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Effects of auditory enrichment and regular human contact on stress response, underlying fearfulness, and growth performance in broiler chickens

Einfluss einer akustisch angereicherten Umgebung und regelmäßigem Personenkontakt auf Stressreaktion, zugrunde liegende Ängstlichkeit und Wachstumsleistung von Masthühnern

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Abstract

A study determined whether providing auditory enrichment to broiler chickens with or without regular human contact influenced stress response, underlying fearfulness, and growth performance. The experiment was conducted in a completely randomised design with a 3 × 2 factorial arrangement consisting of three auditory enrichment (AE) treatments and two human contact (HC) regimens. From days 1 to 35, a total of 480 day-old male broiler chicks were allotted to one of the three auditory enrichment treatments: no auditory enrichment (NE), auditory enrichment with classical music (Vivaldi's Four Season) (ME), or auditory enrichment with Quran recitation (Yaseen chapter) (QE). An equal number of chicks from each AE treatment were subjected to either no-human contact (NC) or regular human contact (the experimenter placed her right hand into the cage for 30 seconds twice daily) (RC). On day 7, the ME birds had significantly higher body weights (BW) than the control and QE birds. The BW of the QE chicks was significantly lower than that of their ME counterparts on day 14. HC had a negligible influence on the birds' BW, feed intake (FI), or feed conversion ratio (FCR). Neither AE nor HC significantly affected tonic immobility (TI) duration or number of induction attempts. ME and QE significantly affected serum levels of corticosterone (CORT) on days 14 and 21. However, HC did not affect CORT. In conclusion, ME has a beneficial effect on the performance of broilers during the early stage of growth. Exposure to ME or QE dampens stress response but not underlying fearfulness in chickens at market age.

Key words

broiler chickens; auditory enrichment; human contact; physiological stress response; tonic immobility; growth performance

Zusammenfassung

In dieser Studie wurde untersucht, ob eine akustisch angereicherte Umwelt mit oder ohne regelmäßigem Personenkontakt die Stressreaktion, die zugrunde liegende Ängstlichkeit und die Wachstumsleistung von Masthühnern beeinflusst. Der Versuch wurde in einem vollständig randomisierten Design mit einer 3 × 2 faktoriellen Anordnung durchgeführt, bestehend aus drei Behandlungen mit akustischer Anreicherung (AE) und zwei Behandlungen mit menschlichem Kontakt (HC). Von Tag 1 bis 35 wurden insgesamt 480 einen Tag alte männliche Mastküken einer der drei Höranreicherungsbehandlungen zugeteilt: keine Höranreicherung (NE), Höranreicherung mit klassischer Musik (Vivaldis Vier Jahreszeiten) (ME) oder Höranreicherung mit Koranrezitation (Kapitel Yaseen) (QE). Eine gleiche Anzahl von Küken aus jeder AE-Behandlung wurde entweder keinem (NC) oder regelmäßigem Personenkontakt (die Versuchsleiterin hielt zweimal täglich ihre rechte Hand für 30 Sekunden in den Käfig) (RC) ausgesetzt. Am 7. Tag errreichten die ME-Vögel ein signifikant höheres Körpergewicht (BW) im Vergleich zu den Kontroll- und QE-Vögeln. Das Körpergewicht der QE-Küken war am Tag 14 signifikant niedriger als das der

ME-Küken. HC hatte einen vernachlässigbaren Einfluss auf das Körpergewicht, die Futteraufnahme (FI) oder die Futterverwertung (FCR) der Tiere. Weder AE noch HC hatten einen signifikanten Einfluss auf die Dauer der tonischen Immobilität (TI) oder die Anzahl der Induktionsversuche. ME und QE wirkten sich signifikant auf die Serumspiegel von Corticosteron (CORT) an den Tagen 14 und 21 aus. HC hatte jedoch keinen Einfluss auf CORT. Zusammenfassend lässt sich sagen, dass ME eine positive Wirkung auf die Leistung von Masthähnchen in der frühen Wachstumsphase hat. Die Exposition gegenüber ME oder QE dämpft die Stressreaktion, nicht aber die zugrunde liegende Ängstlichkeit bei Hühnern im Marktalter.

Stichworte

Masthühner; akustische Anreicherung; Personenkontakt; physiologische Stressreaktion; tonische Immobilität; Wachstumsleistung

List of Abbreviations

AE	Auditory enrichment
ANOVA	Analysis of variance
BW	Body weight
CORT	Serum levels of corticosterone
FCR	Feed conversion ratio
FI	Feed intake
HC	Human contact
HLR	Heterophil-to-lymphocyte ratio
HSP	Heat shock protein
ME	Auditory enrichment with music
NC	Control; no-human contact
NE	Control; no auditory enrichment
QE	Auditory enrichment with Quran recitation
RC	Regular human contact
SE	Standard error
TI	Tonic immobility

Introduction

The environment of broiler chickens in the modern production system is a composite of interacting stressors. The success of birds in coping with its environment depends on its physiological ability to respond efficiently. There is substantial evidence suggesting the benefits of regular contact with humans in dampening stress and fear reactions in pigs (Hemsworth et al., 1981), cattle (Breuer et al., 2003), goats (Lyons et al., 1988), and poultry (Zulkifli et al., 2002; Zulkifli and Siti Nor Azah, 2004; Al-Aqil et al., 2013; Mitin et al., 2023). Mitin et al. (2023) subjected ducks to regular positive physical contact daily for 30 seconds from 1 to 21 or 1 to 42 days of age. The authors found that those ducks had lower serum levels of corticosterone (CORT) and shorter tonic immobility (TI) duration than their neglected counterparts following crating and road transportation as stressors.

Intensively raised chickens are often housed in a barren and visually restricted environment. Such an environment may lead to frustration, boredom, and harmful abnormal behaviour (Petherick and Rushen, 1997). Farm animals will seek environmental challenges because such complexities can be considered enrichment or extra stimulation in the house environment that may enhance the ability to cope with the challenge, health, and behavioural repertoire (Jones, 1996). A practical environmental enrichment approach should stimulate visual, somatosensory, and olfactory systems of the animals. The key idea is that this enrichment should provide novelty to the environment (Nithianantharajah and Hannan, 2006). In poultry, the addition of 'toys', such as mirrors, balls, rubber stoppers, tubing, beads, balls, thimbles, and strings, is a common environmental enrichment approach to reduce and control

the incidence of problematic behaviours such as feather pecking, cannibalism, and aggression (Jones, 2001; Martrenchar et al., 2001; Huff et al., 2003; McAdie et al., 2005). However, chickens may lose interest towards the "toys" within a short period (Newberry, 1995; Jones, 1996; Mench, 1998). There is also the question of whether these "toys" could be considered entirely safe for poultry health (Jones, 1996). Following that, there is an increasing interest in exploring the impact of sound stimulation as a method of environmental enrichment for farm animals.

Beneficial effects of music on the well-being of laying hens (Dávila et al., 2011), broiler chickens (Gvaryahu et al., 1989; Nicol, 1992), dairy cows (Uetake et al., 1997), pigs (Silva et al., 2017; Li et al., 2021), and sheep (Meshabaz et al., 2017) have been reported. Da Cruz et al. (2011) proposed that music can be advantageous by "masking" potentially unexpected artificial noises and transforming a monotonous auditory environment into a pleasant atmosphere, which has been shown to impede the development of normal cognitive abilities. Music has been shown to alleviate anxiety, stress, and pain in humans (Binns-Turner et al., 2011). Others (Ladd et al., 1992; Cloutier et al., 2000; Campo et al., 2005) have found that music has a negative or minimal effect on livestock behaviour, physiological stress response, performance, and health. These inconsistent findings could be attributed to variations in the type of music, pace of melody, rhythm, tone, frequency, and intensity (Alworth and Buerkle, 2013).

A growing body of evidence shows that recitation of the Holy Quran may reduce stress, anxiety and inspire calmness among Muslims (Hasanpoor, 2001; Kazemi et al., 2003; Ansari et al., 2005; Sadeghi, 2011; Abu Bakar, 2015). Mahjoob et al. (2014) suggested that listening to the Quran can improve mental health and achieve greater peace. The question is whether Quran recitation may be an auditory enrichment in animals. Ghazali et al. (2015) reported that broadcasting the sound of Quran verses in poultry houses improved the growth performance of commercial broilers. To the best of our knowledge, the effect of the Quran recital on stress and fear reactions in poultry is unknown.

Therefore, this study aimed to investigate the effects of auditory enrichment employing classical music and Quran recitation, and regular human contact through placing one hand inside the cage on growth performance, TI as a measurement of fear response, and CORT as a physiological stress indicator in broiler chickens.

Materials and Methods

Experimental site, animals, and management

This study was undertaken following the guidelines of the Research Policy on Animal Ethics of the Universiti Putra Malaysia. This experiment was conducted at the Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. A total of 480 one-day-old male broiler chicks (Cobb 500) were obtained from a local commercial hatchery. The chicks were individually weighed, wing tagged, and randomly assigned to 48 (10 chicks per cage) battery cages (90 cm × 56 cm × 50 cm; length × width × height) with stainless steel wire floors. All cages were located in an environmentally controlled room. on day 1, the room's ambient temperature was set at 32°C and was gradually reduced to 24°C by day 21. on days 1 and 2, the birds were provided with continuous lighting. The lighting duration was then reduced to 18 hours per day, starting from day 3 onwards. From day 1 to 21, the birds were fed starter crumble (12.3 MJ ME/kg; 21% crude protein), followed by finisher pellet (12.8 MJ ME/kg; 19% crude protein) from day 22 onwards. on days 7 and 21, all birds were administered with live Newcastle vaccine (Nobilis ND Clone 30, Intervet International, 58300 AA Boxmeer, The Netherlands) via drinking water. Throughout the study period, feed and water were provided *ad libitum*.

Experimental design

The experimental treatments consisted of 3 × 2 factorial arrangements with three auditory enrichment (AE) treatments and two human contact (HC) regimens in a completely randomised design. Each auditory enrichment-human contact subgroup had eight replicate cages, with 10 birds in each replicate. From days 1 to 35, the birds were exposed to the following: (i) no auditory enrichment (NE), (ii) auditory enrichment with classical music (Vivaldi's Four Season) (ME), or (iii) auditory enrichment with Quran recitation (Yaseen chapter) (QE). Equal numbers of chickens in each auditory enrichment were randomly subjected to either no-human contact (NC), or regular human contact (RC). There were two sound-proof rooms for each AE group, each with 16 cages (8 for NC and 8 for RC).

Auditory enrichment was applied for 3 hours daily between 09:00 and 18:30 h at 75dB using a multi-player (Panasonic RX-DU10) equipped with speakers (1-way, two units of 9 cm each). The sound level was measured using a digital meter (TECPEL DSL-330). The regular HC was conducted twice daily between 10:30 and 11:00 h and 16:30

and 17:00 h. The experimenter entered the room gently with minimal noise and placed her right hand into the cage for the RC treatment group. The hand of the experimenter was placed in the centre of the cage for 30 seconds, with no attempt to initiate any physical or eye contact with the birds (JONES, 1993). The same trained experimenter performed the human contact treatment throughout the experiment. The NC birds received no physical contact with humans other than routine husbandry. All cages were provided with two nipple drinkers and a feed trough. To reduce visual contact with other flocks, opaque partitions were erected between adjacent cages.

Tonic immobility test

On day 35, six birds per cage were selected randomly for TI test according to the modified method of BENOFF and SIEGEL (1976). The birds were caught individually with minimal disturbance to flockmates, carried in an inverted manner, and placed for 10 minutes in plastic crates (0.8 m × 0.6 m × 0.3 m; length × width × height) at six birds per crate. Birds from different cages were not mixed in the same crates. The chickens were caught individually inverted after 10 minutes of crating, taken to a separate room (no visual contact with other birds) and subjected to TI test measurements. As soon as the birds were taken to a separate room, TI was induced by gently restraining them on their right side and wings for 15 seconds. The experimenter withdrew approximately 1 m and stood in the bird's sight but did not make unnecessary noise or movement. Direct eye contact was also avoided between the observer and the birds. A stopwatch was used to record latency until the bird righted itself. The TI induction was repeated if the bird righted itself < 10 seconds. If an induction fails after three attempts, the TI duration is considered to be 0 seconds. The maximum permitted TI duration was 600 seconds. The induction number required to achieve TI was recorded.

Growth performance

The individual body weight (BW) and feed intake (FI) (cage basis) were recorded weekly, and feed conversion ratios (FCR, feed per gain) were calculated.

Serum levels of corticosterone

On days 7, 14, 21, and 35, two birds were randomly selected from each cage, and their blood samples (1.5 ml) were collected via the jugular vein. Each bird was caught and sampled, one immediately after another. The chickens were returned to their home cages after blood sampling. Blood samples were centrifuged, and plasma samples were stored at –20°C until further tests were carried out to determine the CORT. The CORT concentration was determined by a high-sensitivity EIA kit (AC-15F1, IDS, Boldon, UK). The variability of inter- and intraassay for this kit was less than 7.8 and 6.7%, respectively, and the detection limit was 27 ng/ml.

Statistical Analysis

All data were analysed using the SAS GLM procedure (SAS Institute Inc., Cary, NC) for analysis of variance (ANOVA). The data were analysed with enrichment, regular human contact and their interactions as the main effects. Comparisons were made within each experimental variable when effects were found to be significant. Data of TI were logarithmically transformed before analysis. Duncan's multiple range test was used to compare the means. The statistical significance is considered at $P \leq 0.05$.

Results and Discussion

The effects of AE and HC on BW, FI, and FCR of chickens are presented in Table 1. There were no significant AE × HC interactions for BW, FI, and FCR. The ME birds had significantly greater BW than the NE and QE birds on day 7. on day 14, the BW of QE chicks were significantly lower than that of their ME counterparts. The QE birds consumed significantly less feed than the ME group from days 1 to 7. Auditory enrichment had no significant influence on FCR throughout the study. Regular human contact did not affect the birds' BW, FI, or FCR.

Table 1. Mean (±SE) body weights, feed intake, and feed conversion ratios in broiler chickens by auditory enrichment and human contact.

Mittlere (±SE) Körpergewichte, Futteraufnahme und Futterverwertung bei Masthühnern nach akustischer Anreicherung der Umgebung und Personenkontakt.

		AE			HC		P value		
Item		NE	ME	QE	RC	NC	AE	HC	AE × HC
Body weight (g)	Day 7	191[b] ± 1	195[a] ± 1	188[b] ± 1	192 ± 1	191 ± 1	0.002	0.759	0.347
	Day 14	508[ab] ± 3	516[a] ± 2	502[b] ± 3	507 ± 3	510 ± 2	0.017	0.564	0.506
	Day 21	976 ± 7	981 ± 6	968 ± 7	971 ± 7	979 ± 4	0.435	0.354	0.124
	Day 28	1416 ± 12	1430 ± 10	1428 ± 18	1421 ± 10	1419 ± 8	0.738	0.752	0.910
	Day 35	1983 ± 20	1991 ± 20	2014 ± 18	1998 ± 17	1993 ± 14	0.509	0.811	0.565
Feed intake (g)	Day 1 – 7	152[ab] ± 1	158[a] ± 1	150[b] ± 3	153 ± 2	154 ± 2	0.041	0.564	0.852
	Day 1 – 14	543 ± 4	555 ± 3	541 ± 6	545 ± 4	548 ± 3	0.074	0.609	0.248
	Day 1 – 21	1174 ± 9	1177 ± 7	1177 ± 9	1176 ± 8	1175 ± 6	0.963	0.915	0.178
	Day 1 – 28	2167 ± 18	2188 ± 18	2158 ± 14	2189 ± 15	2171 ± 12	0.604	0.328	0.365
	Day 1 – 35	3488 ± 30	3490 ± 25	3501 ± 29	3487 ± 27	3499 ± 18	0.943	0.713	0.883
FCR (%)	Day 1 – 7	0.8 ± 0.01	0.81 ± 0.01	0.8 ± 0.02	0.8 ± 0.01	0.81 ± 0.01	0.706	0.492	0.697
	Day 1 – 14	1.07 ± 0.01	1.07 ± 0.01	1.08 ± 0.01	1.07 ± 0.01	1.07 ± 0.01	0.821	0.961	0.459
	Day 1 – 21	1.2 ± 0.01	1.2 ± 0.01	1.22 ± 0.01	1.21 ± 0.01	1.2 ± 0.01	0.167	0.141	0.591
	Day 1 – 28	1.53 ± 0.01	1.54 ± 0.01	1.53 ± 0.01	1.54 ± 0.01	1.53 ± 0.01	0.921	0.541	0.363
	Day 1 – 35	1.76 ± 0.01	1.75 ± 0.02	1.74 ± 0.01	1.75 ± 0.02	1.76 ± 0.02	0.588	0.529	0.558

[a, b] Means within a row-subgroup with no common superscripts differ at $P \leq 0.05$.
SE = standard error; AE = auditory enrichment; HC = human contact; NE = no auditory enrichment; ME = auditory enrichment with music; QE = auditory enrichment with Quran recitation; RC = regular human contact; NC = no-human contact; FCR = feed conversion ratio.

The improved BW in the ME group during the early age period may be attributed to the numerically higher FI compared to the NE birds. Woodcock et al. (2004) reported that auditory stimuli attracted chicks to feeders, increasing feed intake. Jones and Rayner (1999) indicated that higher feed intake associated with music could be due to the calming effect of music on animals. However, the positive response to music at a later age is not evident in the present study. It appears chickens are quickly habituated to a similar auditory stimulus when the stimuli are exposed repeatedly or for a long time. This condition has been well-documented in human and animal learning (Gallistel, 1990; Domjan, 2005 Kuczaj et al., 2002). It could be associated with the familiarisation of the birds with the stimuli, whereby the novelty factor of the enrichment has lost its positive stimulatory effects. A study by Fox and Millam (2007) has shown that birds responded effectively to different enrichment tools (enrichment tools being changed weekly) throughout the experiment rather than being provided with the same enrichment tools. on the contrary, Ladd et al. (1992) reported that exposure to country music for 8 hours accelerated the feeding of the hens.

The present findings suggest that the QE was less effective than ME in improving early-age weight gain. It appears that QE, which consisted of the human voice, could not enrich chickens. on the contrary, Ghazali et al. (2015) demonstrated that broilers exposed daily to Quran recitation for 8 hours had more significant body weight gains than controls on day 39. They postulated that the sound of Quran verses helped to soothe the chicken from the stress caused by the environment and temperature, thereby increasing the chickens' feeding. The discrepancies between our findings and Ghazali et al. (2015) could be associated with breed differences, as genetic makeup plays a vital role in response to enrichment stimulation (Hill et al., 1998).

The present results suggested that regular human contact through placing one hand inside the cage had a negligible effect on broiler chickens' BW, FI, and FCR. In agreement with our results, previous studies by Cransberg et al. (2000), Türkyilmaz et al. (2010), and Fidan et al. (2014) also reported no changes in the growth performance of broilers subjected to regular visual contact with human beings. Zulkifli and Siti Nor Azah (2004) subjected chicks to physical contact by picking them up individually and stroking them gently for 30 seconds daily in their home pens. The authors reported that broilers exposed to regular physical contact with humans had better body weight gain and FCR than controls. It is possible that the human contact treatment practised in the current study did not create an active interaction with the birds as compared to the physical contact approach reported by Zulkifli and Siti Nor Azah (2004).

The effects of auditory enrichment and regular human contact on TI response of the birds are shown in Table 2. Neither AE nor HC significantly affected TI duration or the number of induction attempts. These results support DÁVILA et al. (2011) findings. They reported that auditory enrichment through classical music resulted in inconsistent TI duration in eight breeds of 8-week-old layer chickens. In the study, only Black Castellana hens showed significantly shorter TI durations than the controls. GVARYAHU et al. (1989) found that combining auditory enrichment with classical music and physical enrichment reduced TI duration in 6-week-old broilers.

Table 2. Mean (±SE) tonic immobility duration and number of inductions in broiler chickens by auditory enrichment and human contact.

Mittlere (±SE) Dauer der tonischen Immobilität und Anzahl der Induktionen bei Masthühnern mit akustischer Anreicherung der Umgebung und Personenkontakt.

	AE			HC		P value		
Item	NE	ME	QE	RC	NC	AE	HC	AE × HC
Duration (s)	129 ± 19	135 ± 21	149 ± 25	148 ± 18	127 ± 17	0.807	0.459	0.925
Induction (No.)	1.84 ± 0.16	1.75 ± 0.17	1.80 ± 0.13	1.80 ± 0.13	1.54 ± 0.12	0.098	0.096	0.444

Means are not significantly different (P > 0.05).
SE = standard error; AE = auditory enrichment; HC = human contact; NE = no auditory enrichment; ME = auditory enrichment with music; QE = auditory enrichment with Quran recitation; RC = regular human contact; NC = no-human contact.

On the contrary, TI durations were significantly longer in two Spanish layer breeds exposed to a specific noise or classical music stimulus at 36 weeks of age (CAMPO et al., 2005). According to FORKMAN et al. (2007), TI may be influenced by age and previous experience. Thus, discrepancies between the present and previous studies could be due to variations in the age of birds and duration of stimuli exposure. on the contrary, JONES (1993) found that placing a hand into a bird's cage twice daily reduced TI and their avoidance of humans. These inconsistencies could be attributed to the variations in the number of birds allocated to a cage as an experimental unit. JONES (1993) had a pair of layer chicks per cage, but the present study assigned ten broiler chicks to a cage. LEONE et al. (2007) reported that chickens in a smaller group were more affected by enrichment than those in a larger flock. Furthermore, the different breeds of chickens used in the two studies may have contributed to the observed discrepancies. As indicated earlier, the response of farm animals to enrichment stimuli may vary according to their genetic makeup (HILL et al., 1998).

There were no significant interactions between AE and HC for CORT (Table 3). However, the response to auditory enrichment varied with the age of the birds. While ME and QE significantly reduced CORT on days 14 and 21, it had no significant effect on days 7 and 35. Previous studies on auditory enrichment in poultry did not measure CORT. LADD et al. (1992) and DÁVILA et al. (2011) reported that auditory enrichment with classical music significantly reduced heterophil-to-lymphocyte ratios (HLR) in laying hens. Stress-related behaviour in laying hens was also reduced by auditory enrichment (CHRISTENSEN and KNIGHT, 1975). However, LADD et al. (1992) reported that auditory enrichment using country music (LADD et al., 1992) and classical music (CAMPO et al., 2005) did not affect HLR in laying hens. The discrepancies might be due to the variations in the previous experience of the birds and age or the type and intensity of the music enrichment. Both acute and chronic noise exposures above 80 dB were noted to increase CORT levels in broiler chickens and caused significant changes in their adrenal glands histological structure (CHLOUPEK et al., 2009; ŽIKIĆ et al., 2011).

Table 3. Mean (±SE) serum levels of corticosterone (mg/ml) in broiler chickens by auditory enrichment and human contact.

Mittlere (±SE) Serumspiegel von Corticosteron (mg/ml) bei Masthühnern nach akustischer Anreicherung der Umgebung und Personenkontakt.

	AE			HC		P value		
Item	NE	ME	QE	RC	NC	AE	HC	AE × HC
Day 7	12.67 ± 0.89	14.98 ± 1.14	14.37 ± 1.10	13.74 ± 0.91	14.29 ± 0.83	0.283	0.712	0.451
Day 14	8.99[a] ± 1.06	6.66[b] ± 0.56	6.24[b] ± 0.54	7.36 ± 0.62	7.07 ± 0.62	0.024	0.931	0.793
Day 21	2.70[a] ± 0.29	1.98[b] ± 0.15	1.93[b] ± 0.18	2.09 ± 0.19	2.32 ± 0.17	0.019	0.339	0.291
Day 35	0.81 ± 0.25	0.78 ± 0.10	0.58 ± 0.08	0.76 ± 0.16	0.69 ± 0.07	0.542	0.632	0.226

[a,b] Means within a column-subgroup with no common superscripts differ at $P \leq 0.05$.
SE = standard error; AE = auditory enrichment; HC = human contact; NE = no auditory enrichment; ME = auditory enrichment with music; QE = auditory enrichment with Quran recitation; RC = regular human contact; NC = no-human contact.

As practised in the current study, ME and QE in a lower volume (75 dB) may have calming and relaxing effects. Hence, ME and QE could potentially mitigate the stress in broilers. Auditory enrichment may achieve this by diminishing the stress response in the hypothalamic-pituitary-adrenal axis by stimulating hippocampal activity in the brain (KOELSCH and SKOURAS, 2014). There is the possibility that the stress-reducing impact of ME and QE on broiler chicks might be linked to their capacity to modulate neuronal activity within these brain regions. HANAFI et al. (2023) showed that classical music enhanced the ability of embryos and neonatal chicks to express heat shock protein (HSP) 70. The profound role of HSP 70 in the acquisition of stress resilience in poultry has been well-documented (BALAKRISHNAN et al., 2023).

Notably, the lack of enrichment effect on CORT at day 35 might be attributed to adaptation. Although it is reported that birds constantly respond to stimuli within their microenvironment (COCKREM, 2007), monotonous and continuous environmental stimuli may lead to boredom and adaptation (GVARYAHU et al., 1994). The regular human contact in the current study did not affect CORT. on the contrary, ZULKIFLI and SITI NOR AZAH (2004) reported that regular physical contact with humans during the first three weeks reduced stress and fear reaction to translocation from floor pens to cages later in life in broiler chickens. The discrepancies could be attributed to the differences in the procedure of human contact applied. The work of ZULKIFLI and SITI NOR AZAH (2004) involved an experimenter who handled each bird individually for 30 seconds daily, while in the current study, one hand was placed in the bird's cage for 30 seconds twice a day. It is possible that the human contact treatment practised in the current study is not interactive and engaging enough for the birds.

Conclusions

Auditory enrichment through classical music may have a beneficial effect on the performance of broilers during the early stage of growth. Exposure to music or Quran recital dampened stress response but not underlying fearfulness in chickens at market age. Prolonged exposure to a similar auditory stimulus may lead to habituation, and thus the effectiveness may be reduced. Presenting different enrichment tools intermittently may overcome the problem.

Authors contribution

H.M.T. sampling, animal care, handling and laboratory work and writing. Z.I. funding acquisition, experimental design, supervision, and writing. A.S.F. supervision and facilitating the research work. E.A.A laboratory work, statistical analysis, and monitoring of research work. A.N.H. writing, review, and editing. All the authors read and approved the final manuscript.

Conflict of interest

The authors disclose that there is no conflict of interest.

References

ABU BAKAR, S.A., 2015: Effects of Holy Quran listening on physiological stress response among muslim patients in intensive care unit. J. Manag. Muamalah. **5(1)**, 1-11.

AL-AQIL, A., I. ZULKIFLI, M. HAIR BEJO, A.Q. SAZILI, M.A. RAJION, M.N. SOMCHIT, 2013: Changes in heat shock protein 70, blood parameters and fear-related behavior in broiler chickens as affected by pleasant and unpleasant human contact. Poult. Sci. **93**, 33-40.

ALWORTH, L., S. BUERKLE, 2013: The effects of music on animal physiology, behavior and welfare. Lab. Anim. **42**, 54-61.

ANSARI, J. A., T. NEGAHBAN, A.R. SAYYADI ANAR, P. AGHA MOHAMMAD HOSAYNI, 2005: Reciting the Quran on depression in Moradi hospital patients. Kordestan. Univ. Med. Sci. J. **10(2)**, 42-8.

BALAKRISHNAN, K.N., S.K. RAMIAH, I. ZULKIFLI, 2023: Heat shock protein response to stress in poultry: A review. Animals. **13(2)**, 317.

BENOFF, F.H., P.B. SIEGEL, 1976: Genetic analysis of tonic immobility in young Japanese quail (*Coturnix cotunix japonica*). Ani. Learning Behav. **4(2)**, 160-162.

BINNS-TURNER, P.G., L.L. WILSON, E.R. PRYOR, G.L. BOYD, C.A. PRICKETT, 2011: Perioperative music and its effects on anxiety, hemodynamics, and pain in women undergoing mastectomy. J. Am. Assoc. Nurse Anesth. **79**, S21–S27.

BREUER, K., P.H. HEMSWORTH, G.J. COLEMAN, 2003: The effect of positive or negative handling on the behavioural and physiological responses of nonlactating heifers. Appl. Anim. Behav. Sci. **84**, 3-22.

CAMPO, J.L., M.G. GIL, S.G. DÁVILA, 2005: Effects of specific noise and music stimuli on stress and fear levels of laying hens of several breeds. Appl. Anim. Behav. Sci. **91(1-2)**, 75-84.

CHLOUPEK, P., E. VOSLAROVA, J. CHLOUPEK, I. BEDANOVA, V. PISTEKOVA, V. VECEREK, 2009: Stress in broiler chickens due to acute noise exposure. Acta Vet. Brno. **78**, 93-98.

CHRISTENSEN, A.C., A.D. KNIGHT, 1975: Observations on the effects of music exposure to growing performance of meat-type chickens. Poult. Sci. **54**, 619-621.

CLOUTIER, S., D.M. WEARY, D. FRASER, 2000: Can ambient sound reduce distress in piglets during weaning and restraint? J. Appl. Anim. Welfare. Sci. **3**, 107-116.

COCKREM, J.F., 2007: Stress, corticosterone responses and avian personalities. J. Ornithol. **148**, 169-178.

CRANSBERG, P.H., P.H. HEMSWORTH, G.J. COLEMAN, 2000: Human factors affecting the behavior and productivity of commercial broiler chickens. Brit. Poultry Sci. **41**, 272-279.

DA CRUZ, J.N., D.D. DE LIMA, D.D. DAL MAGRO, J.G.P. DA CRUZ, 2011: The power of classic music to reduce anxiety in rats treated with simvastatin. Basic Clin. Neurosci. **2(4)**, 5.

DÁVILA, S.G., J.L. CAMPO, M.G. GILL, M.T. PRIETO, O. TORRES, 2011: Effects of auditory and physical enrichment on 3 measurements of fear and stress (tonic immobility duration, heterophil to lymphocyte ratio, and fluctuating asymmetry) in several breeds of layer chicks. Poult. Sci. **90**, 2459-2466.

DOMJAN, M., 2005: Pavlovian conditioning: A functional perspective. Annu. Rev. Psychol. **56**, 179-206.

FIDAN, E.D., M.K. TURKYILMAZ, S.U. AYPAK, 2014: Effect of differently coloured clothes on fear and stress responses, some meat quality traits and performance in broilers. Ital. J. Anim. Sci. **13(3)**, 3453.

FORKMAN, B., A. BOISSY, M.C. MEUNIER-SALAU¨N, E. CANALI, R.B. JONES, 2007: A critical review of fear tests used on cattle, pigs, sheep, poultry andhorses. Physiol. Behav. **92**, 340-374.

FOX, R.A., J.R. MILLAM, 2007: Novelty and individual differences influence neophobia in orange-winged Amazon parrots (*Amazona amazonica*). Appl. Anim. Behav. Sci. **104(1-2)**, 107-115.

GALLISTEL, C.R., 1990: The organisation of learning. Chapter 14. The MIT Press.

GHAZALI, R., H. ABDUL RAHIM, M. SHIKIN MAIDIN, S. SAHLAN, N. ABDUL RAZAK, 2015: Sound exposure towards Ross broilers growth rate. Int. J. Biol. Biomol. Agri. Food Biotech. Engin. **9(1)**, 101-105.

GVARYAHU, G., D.L. CUNNINGHAM, A. TIENHOVEN, 1989: Filial imprinting, environmental enrichment and music application. Poult. Sci. **68**, 211-217.

GVARYAHU, G.E., E. ARARAT, M. ASAF, J.I. LEV, B. WELLER, N. ROBINSON, SNAPIR, 1994: An enrichment object that reduces aggressiveness and mortality in caged laying hens. Psych. Behav. **55**, 313-316.

HANAFI, S.A., I. ZULKIFLI, S.K. RAMIAH, E.L.T. CHUNG, R. KAMIL, E.A. AWAD, 2023: Prenatal auditory stimulation induces physiological stress responses in developing embryos and newly hatched chicks. Poult. Sci. **102(2)**, 102390.

HASANPOOR, G., 2001: Quran recitation effect on reducing anxiety and depression. Tehran, First Conference of the Religion Role in Mental Health.

HEMSWORTH, P.H., J.L. BARNETT, C. HANSEN, 1981: The influence of handling by humans on the behavior, growth, and corticosteroids in the juvenile female pig. Horm. Behav. **15(4)**, 396-403.

HILL, J.D., J.J. MCGLONE, S.D. FULLWOOD, M.F. MILLER, 1998: Environmental enrichment influences on pig behavior, performance and meat quality. Appl. Anim. Behav. Sci. **57**, 51-68.

HUFF, G.R., W.E. HUFF, J.M. BALOG, N.C. RATH, 2003: The effects of behavior and environmental enrichment on disease resistance of turkeys. Brain. Behav. Immun. **17(5)**, 339-349.

JONES, R.B., 1993: Reduction of the domestic chick's fear of humans by regular handling and related treatments. Anim. Behav. **46**, 991-998.

JONES, R.B., 1996: Fear and adaptability in poultry: insights, implications and imperatives. Worlds. Poult. Sci. J. **52**, 131-174.

JONES, R.B., S. RAYNER, 1999: Music in the hen house: a survey of its incidence and perceived benefits. Poult. Sci. **78 (S1)**, 110.

JONES, R.B., 2001: Environmental enrichment for poultry welfare. BSAP Occasional Publication, **28**, 125-131.

KAZEMI, M., A. ANSARI, M. ALLAH TAVAKOLI, S. KARIMI, 2003: Impact on mental health nursing students reciting the Quran. Rafsanjan. Univ. Med. Sci. J. **3(1)**,52-7.

KOELSCH, S., S. SKOURAS, 2014: Functional centrality of amygdala, striatum and hypothalamus in a "small-world" network underlying joy: An fMRI study with music. Hum. Brain Mapp. **35(7)**, 3485-3498.

KUCZAJ, S., T. LACINAK, O. FAD, M. TRONE, 2002: Keeping environmental enriching. Int. J. Comp. Psych. **15**, 127-137.

LADD, J.K., J.L. ALBRIGHT, A.M. BECK, B.T. LADD, 1992: Behavioural and physiological studies on the effect of music on animals. J. Anim. Sci. **70**, 170.

LEONE, E.H., I. ESTEVEZ, M.C. CHRISTMAN, 2007: Environmental complexity and group size: Immediate effects on use of space by domestic fowl. Appl. Anim. Behav. Sci. **102(1-2)**, 39-52.

LI, J., X. LI, H. LIU, J. LI, Q. HAN, C. WANG, X. ZENG, Y. LI, W. JI, R. ZHANG, J. BAO, 2021: Effects of music stimulus on behavior response, cortisol level, and horizontal immunity of growing pigs. J. Anim. Sci. **99(5)**, skab043.

LYONS, D.M., E.O. PRICE, G.P. MOBERG, 1988: Individual differences in temperament of domestic dairy goats: Constancy and change. Anim. Behav. **36**, 1323-1333.

MITIN, H., I. ZULKIFLI, G.Y. MENG, A.Q. SAZILI, E.A. AWAD, 2023: Effects of positive human contact on fear and physiological stress responses in Pekin ducks (*Anas platyrhynchos domesticus*) subjected to crating and transport. Appl. Anim. Behav. Sci. **269**, 106108.

MAHJOOB, M., J. NEJATI, A. HOSSEINI, N.M. BAKHSHANI, 2014: The effect of Holy Quran voice on mental health. J. Relig. Health. 1-5.

MARTRENCHAR, A., D. HUONNIC, J.P. COTTE, 2001: Influence of environmental enrichment on injurious pecking and perching behaviour in young turkeys. Brit. Poult. Sci. **42(2)**, 161-170.

MCADIE, T.M., L. J. KEELING, H. J. BLOKHUIS, R.B. JONES, 2005: Reduction in feather pecking and improvement of feather condition with the presentation of a string device to chickens. Appl. Anim. Behav. Sci. **93(1-2)**, 67-80.

MENCH J.A., 1998: Environmental enrichment and the importance of exploratory behavior. In: Shepherdson D.J., J.D. Mellen, M. Hutchins, editors. Second Nature: Environmental Enrichment for Captive Animals.

MESHABAZ, R.A., N.J. HUSSEIN, M.A. MERSHAM, M.S. MHAMED, 2017: Effect of using two music types on non-pregnant non-lactating Arabi ewes behaviour as a tool for welfare improvement. Sci. J. Uni. Zakho. **5(4)**, 301-306.

NEWBERRY, R.C., 1995: Environmental enrichment: Increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci. **44**, 229-243.

NICOL, C.J., 1992: Effects of environmental enrichment and gentle handling on behavior and fear responses of transported broilers. Appl. Anim. Behav. Sci. **33**, 367-380.

NITHIANANTHARAJAH, J., A.J. HANNAN, 2006: Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat. Rev. Neurosci. **7(9)**, 697-709.

PETHERICK, J.C., J. RUSHEN, 1997: Behavioural restriction: Appbleby M.C., B.O. Hughes (editors). Animal welfare. Cambridge: CABI Publishing.

SADEGHI, H., 2011: Voice of Quran and health: A review of performed studies in Iran. Quart. Quran. Med. **1(1)**, 33-337.

SILVA, F.R., K.O.D.S. MIRANDA, S.M.D.S. PIEDADE, D.D.A. SALGADO, 2017: Effect of auditory enrichment (music) in pregnant sows welfare. Eng. Agrí. **37**, 215-225.

TÜRKYILMAZ, M.K., A. NAZLIGÜL, M. BALKAYA, E.F. DERELI, 2010: Effect of human factor on fear and stress reactions and some performance parameters in broiler chickens. Int. J. Poult. Sci. **9(1)**, 59-62.

UETAKE, K., J.F. HURNIK, L. JOHNSON, 1997: Effect of music on voluntary approach of dairy cows to an automatic milking system. Appl. Anim. Behav. Sci. **53**, 175-182.

WOODCOCK, M.B., E.A. PAJOR, M.A. LATOUR, 2004: The effects of hen vocalisations on chick feeding behaviour. Poult. Sci. **83**, 1940-1943.

ŽIKIĆ, D., G. USCEBRAKA, D. GLEDIC, M. LAZAREVIC, S. STOJANOVIC, Z. KANACKI, 2011: The influence of long term sound stress on histological structure of broiler's adrenal glands. Biotech. Anim. Husb. **27(4)**, 1613-1619.

ZULKIFLI, I., J. GILBERT, P.K. LIEW, J. GINSOS, 2002: The effects of regular visual contact on tonic immobility, heterophil/lymphocyte ratio, antibody and growth responses in broiler chickens. Appl. Anim. Behav. Sci. **79**, 103-112.

ZULKIFLI, I, A. SITI NOR AZAH, 2004: Fear and stress reactions, and the performance of commercial broiler chickens subjected to regular pleasant and unpleasant contacts with human beings. Appl. Anim. Behav. Sci. 2004, **88**: 77-87.