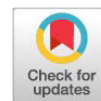


Research Article



Physiological Stress Responses of Broiler Chickens Exposed to Prenatal and Neonatal Auditory Stimulation during Road Transport in the Tropics

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Abstract | Road transportation exposes broiler chickens to multiple stressors, particularly in hot, humid tropical conditions. This study examined whether prenatal and neonatal auditory stimulation influences selected physiological stress markers in broilers subjected to road transportation at 35 days of age. Fertile eggs were either unexposed (prenatal control) or exposed to traffic noise at 90 dB for 20 min/h from embryonic days 12 to 21. After hatching, chicks from each prenatal group were either not exposed to sound or exposed to the same auditory stimulus on days 4–6 post-hatch, forming four treatment groups: prenatal control–neonatal control (PC-NC), prenatal control–neonatal noise (PC-NN), prenatal noise–neonatal control (PN-NC), and prenatal noise–neonatal noise (PN-NN). On day 35, birds were either not transported or subjected to 3 h of road transportation. Serum concentrations of corticosterone (CORT), glucose (GLU), creatine kinase (CK), and alanine aminotransferase (ALT) were determined. Road transportation significantly increased CORT ($p < 0.001$), CK ($p = 0.008$) and ALT ($p < 0.001$) while reducing GLU ($p < 0.001$). Overall, PN-NC birds exhibited lower CK than PC-NC birds ($p = 0.026$) and lower ALT than the other treatment groups ($p = 0.006$). For GLU, the transported PN-NC birds had higher glucose concentrations than the other transported groups ($p = 0.001$). Combined prenatal and neonatal auditory stimulation did not confer additional physiological effects. These findings indicate that early-life auditory exposure may influence physiological stress responses during transportation in broilers under tropical conditions.

Keywords | Auditory stimulation, Physiological stress markers, Road transportation stress, Broiler chickens, Tropical poultry production, Prenatal and neonatal conditioning

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INTRODUCTION

Catching, crating, and transportation, particularly under hot and humid tropical conditions, although brief in a broiler chicken's life, can cause significant physical and psychological distress due to handling, feed withdrawal, vibration, noise, temperature fluctuations, overcrowding, and restricted movement (Broom, 2005; Zheng *et al.*, 2020). These stressors are associated with negative impacts

on animal welfare, carcass and meat quality, and economic performance. Hence, alleviating transportation-related stress is crucial to ensuring animal welfare and promoting the sustainability of the poultry sector.

Early-life challenges, including the prenatal and neonatal periods, can shape resilience, with a certain magnitude of stress promoting coping ability later in life (Macri *et al.*, 2007; Colditz and Hine, 2016). Earlier studies have

shown that prenatal auditory stimulation enhanced brain development, memory, and fear reduction in chicks (Chaudhury *et al.*, 2006, 2009; Sanyal *et al.*, 2013; Zhao *et al.*, 2022) and improved the tolerance of broilers to feed restriction at market age (Ahmad-Hanafi *et al.*, 2024). It remains unclear whether combining prenatal and neonatal auditory stimulation can further enhance stress resilience in broilers at market age. This study investigated whether prenatal and neonatal auditory stimulation influences selected physiological stress markers (corticosterone, glucose, creatine kinase, and alanine aminotransferase) in broilers exposed to road transportation under hot, humid tropical conditions at 35 days of age.

MATERIALS AND METHODS

EXPERIMENTAL INCUBATORS

Two Brinsea Ovo-Easy incubators (Brinsea Products Inc., Titusville, FL) were used. The preset incubation temperature was 37.8°C for each incubator (precision of 0.5°C), and the relative humidity ranged from 55% on embryonic day (ED) 1 to ED 17, and from 65% on ED 18 and beyond, until hatching. The eggs were automatically turned every 2 h until ED 18. From ED 18, eggs were placed in hatching trays without further turning. Incubators were kept in a ventilated room at 24–25 °C (± 2 °C) and fitted with double-walled, sound-insulated MDF to prevent acoustic interference. SPL was maintained at 90 dB inside and below 50 dB outside the incubators; forced-air ventilation ensured uniform conditions. A total of 256 Cobb 500 eggs (~65 g each) from a commercial hatchery in Negeri Sembilan were fumigated and set (128 eggs per incubator). Hatch was monitored hourly between incubation days 20 and 21, and hatchability (%) was calculated as the number of chicks hatched per fertile eggs.

AUDITORY STIMULATION PROTOCOLS

The auditory stimulation protocol used in the current study was based on that of Hanafi *et al.* (2023).

EXPERIMENTAL GROUPS

PRENATAL AUDITORY STIMULATION

The hatching eggs were subjected to the following prenatal auditory stimulation treatments:

- No sound stimulation: The eggs were incubated and received no additional sound stimuli except the unavoidable sound of the compressor (40 dB). This treatment was termed the “Prenatal Control” (PC) group.
- Noise stimulation: The eggs were incubated under conditions similar to those of the PC group. From ED 12 to ED 21, the eggs were exposed to pre-recorded traffic noise at 90 dB (± 2 dB) for 20 minutes per hour over 24 hours (a total of 8 hours per day). The last

cycle of noise stimulation occurred approximately at 09:00 h. This treatment was referred to as the “Prenatal Noise” (PN) group.

NEONATAL AUDITORY STIMULATION

During the neonatal stage, chicks from the PC and PN groups were subjected to the following treatment:

- The PC chicks were not exposed to sound stimulation on days 4, 5, and 6 post-hatch, except for the unavoidable sound of the compressor and fan in the chamber (55 dB). This treatment was referred to as the “Prenatal Control – Neonatal Control” (PC-NC) group.
- The PC chicks were exposed to pre-recorded traffic noise at 90 dB (± 2 dB) for 20 minutes per hour over 24 hours (a total of 8 hours per day) on days 4, 5, and 6 post-hatch. The last cycle of noise stimulation occurred approximately at 09:00 h. This treatment was referred to as the “Prenatal Control – Neonatal Noise” (PC-NN) group.
- The PN chicks were not exposed to sound stimulation on days 4, 5, and 6 post-hatch, except for the unavoidable sound of the compressor and fan in the chamber (55 dB). This treatment was referred to as the “Prenatal Noise – Neonatal Control” (PN-NC) group.
- The PN chicks were exposed to pre-recorded traffic noise at 90 dB (± 2 dB) for 20 minutes per hour over 24 hours (a total of 8 hours per day) on days 4, 5, and 6 post-hatch. The last cycle of noise stimulation occurred approximately at 09:00 h. This treatment was termed the “Prenatal Noise – Neonatal Noise” (PN-NN) group.

HOUSING AND DIET

At hatch, 168 chicks were subjected to one of the four treatment groups: PC-NC (n=42), PC-NN (n=42), PN-NC (n=42), and PN-NN (n=42). The chicks were allocated to groups of seven to 24 battery cages, evenly distributed across two soundproof, environmentally controlled rooms, with 12 cages per room. The PC-NC and PN-NC groups were housed together in one room, while the PC-NN and PN-NN groups were housed in the other. The ambient temperature was initially set at 32 $\pm$ 1 °C on day 1 and gradually reduced to 24 $\pm$ 1 °C by day 21. The birds were fed with commercial broiler diets.

ROAD TRANSPORTATION

On day 35 (06:30 h), 36 birds from each of the four subgroups were assigned to either (i) no transport or (ii) 3 h road transport (Table 1). Transported birds were moved from cages into plastic crates (0.80 $\times$ 0.60 $\times$ 0.31 m), with six birds per crate. Each bird had 486 cm² of floor space, provided by cardboard and cable-tie modifications. Crates were loaded onto an open truck and transported for 3 h at ~80 km/h along highways, busy roads, and intersections. Non-transported birds remained undisturbed in their

home cages. During transport (06:30–09:30 h), the ambient temperature ranged from 26.1 to 29.4 °C, and the relative humidity ranged from 69% to 85%.

BLOOD SAMPLING

On day 35, body weights were recorded before transport. Blood was collected (via the wing vein) post-transport from 12 transported birds per subgroup (randomly selected) and from 12 non-transported birds per subgroup (randomly selected) for comparison. Samples were centrifuged at $3,000 \times g$ for 10 min at room temperature, and serum was stored at -80°C for analysis.

ANALYSIS OF BLOOD PARAMETERS

Serum levels of corticosterone (CORT) were determined using commercially available high-sensitivity EIA kits (AC-15F1, IDS, Boldon, UK). An automatic analyser (Hitachi 902, Tokyo, Japan) was used to determine serum levels of creatine kinase (CK), glucose (GLU), and alanine aminotransferase (ALT). All the reagents used

were obtained from Roche (Roche Diagnostics, Basel, Switzerland).

STATISTICAL ANALYSIS

All analyses were conducted using SAS (SAS Institute, Cary, NC). The CORT, GLU, CK, and ALT data were normalised by square root or log transformation. Two-way ANOVA analysed blood data with prenatal-neonatal auditory stimulation, transportation, and their interaction as factors, and means were compared using Duncan's test at $p \leq 0.05$.

RESULTS

Hatchability rate was not significantly affected by PAS (PC 81.8%; PN, 73.4%). On day 35, prior to transportation, all the birds had similar mean body weights (PC-NC, 2.11 kg/bird; PC-NN, 2.21 kg/bird; PN-NC, 2.19 kg/bird; PN-NN, 2.28 kg/bird). Table 2 presents the effects of prenatal-

Table 1: Experimental design.

Prenatal auditory stimulation (embryonic days 12 to 21)	Neonatal auditory stimulation (days 4, 5 and 6 post-hatch)	Experimental subgroup	Road transportation (Day 35)
No exposure to auditory stimulation (128 eggs)	No exposure to auditory stimulation (42 birds)	Prenatal Control - Neonatal Control (PC-NC)	Not transported (18 birds) Transported (18 birds)
	Exposure to auditory stimulation (42 birds)	Prenatal Control - Neonatal Noise (PC-NN)	Not transported (18 birds) Transported (18 birds)
Exposure to auditory stimulation (128 eggs)	Exposure to auditory stimulation (42 birds)	Prenatal Noise - Neonatal Control (PN-NC)	Not transported (18 birds) Transported (18 birds)
	Exposure to auditory stimulation (42 birds)	Prenatal Noise - Neonatal Noise (PN-NN)	Not transported (18 birds) Transported (18 birds)

Table 2: The effects of prenatal-neonatal auditory stimulation and transport on serum levels of corticosterone (CORT), creatine kinase (CK), glucose (GLU), and alanine aminotransferase (ALT) in broiler chickens on day 35 (Means $\pm$ SEM).

Treatment	Parameters			
	CORT (ng/ml)	CK (U/L)	GLU (mmol/L)	ALT (U/L)
Prenatal and Neonatal Auditory Stimulation (PNAS)				
PC-NC	0.81 ± 0.31	7.08 ± 2.30^a	11.86 ± 0.37^{ab}	4.71 ± 0.37^a
PC-NN	1.07 ± 0.59	6.01 ± 1.30^{ab}	12.55 ± 0.88^{ab}	5.34 ± 0.96^a
PN-NC	1.78 ± 0.48	3.63 ± 0.51^b	13.21 ± 0.49^a	3.19 ± 0.48^b
PN-NN	0.89 ± 0.47	4.26 ± 0.55^{ab}	11.41 ± 0.70^b	6.00 ± 0.99^a
Road Transportation (RT)				
NT	0.15 ± 0.03^b	3.97 ± 0.30^b	13.17 ± 0.32^a	3.33 ± 0.30^b
T	2.62 ± 0.41^a	6.20 ± 1.15^a	10.93 ± 0.50^b	6.20 ± 0.60^a
Analysis of Variance				
PNAS	0.169	0.026	0.007	0.006
RT	<.001	0.008	<.001	<.001
PNAS $\times$ RT	0.388	0.274	0.001	0.310

PC-NC = Prenatal Control – Neonatal Control; PC-NN = Prenatal Control – Neonatal Noise; PN-NC = Prenatal Noise – Neonatal Control; PN-NN = Prenatal Noise – Neonatal Noise; NT = Not transported; T = Transported. Means with different letters are significantly different ($p \leq 0.05$).

Table 3: Mean ($\pm$ SEM) serum levels of glucose (GLU) (mmol/L), where prenatal-neonatal auditory stimulation x transportation interactions were significant in broiler chickens on day 35.

Road transportation	Prenatal and neonatal auditory stimulation			
	PC-NC	PC-NN	PN-NC	PN-NN
NT	12.78 $\pm$ 0.47 ^{ax}	14.21 $\pm$ 0.99 ^{ax}	12.93 $\pm$ 0.30 ^{ax}	12.67 $\pm$ 0.51 ^{ax}
T	11.03 $\pm$ 0.41 ^{by}	9.57 $\pm$ 0.34 ^{by}	13.71 $\pm$ 1.34 ^{ax}	8.90 $\pm$ 1.01 ^{by}

PC-NC = Prenatal Control – Neonatal Control; PC-NN = Prenatal Control – Neonatal Noise; PN-NC = Prenatal Noise – Neonatal Control; PN-NN = Prenatal Noise – Neonatal Noise; NT = Not transported; T = Transported. ^{a,b}Means within a column-subgroup with different letters are significantly different ($p \leq 0.05$). ^{x,y}Means within a row-subgroup with different letters are significantly different ($p \leq 0.05$).

neonatal auditory stimulation and transportation on blood parameters on day 35. There were no significant prenatal-neonatal auditory stimulation x transport interactions for CORT ($p=0.3882$), CK ($p=0.2738$), and ALT ($p=0.3099$) on day 35. CORT was not significantly ($p=0.169$) affected by prenatal-neonatal auditory stimulation on day 35. Irrespective of prenatal-neonatal auditory stimulation, road transportation significantly ($p < 0.0001$) increased CORT in broilers. The PN-NC (3.63 ± 0.51 U/L) broilers showed significantly ($p=0.026$) lower CK than their PC-NC (7.08 ± 2.30 U/L) counterparts on day 35 (Table 2). The CK of PC-NN (6.01 ± 1.30 U/L) and PN-NN (4.26 ± 0.55 U/L) birds were similar to those of the other groups. Following road transportation, all the birds had significantly elevated CK ($p = 0.0081$). The ALT of PN-NC (3.19 ± 0.48 U/L) birds were significantly ($p=0.0055$) lower than that of PC-NC (4.71 ± 0.37 U/L), PC-NN (5.34 ± 0.96 U/L), and PN-NN (6.00 ± 0.99 U/L) on day 35 (Table 2). Road transportation was associated with a significant increase in ALT ($p < 0.0001$). On day 35, there were significant prenatal-neonatal auditory stimulation and transportation interactions for GLU ($p = 0.0014$) (Table 2). All non-transported birds, regardless of prenatal-neonatal auditory stimulation, had similar GLU levels on day 35 (Table 3). However, following transportation, the PN-NC (13.71 ± 1.34 mmol/L) showed significantly higher glucose levels than their PC-NC (11.03 ± 0.41 mmol/L), PC-NN (9.57 ± 0.34 mmol/L), and PN-NN (8.90 ± 1.01 mmol/L) counterparts.

DISCUSSION

Road transportation under hot, humid tropical conditions elicited clear physiological stress responses in broiler chickens, as indicated by increased CORT, CK and ALT, and reduced GLU. These responses confirm that the transport protocol imposed an acute, multifactorial stress challenge involving handling, confinement, vibration, and thermal load, consistent with previous reports in poultry subjected to road transportation (Al-Aqil and Zulkifli, 2009; Jacobs *et al.*, 2017; Zheng *et al.*, 2020).

Prenatal and neonatal auditory stimulation did not

adversely affect hatchability or body weight at market age, indicating that the auditory exposure protocol applied in this study did not disrupt embryonic development or post-hatch growth. Similarly, Ahmad-Hanafi *et al.* (2024) reported that prenatal auditory stimulation had no adverse effect on broiler growth performance when applied within controlled limits.

Corticosterone concentrations were primarily influenced by transportation itself and were not significantly modified by early-life auditory treatments. This suggests that corticosterone reflects acute activation of the hypothalamic–pituitary–adrenal axis in response to transport stress but may be less sensitive to detecting subtle differences in coping capacity induced by early-life conditioning. The limitations of glucocorticoids as the sole indicators of stress resilience have been discussed previously (Ralph and Tilbrook, 2016; Scanes, 2016).

In contrast, treatment-related differences were more evident in biochemical markers associated with tissue integrity and energy metabolism. Overall, PN-NC broilers showed lower CK concentrations than PC-NC birds and lower ALT concentrations than the other treatment groups. Elevated ALT is commonly associated with hepatic strain and altered metabolic processing, whereas increased CK is frequently linked to muscle membrane disruption and heightened muscular activity during stress (Tang *et al.*, 2018; Zheng *et al.*, 2020). The lower values observed in PN-NC birds, therefore, suggest reduced tissue disturbance during translocation.

Transported PN-NC birds also maintained higher circulating glucose concentrations compared with other treatment groups. Transport stress is frequently associated with increased energy demand and reduced circulating glucose levels due to heightened muscular activity and stress-related metabolic shifts (Mitchell, 2009; Scanes, 2016). Maintenance of higher glucose concentrations in PN-NC birds may indicate improved regulation of energy metabolism during acute stress.

Interestingly, combined prenatal and neonatal auditory

stimulation did not confer additional physiological benefits. This suggests that the timing and magnitude of early-life auditory exposure are critical, and that moderate prenatal stimulation may be sufficient to induce adaptive physiological adjustments, whereas additional neonatal exposure may not further enhance these responses and could exceed the optimal range for beneficial conditioning (Macri *et al.*, 2007; Hartmann and Schmidt, 2020). The present study focused on selected biochemical indicators of stress physiology and did not assess behavioural responses, carcass characteristics, or mortality outcomes. Accordingly, the present findings should be interpreted as evidence of altered physiological stress responses rather than definitive proof of improved welfare outcomes. In conclusion, road transportation under tropical conditions induced significant physiological stress in broiler chickens. Prenatal auditory stimulation alone was associated with lower CK and ALT and higher glucose levels following transportation, suggesting improved adaptation to transport stress. These findings indicate that moderate prenatal auditory stimulation may enhance physiological resilience in broilers. This study is among the first to investigate the effects of prenatal and neonatal auditory stimulation on physiological responses to transportation stress in broilers under tropical conditions. The findings suggest that prenatal auditory stimulation may enhance physiological resilience at market age.

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NOVELTY STATEMENT

Among the first studies to evaluate combined prenatal and neonatal auditory stimulation in poultry.

AUTHOR'S CONTRIBUTION

Dineshvaran Puvanesvaran, A'ina Nafisah Mohd Nizam, Pi Yuan Chee, and Zulkifli Idrus conceived and designed the research and conducted the experiment. Zulkifli Idrus performed the statistical analysis. All authors prepared the manuscript. Pi Yuan Chee and Zulkifli Idrus reviewed the manuscript upon submission.

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DATA AVAILABILITY

Data are available from the corresponding author on request.

ETHICS APPROVAL

All experimental methods were approved by the Institutional Animal Care and Use Committee, Universiti Putra Malaysia (AUP-R035/2023).

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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