Najafi P, Zulkifli I, Amat Jajuli N, Soleimani AF, Ramiah SK, Amir AA, O'Reilly EL, Eckersall PD (2015) Environmental temperature and stocking density effects on acute phase proteins, heat shock protein 70, circulating corticosterone and performance in broiler chickens. Int J Biometeorol 59:1577–1583.
10. Navidshad B, Liang JB, Jahromi MF (2012) Correlation coefficients between different methods of expressing bacterial quantification using real-time PCR. Int J Mol Sci 13:2119–2132.
11. Nijdam E, Delezie E, Lambooij E, Nabuurs MJ, Decuypere E, Stegeman JA (2005) Feed withdrawal of broilers before transport changes plasma hormone and metabolite concentrations. Poult Sci 84:1146–1152.
12. Pourabedin M, Zhao X (2015) Prebiotics and gut microbiota in chickens. FEMS Microbiol Lett 362:fnv122
13. Renaudeau D, Collin A, Yahav S, de Basilio V, Gourdine JL, Collier RJ (2012) Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal 6:707–728.
14. Scanes CG (2018) Animal agriculture: livestock, poultry, and fish aquaculture. In: Scanes CG, Toukhsati SR (eds) Animals and human society. Academic Press, London, pp 133–179.
15. Sohail MU, Ijaz A, Yousaf MS, Ashraf K, Zaneb H, Aleem M, Rehman H (2010) Alleviation of cyclic heat stress in broilers by dietary supplementation of mannan-oligosaccharide and Lactobacillus-based probiotic: dynamics of cortisol, thyroid hormones, cholesterol, C-reactive protein, and humoral immunity. Poult Sci 89:1934–1938.
16. Sohail MU, Hume ME, Byrd JA, Nisbet DJ, Ijaz A, Sohail A, Shabbir MZ, Rehman H (2012) Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult Sci 91:2235–2240.
17. Song J, Xiao K, Ke YL, Jiao LF, Hu CH, Diao QY, Shi B, Zou XT (2014) Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress. Poult Sci 93:581–588.
18. Spring P, Wenk C, Dawson KA, Newman KE (2000) The effects of dietary mannaoligosaccharides on caecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. Poult Sci 79:205–211.
19. Tannock GW (2016) Understanding the gut microbiota. John Wiley & Sons.
20. Tawfeek SS, Hassanin KMA, Youssef IMI (2014) The effect of dietary supplementation of some antioxidants on performance, oxidative stress, and blood parameters in broilers under natural summer conditions. J World Poult Res 4:10–19.

21. Wasti S, Sah N, Mishra B (2020) Impact of heat stress on poultry health and performances, and potential mitigation strategies. Animals 10:1266.
22. Whyte P, Collins JD, McGill K, Monahan C, O'Mahony H (2001) The effect of transportation stress on excretion rates of campylobacters in market-age broilers. Poult Sci 80:817–820.
23. Zhao ZG, Li JH, Li X, Bao J (2014) Effects of housing systems on behaviour, performance and welfare of fast-growing broilers. Asian-Australas J Anim Sci 27:140–146.
24. Zulkifli I, Al-Aqil A, Omar AR, Sazili AQ, Rajion MA (2009) Crating and heat stress influence blood parameters and heat shock protein 70 expression in broiler chickens showing short or long tonic immobility reactions. Poult Sci 88:471–476.
25. Zulkifli I, Najafi P, Nurfarahin AJ, Soleimani AF, Kumari S, Aryani AA, O'Reilly EL, Eckersall PD (2014) Acute phase proteins, interleukin 6, and heat shock protein 70 in broiler chickens administered with corticosterone. Poult Sci 93:3112–3118.