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Breed differences in tonic immobility and emergence of commonly raised broiler chicken

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ABSTRACT: The impact of breed on various behavioural traits has been documented across numerous avian species; however, there exists a scarcity of studies focusing on commonly raised broiler chickens. Fear, characterised as a detrimental affective state, serves as an indicator of distress and is correlated with adverse implications for the welfare and productivity of livestock. This investigation sought to evaluate fear-related behaviours in commonly raised broiler chickens of four distinct genotypes (Arbor Acres, Hubbard, Ross, and Cobb) at weeks 3, 5, 7, and 9 of age. A total of 140 birds (35 individuals per breed) were utilized, all derived from a common genetic lineage. Tonic immobility and emergence tests were conducted at weeks 3, 5, 7, and 9, respectively. Statistical analyses were performed employing Kruskal-Wallis tests and Mann-Whitney U tests. At the 3rd week of observation, the duration of tonic immobility exhibited a significant association with the breed ($\chi2=10.686$, DF=3, p=0.014); however, this association was not significant by the 7th week ($\chi2=6.349$, DF=3, p=0.096). Moreover, the latency to emerge from a dark enclosure was significantly influenced by plumage colour ($\chi2 =8.9229$, DF=2, p = 0.01), with the shortest emergence latency recorded in black NIT. Breed significantly affected the latency to emerge from the box at both observation weeks ($\chi2=20.224$, DF=3, p= 0.00 and $\chi2=19.419$, DF=3, p=0.00). Conversely, no significant effects of sex were observed on the duration of tonic immobility at either observation week (Ų =2344.500, ž=-0.366, p=0.714 and Ų=2135.500, ž=-1.094, p=0.274). While the latency to emerge from the box was not significantly associated with sex at the 7th week of observation, a significant association was noted with the breed at the 9th week (Ų =2285.000, ž=-0.673, p=.501 and Ų=1883.500, ž=-2.230, p=.026). Remarkably, at the 9th week, females displayed a shorter latency to emerge from the box compared to males, indicating varying fear responses. In conclusion, breed exerted a discernible influence on fear levels in commonly raised broiler chickens, with Hubbard birds demonstrating the highest level of fear across two statistically significant fear indicators (prolonged emergence duration and tonic immobility).

Keywords: Behavioural traits, broiler chicken, fear, emergence, tonic immobility,

INTRODUCTION

Global animal production has experienced significant growth over the past four decades, albeit with disparate distribution patterns worldwide (Sims *et al*., 2005). Notably, discernible long-term trends in animal production emerge when considering developed and developing countries separately, as initial intensification efforts were predominantly observed in developed nations. However, there is a notable surge in animal production in many developing countries, while in most developed nations, the growth in animal production has plateaued. This trend is attributed to the economic development of poorer nations, leading to increased disposable income and enhanced accessibility to animal protein.

Among livestock species, chickens hold a paramount

position, boasting a global population of 166 million birds (FAOSTAT, 2007) and serving as a principal source of animal protein, comprising both meat and eggs, for the burgeoning global population (FAOSTAT, 2020). Poultry products, including fresh and frozen meat, feathers, eggs, and poultry manure, are widely recognized for their nutritional value and contribute significantly to dietary protein intake. The economic importance of poultry meat is substantial, influencing the economies of both developed and developing nations.

Broiler production entails rearing chickens of heavy meat breeds with the aim of obtaining high-quality meat products, typically marketed live or processed at ten to twelve weeks of age (Amos, 2006). This practice is ubiquitous across various regions, devoid of known religious, social, or cultural constraints associated with consumption (Emaikwu *et al*., 2011). Notably, investment in broiler production is appealing due to its comparatively low production costs per unit in comparison to other livestock sectors.

The concept of animal welfare encompasses the harmonious relationship between an animal and its environment, entailing physical and mental equilibrium and freedom from pain, injury, disease, fear, distress, hunger, thirst, and discomfort, while also enabling the expression of natural behaviours (Brambell, 1965; Bracke and Hopster, 2006). Assessment of animal welfare can be predicated on physiological indicators (such as body weight and nasal discharge) as well as natural behavioural indices (including feeding and drinking patterns, body posture, flock distribution, and gaits) (IFC, 2014). Notably, an animal's welfare and its behaviour are intricately linked, with behaviour being a response to environmental stimuli (Jensen and Toates, 1993). However, defining and assessing animal welfare have been subjects of considerable debate, yielding diverse methodologies and interpretations across various farmed species (Fraser, 2003; Hemsworth and Coleman, 1998).

To evaluate bird welfare effectively, it is imperative to conduct observations and measurements on individual animals. For instance, the poultry welfare quality project (Laywel, 2006) utilized welfare freedoms as a framework for assessing bird welfare, focusing on four key categories: injury, disease, and pain; hunger, thirst, and productivity behaviour; and fear, stress, and discomfort. Alongside the rise in turkey production, welfare concerns have also escalated, with major issues including injurious pecking, footpad dermatitis, and leg and skeletal abnormalities.

Individual variations in fearfulness play a crucial role in determining an animal's ability to cope with environmental challenges. Highly fearful animals exhibit heightened physiological responses to stressors and demonstrate divergent behaviours with significant economic implications. Excessive fear in broilers, for instance, can trigger maladaptive behaviours, leading to injury, pain, and suffocation (Jones, 1996). Moreover, heightened fear and anxiety impair birds' ability to adapt to environmental changes, ultimately impacting factors such as handling, transport, feed conversion ratio, and overall productivity (Hemsworth *et al*., 1994; Altan *et al*., 2003). While fear is deemed an adaptive state essential for protecting animals from harm, chronic fear represents a painful emotion associated with suffering and detrimental effects on productivity and welfare. Nonetheless, a certain level of fear is considered beneficial, aiding animals in avoiding potential threats (Durosaro *et al*., 2021).

The welfare and productivity of growing broilers at the time of harvesting (35-42 days) have garnered considerable attention in recent times. Broiler farmers prioritize achieving higher body weight gain and reduced feed consumption, particularly in light of soaring cereal grain prices in recent years. Conversely, poultry welfare scientists and behaviourists focus on optimizing broiler welfare and mitigating fearfulness, recognizing that intense and prolonged fear can significantly impair performance and welfare. Indeed, mounting evidence suggests a negative association between fear-related behaviours and several performance indicators in poultry, including growth, feed conversion efficiency, egg production, and product quality (Jones, 1996).

While numerous studies have explored fear levels in broiler chickens, there remains a dearth of systematic investigations aimed at comparing fear responses across diverse broiler breeds. Thus, the rationale for conducting this study on "breed differences in fear behaviour of commonly raised broiler chickens" is substantiated.

MATERIALS AND METHODS

The experimental protocol employed in this study received approval from the Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.

Experimental site

The research was conducted at the Poultry Breeding Unit within the College of Animal Science and Livestock Production at the Federal University of Agriculture, located in Odeda Local Government Area of Ogun State, Nigeria. The university is situated between latitudes 7°20' and 7°32' N and longitudes 3°35' and 3°47' E, with an elevation of approximately 76 meters above sea level. It is positioned in the southwestern region of Nigeria, characterized by a tropical climate. The area experiences a mean annual rainfall of approximately 1037 mm. The average monthly ambient temperature ranges from 28°C in December to 36°C in February, with an annual average relative humidity of around 82%. The vegetation surrounding the university exhibits characteristics of both tropical rainforest and derived savannah ecosystems.

Experimental birds and management

In this study, a total of 140 broiler chickens representing four different breeds were utilized, and they were managed under an intensive system. Each pen was delineated using wire gauze, and dry wood shavings were employed as litter material, spread across the floor of each pen. The birds were provided with feeders, drinkers, access to clean water, and ample feed. Drinkers were cleaned daily, and litter was regularly changed to mitigate the accumulation of microorganisms. Each bird was tagged on its wing to facilitate easy identification throughout the study. Stringent sanitation measures and vaccination programs were implemented to prevent the occurrence of diseases.

Experimental procedures

A total of 140 broiler chickens were utilized in this study, comprising 35 Hubbard, 35 Ross, 35 Arbor Acres, and 35 Cobb breeds. These birds were reared under intensive management conditions from day one until 10 weeks of age. All chickens were sourced from the same genetic lineage and procured from a reputable hatchery located in Ibadan, Oyo State, Nigeria. All chicks were hatched simultaneously and transported to the brooding pen on the day of hatching. Upon arrival, they were housed together in the same brooding pen, which measured 3.6 m × 3.6 m and was equipped with 200-watt bulbs for heating.

The floor of the brooding pen was layered with 5 cm of wood shavings as litter substrate. Throughout the experimental period, the birds remained in the brooding pen, with a lighting regimen set at 12 hours of light and 12 hours of darkness. The brooding temperature was maintained at 34°C during days 1–7, 30°C during days 8–14, and 28°C during days 15–16.

The birds were provided with a chicken starter diet containing 28% crude protein and 2860 kcal/MEkg metabolizable energy, offered in plastic trays (40 cm diameter). Clean water was provided *ad libitum* using bell drinkers (25 cm diameter and 30 cm height). Strict adherence to a vaccination schedule and meticulous sanitation practices were observed to prevent disease outbreaks.

Throughout the duration of the experiment, the birds were solely subjected to brooding, feeding, and provision of water, vaccination, medication, and experimental handling. The study spanned the entire 10 weeks' lifespan of the birds.

Assessment of fear behaviour

All the tests of fear were performed in a sound-proof separate room (3.6 m × 3.6 m) adjacent to the brooding pen. Three people were involved in the conduct of the fear tests; the person bringing the chickens from the brooding pen, the person conducting the fear test and the timekeeper. All the fear tests were conducted on all the 140 chicken randomly without any preference for a particular breed. The fear test was conducted on a bird at a time.

Emergence

The emergence test was conducted when the birds reached 5 and 9 weeks of age. Each bird was individually placed through an inlet opening measuring 10 cm × 10 cm into a dark box measuring 48 cm × 32 cm × 26 cm, equipped with an exit opening measuring 18 cm × 15 cm. The time taken by each bird to emerge from the box was recorded in seconds, with a maximum time allowance of 300 seconds

Tonic immobility

At the age of 3 and 7 weeks, tonic immobility was induced in the birds. This involved gently placing each bird on its back and applying slight pressure for 10 seconds, with one hand on the chest and the other over the head. Following this, the experimenter withdrew their hands and moved away without further disturbance to the bird. The test concluded either after 300 seconds of immobility or if the bird spontaneously righted itself after remaining motionless for at least 10 seconds. The duration of tonic immobility was meticulously measured in seconds for each bird, with all individuals successfully entering into tonic immobility.

Statistical analysis

All data were tested for normality using Shapiro-Wilks tests. As the data will not be normally distributed, even after transformation, the effects of breed differences and sex on behavioural fear responses were analysed using non-parametric tests (Kruskal-Wallis and Mann- Whitney U tests). The Shapiro-Wilks tests were done using PROC UNIVATIATE, while the Kruskal-Wallis tests and Mann-Whitney U tests were done using PROC NPAR1WAY, both in SAS v9 (2002).

RESULTS

The effect of breed differences on the tonic immobility of broiler chickens at week three of observation is presented in Figure 1A, while the effect of breed differences on the tonic immobility of broiler chickens at week seven of observation is presented in Figure 1B. The duration of tonic immobility was significantly associated with the breed at the 3rd week of observation but not significantly

Figure 1A. The effect of breed on Tonic immobility at the 3rd week of observation. Means for different letters are significantly ($p < 0.05$) different.

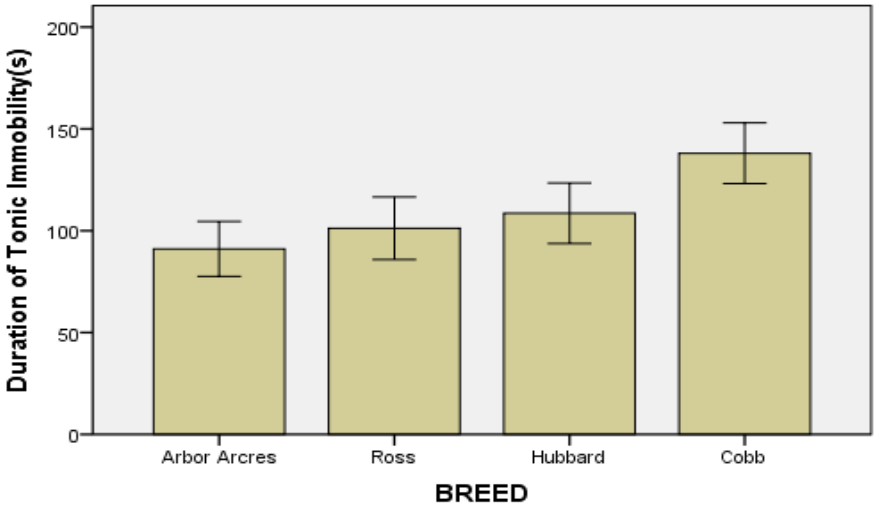

Figure 1B. The effect of breed on tonic immobility at the 7th week of observation.

associated with the breed at the 7th week of observation ($\chi2=10.686$, DF=3, p=0.014 and $\chi2=6.349$, DF=3, p=0.096). At the 3rd week of observation, the shortest duration of tonic immobility was observed in Ross (Figure 1A), while the highest duration of tonic immobility was recorded in Hubbard.

Figure 2A presents the effect of breed differences on duration to emerge at the 5th week of observation, while Figure 2B shows the effect of breed on duration to emerge at the 9th week of observation. The latency to emerge from a box was significantly associated with the breed at both weeks of observations ($\chi2=20.224$, DF=3, p= 0.00 and $\chi2=19.419$, DF=3, p=0.00). At the 5th and 9th week of observation, the shortest latency to emerge from a box was observed in Arbor arcres and Ross respectively. While Hubbard had the longest time to emerge from the box at the 3rd and 7th weeks of observation.

Figure 3A presents the effect of sex on the duration of tonic immobility at the 3rd week of observation, while Figure 3B shows the sex effect on the duration of tonic immobility at the 7th week of observation. No significant effect was observed at both weeks of observation for the effect of sex on the duration of tonic immobility (Ų =2344.500, ž=-0.366 p=0.714 and Ų=2135.500, ž=-1.094, p=0.274).

Figure 4A shows the effect of sex on the duration to emerge at week 5, while Figure 4B presents the effect of

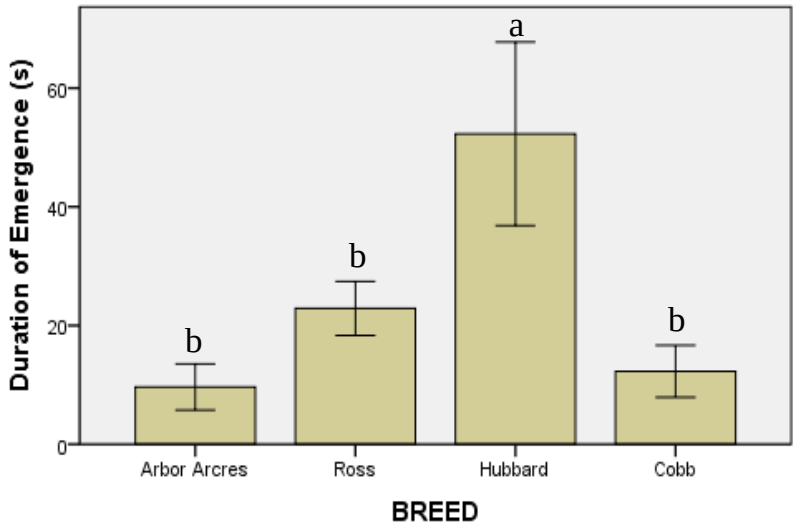

Figure 2A. The effect of breed on latency to emerge at the 5th week of observation. Means for different letters are significantly (p<0.05) different.

Figure 2B. The effect of breed on latency to emerge at the 9th week of observation. Means for different letters are significantly (p<0.05) different.

sex on duration to emerge at week 9. The latency to emerge from a box was not significantly associated with sex at the 5th week of observation but was found to be significantly associated with sex at the 9th week of observation (Ų =2285.000, ž=-0.673 p= 0.501 and Ų=1883.500, ž=-2.230, p= 0.06). At the 9th week of observation, the shortest latency to emerge from a box was observed in the female, while the male took a longer time to emerge from the box.

DISCUSSION

This study aimed to investigate the influence of breed on fear behaviour in broiler chickens, utilizing tonic immobility and emergence from a dark box as fear tests. To avoid inducing stress in the birds, a three-week interval was allowed between fear tests. Two fear tests were employed to validate fear levels and prevent habituation to the same test. Given the use of Kruskal-Wallis and Mann-Whitney U tests for analysis, it is worth noting that the outcomes across tests may be more comparable, potentially resulting in overestimated p-values.

Excessive fear in animals has been associated with heightened stress levels, reduced production, lower body weight, and diminished feed intake (De Haas *et al*., 2013). In this study, breed differences significantly influenced fear levels, with Hubbard chickens exhibiting greater fearfulness

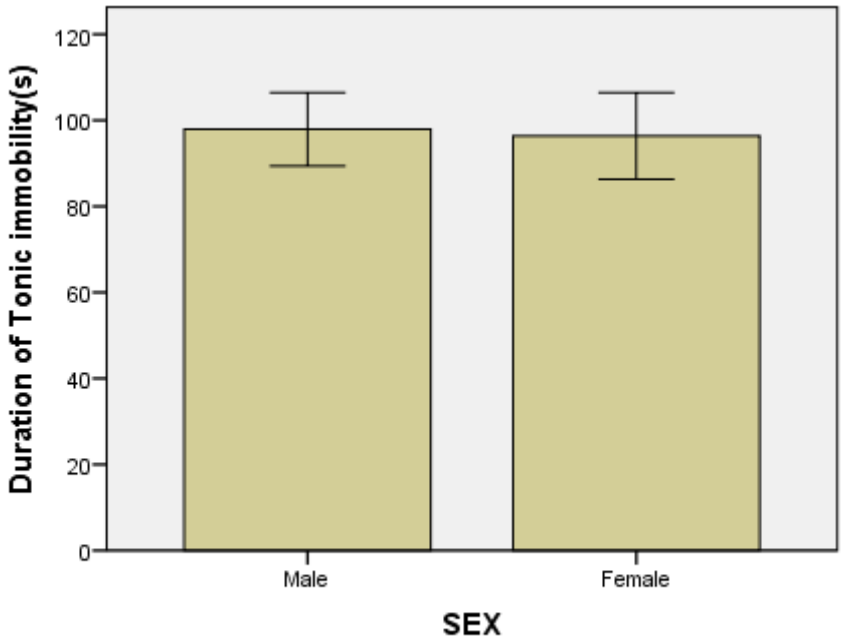

Figure 3A. The effect of sex on duration of tonic immobility at the 3rd week of observation.

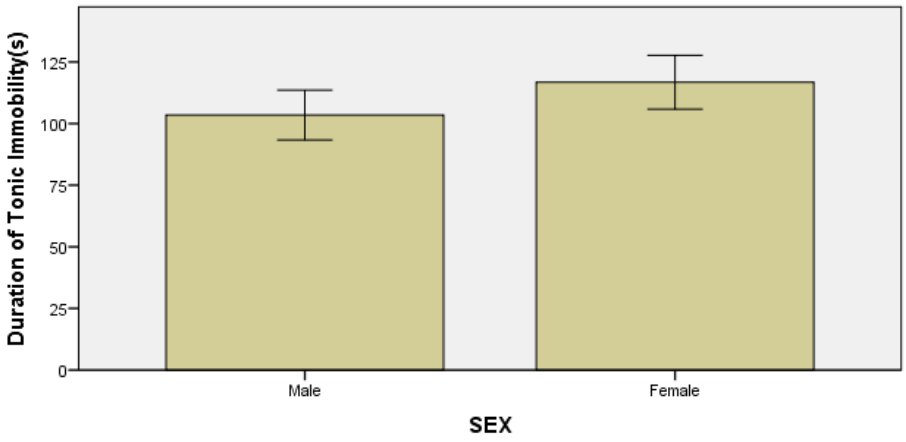

Figure 3B. The effect of sex on duration of tonic immobility at the 7th week of observation.

fearfulness compared to other genotypes, as evidenced by longer latencies to emerge and increased durations of tonic immobility.

Affective states, including fear and anxiety, play a pivotal role in animal welfare assessment (Duncan, 2005). Tonic immobility, a widely recognized fear test, was utilized in this study. Characterized by rigidity and temporary motor inhibition, tonic immobility is induced by brief restraint and represents an unlearned response (Gentle *et al*., 1989). The duration of tonic immobility is believed to be positively correlated with the preceding fear state (Gallup, 1979; Jones, 1986, 1987).

While tonic immobility is a valuable tool for assessing fear, it is not exhaustive, and other behavioural tests such as startle reactions and conditioned avoidance can provide additional insights. However, physiological indices alone may not provide straightforward interpretations and are most effective when combined with behavioural observations and other physiological measurements.

Taskin *et al*. (2018) observed differences in plumage colour affecting the duration of tonic immobility in turkeys, contrasting with the findings of Nakasai *et al*. (2013) who

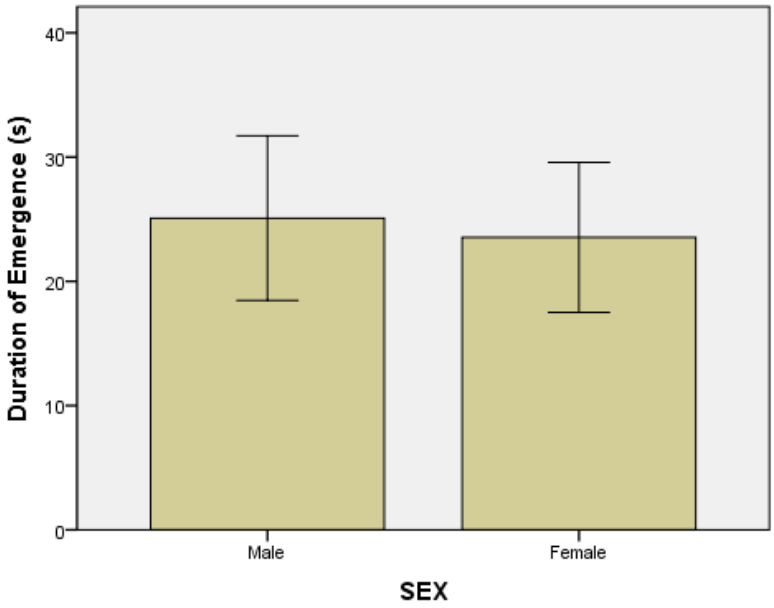

Figure 4A. The effect of sex on duration to emerge at the 5th week of observation.

Figure 4B. The effect of sex on duration to emerge at the 9th week of observation. Means for different letters are significantly ($p<0.05$) different.

observed an increase in tonic immobility duration with age in male Tosa-Jidori chickens. In our study, breed significantly influenced tonic immobility duration at the 3rd week but not the 7th week, suggesting temporal variations in fear responses.

In this study, we found that the latency to emerge from a box was significantly associated with breed at both weeks of observations. The result of this findings is in contrast with the findings of (Durosaro *et al.,* 2023) who reported that breed effect was not significant on latency to emerge from a dark box in FUNAAB Alpha broiler chickens. Though, the shortest latency to emerge from a box observed in this study is in line with their study, as the naked neck FUNAAB Alpha broiler took a longer time to emerge from the box.

The longer emergence times observed for Hubbard and Ross breeds in this study indicate greater fearfulness compared to Arbor Acres and Cobb. This aligns with the premise of the emergence test, where timid birds take longer to emerge from sheltered areas into lit ones

(Russell, 1979; Durosaro *et al*., 2023). In their study, Durosaro *et al*. (2023) reported no significant effect of breed on tonic immobility in the FUNAAB Alpha breed of chicken, which is in line with the result obtained in this study at week seven, where the breeds were similar for tonic immobility.

In conclusion, breed significantly affects fear behaviour in broiler chickens at the 3rd week but not the 5th week of observation. Moreover, breed significantly influences the duration to emerge from a dark box at both observation weeks. These findings highlight the importance of considering breed-specific differences in fear responses when evaluating welfare and managing broiler chicken populations.

CONFLICT OF INTEREST

The authors confirmed that there is no conflict of interest regarding the publication of this paper.

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