



**Research article**

## Open field and tonic immobility responses in broiler chickens administered lycopene

Tunde Ogundehi<sup>1,\*</sup> and Joseph Olusegun Ayo<sup>2</sup>

<sup>1</sup>Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Benin, PMB 1154, Benin City, Nigeria.

<sup>2</sup>Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, P. O. Box 2555, Zaria, Nigeria.

### Abstract

The study aimed to evaluate behavioural responses in broiler chickens administered with lycopene and subjected to open field (OF) and tonic immobility (TI) tests. A total of 100 broiler chicks, divided into five groups of 20 chickens each, were used in experiments I and II. Experiment I had control A and lycopene groups, and Experiment II involved control B, habituation (HB), and habituation+lycopene (HB+Lyco) groups. The olive oil or mixed lycopene was administered by oral gavage once daily, commencing at 07:00 h (GMT+1), to each bird for 28 days for Experiment I and 21 days for Experiment II. In Experiment I, the OF test was conducted on days 14 and 28, while in Experiment II, 4-day habituation training was carried out in the HB and HB+Lyco groups from days 17 to 20, followed by the TI test in each of the three groups on day 21. The results of the OF test indicated a reduced ( $P < 0.05$ ) number of lines crossed, vocalisation, number of jumps, and defecation in the lycopene group compared to control A. An increased ( $P < 0.05$ ) TI duration was recorded in control B compared to the HB group. Lycopene administration for 21 days increased ( $P < 0.05$ ) the TI duration value in the HB+Lyco group compared to the HB group. In conclusion, lycopene administration increased fearfulness in broiler chickens, as evidenced by high fear responses during OF and TI tests. This may be due to the decreased ability to habituate to fear-eliciting stimuli in broiler chickens administered lycopene.

**Keywords:** Animal welfare, Behavioural observation, Fearfulness, Habituation, Nutritional supplements.

**Corresponding author:** Tunde Ogundehi, Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Benin, PMB 1154, Benin City, Nigeria. E-mail: tundeogundehi12@gmail.com

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## INTRODUCTION

The increasing amount of research on antioxidant supplementation in broiler chickens is driven by both the desire to improve productivity and health and the ethical obligation to ensure the welfare of the birds on our farms (Wilcox et al., 2023). Generally, nutritional supplements that can modulate physiological and behavioural responses are of definite concern in good husbandry practice and animal welfare considerations (Dawkins, 2015; Hernandez and Turner, 2022). Lycopene is a potent antioxidant and a naturally occurring pigment belonging to the carotenoid group of phytochemicals (Joshi et al., 2020). Chickens do not consume natural sources of lycopene, but it can be added to their diet as a feed additive (Mezbani et al., 2019; Wang et al., 2022) or administered orally as a supplement (Ayo et al., 2022). The beneficial effects of lycopene in broiler chickens included mitigating the adverse effects of heat stress and improving growth performance (Arain et al., 2018; Ogundesi et al., 2023). Most of the research on the effects of lycopene on behaviour has been conducted in rats and mice, and its protective effects were reflected in the behavioural tests (Chen et al., 2019; El Morsy and Ahmed, 2020). Many studies using mice and rats provide some understanding of the potential effects of lycopene on behavioural responses. According to Bala et al. (2015), lycopene enhanced learning and memory in mice, while Ogundesi et al. (2013) found that it mitigated the effects of psychological stress on excitability and erythrocytes in rats. Zhao et al. (2017) showed that lycopene supplementation decreases oxidative stress and neuroinflammation, improving cognitive function in mice. Wang et al. (2016) further supported these findings by demonstrating that lycopene prevented rats from experiencing cognitive decline caused by a high-fat diet. These studies collectively suggest that lycopene may have a positive impact on behavioural responses. However, the potential for negative or positive behavioural effects of lycopene in broiler chickens has not been specifically addressed in these studies (Dawkins, 2015).

As an early predictor of health and welfare status, behaviour plays a crucial role when evaluating the well-being of broiler chickens (Lourenço da Silva et al., 2021). The open field (OF) and tonic immobility (TI) tests are well-validated fear-related behavioural tests in broiler chickens (Forkman et al., 2007). The behavioural responses of birds subjected to these tests are useful in evaluating health and welfare under different conditions. The TI test is a fear-eliciting behavioural test. Fear habituation in broiler chickens has a cognitive effect, and it is induced using a modified TI test (Nash and Gallup, 1976; Nielsen, 2020). Habituation is a behavioural response deficit whereby animals receive sensation but choose not to respond due to several presentations of the stimulus (Rankin, 2009; Domjan, 2010). The ability to habituate in an environment is a desirable trait in broiler chickens, and efforts aimed at enhancing this ability promote welfare (Jones, 1996).

This study aims to evaluate OF and TI responses in broiler chickens administered lycopene.

## MATERIALS AND METHODS

### Ethical approval

The experiment was approved by the Ethical Committee on Animal Use and Care of Ahmadu Bello University, Zaria (ABUCAUC/2021/025).

### Experimental site and location

The experiment was performed in the poultry house of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria ( $11^{\circ} 10' N$ ,  $07^{\circ} 38' E$ ). The birds were reared under natural thermal environmental conditions. The ambient temperature during the study period was  $38.65 \pm 1.18^{\circ} C$ . The dimensions of the

broiler chicken house were  $8.0 \times 4.5 \times 3.0$  meters. The broiler chicken house from the ground to a height of about 0.5 m was made of cement blocks, and wire mesh was covered from the block to the zinc roof to allow for proper ventilation.

## Experimental flock and management

A total of 100 broiler chickens with an average weight of  $38.65 \pm 1.18$  g were used for this study. They were obtained at a young age from a reputable sales outlet. On arrival at the pens, they were assigned to five different groups. The chickens were raised on a deep litter system. All birds were properly identified using tags. Standard starter diets were fed to the birds from day 1 to day 21, after which they were placed on a standard finisher diet up to the end of the experiment. Clean drinking water was provided *ad libitum*. Proximate analysis of commercial feed was conducted as indicated in Table 1. Biosecurity measures were followed throughout the experiment.

**Table 1** Nutrient contents of the ration fed to the broiler chickens.

Nutrient contents	Amount in % by weight	
	Starter feed (1 - 21 days)	Finisher feed (22 - 28 days)
Calcium (%)	1.20	1.00
Available phosphorous (%)	0.45	0.40
Metabolisable energy (kCal/kg)	2800	2900
Proximate analysis* (%):		
a. Dry matter	97.50	94.00
b. Crude protein	24.74	21.35
c. Crude fibre	4.61	5.35
d. Oil	3.40	3.10
e. Ash	4.50	5.50
f. Nitrogen-free extract	62.75	65.05

\* Analysed in the biochemistry laboratory, Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria.

## Experimental design and groupings

Experiment I: Simple random sampling was used to assign 40-day-old broiler chicks into two groups of 20 chickens each. The Control A group was administered with olive oil (1 ml/kg), and the Lycopene group was administered with lycopene mixed with olive oil. A gelatin capsule containing 10 mg of lycopene (General Nutrition Corporation, Pittsburgh, U.S.A.) was reconstituted in olive oil (Goya en espana, S.A.U., Sevilla, Spain) to a suitable working concentration (Ogundehi et al., 2013). Lycopene was administered at 10 mg/kg (Wang et al., 2022). Lycopene, mixed with olive oil, was administered to each bird by oral gavage once daily for 28 days, commencing at 7:00 h (GMT+1). Each broiler chicken in both groups was subjected to OF tests on days 14 and 28. It was conducted once on each of the designated days, commencing from 08:00 h to 13:00 h (GMT+1).

Experiment II: Simple random sampling was used to assign 60-day-old broiler chicks into three groups of 20 chickens each. The Control B group was administered olive oil with no habituation training; the HB group was administered

olive oil and subjected to habituation training; and the HB+Lyco group was administered lycopene mixed with olive oil and subjected to habituation training. Lycopene was administered at 10 mg/kg. Lycopene, mixed with olive oil, was administered to each bird by oral gavage once daily for 21 days, commencing at 7:00 h (GMT+1). Broiler chickens in the HB and HB+Lyco groups were subjected to 4-day habituation training as described by [Nash and Gallup \(1976\)](#), from days 17 to 20. Briefly, habituation training consists of manually restraining a bird on dorsal recumbency for 15 seconds in a U-shaped cradle covered with cloth by trained personnel. The duration of each resulting immobility episode was terminated after about 15 seconds by gentle prodding. Each bird was given five inductions per day with a 15-second intertrial interval. On day 21, all broiler chickens in the three groups were subjected to a TI test. The habituation training and TI test were conducted once on each of the designated days, commencing from 08:00 h to 13:00 h (GMT+1).

### Open field test

Behavioural data were obtained using the OF test apparatus as described by [Gallup Jr. and Suarez \(1980\)](#), with modifications in the dimensions of the test arena ([Balážová and Baranyiová, 2010](#)). The apparatus was located in the same room in which the birds were housed. On day 14, the test arena of the OF apparatus had dimensions of 80 × 40 cm with square side lengths of 10 cm. The arena was enlarged to 100 × 100 cm on day 28, with a side length of 20 cm. Each chicken was carried from the home cage to the test arena in an upright position with hands around the body. Exposure to the OF test involved placing each broiler chicken alone in the centre of the arena. The duration each bird spent in the test arena was 5 minutes. The 5-minute period started when the bird was placed in the centre of the arena. The handler remained quiet during the period. Thereafter, the bird was returned to its pen. The following behavioural events were assessed: ambulation (the number of times the lines and central lines marked out on the floor of the OF box were crossed); duration of latency (the amount of time the bird remains motionless after being placed in the centre of the OF box); the number of vocalisations (the number of times the bird makes a sound); the number of jumps (the number of times the bird tries to jump out of the box); and defecation (the number of times faecal boluses were excreted).

### Tonic immobility test

The TI test was carried out as described by [Benoff and Siegel \(1976\)](#). Each broiler chicken was gently caught with both hands and prevented from having auditory and visual contact with other birds. TI was induced as soon as the broiler chicken arrived in the separate room by gently restraining it on dorsal recumbency for 15 seconds in a U-shaped cradle covered with form and cloth ([Sinkalu et al., 2016](#)). The birds were then observed from a position about one metre away without making any unnecessary noise or movement. Direct eye contact between the observer and the broiler chicken was avoided as it may prolong TI duration ([Jones, 1986](#)). A stopwatch was started to record latencies until the bird righted itself. If it was righted in less than 10 seconds, the restraining procedure was repeated. When TI was not induced after three attempts, the duration of TI was considered zero. The maximum duration of TI allowed was 600 seconds ([Zulkifli et al., 2000](#)). It was assumed that the catching and returning of the birds did not disturb the other members of the flock ([Harvey et al., 1980; Lagadic et al., 1990](#)).

### Data analysis

The behavioural data obtained were expressed as the mean ± standard error of the mean (mean ± SEM). The OF behaviour was analysed using the Mann-Whitney test. The TI durations were analysed using Kruskal-Wallis' test, followed by Dunn's multiple comparison test to compare means. Values of  $P < 0.05$  were

considered significant. The analyses were performed using GraphPad 8.02 for Windows (San Diego, CA, USA).

## RESULTS

### Behavioural responses in broiler chickens subjected to open field test

The effects of lycopene on behavioural responses in broiler chickens subjected to the OF test during the study period are presented in [Table 2](#). There was no significant difference between both groups in all the behavioural parameters assessed during the OF test on day 14. The number of lines and central lines crossed by the broiler chickens in the control group was higher ( $P < 0.05$ ) than the value obtained in the lycopene group on day 28. The number of jumps, vocalisations, and defecations in the control group was higher ( $P < 0.05$ ) than the value obtained in the lycopene group on day 28.

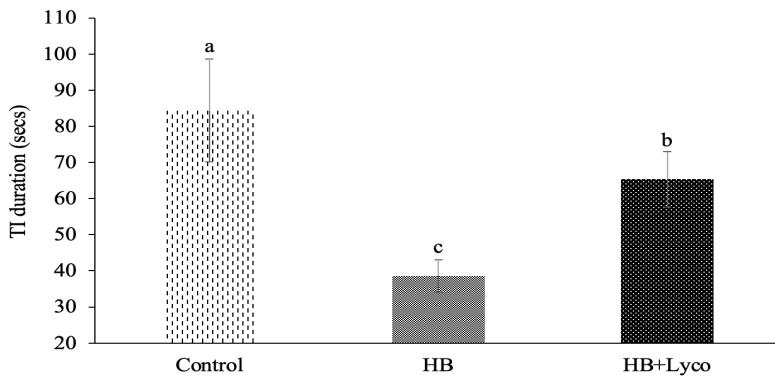
**Table 2** Behavioural responses of broiler chickens administered lycopene and exposed to open field test (n=20).

Parameters	Days of OF test	Control	Lycopene
Number of lines crossed	14	$17.24 \pm 4.88$	$15.89 \pm 4.23$
	28	$16.65 \pm 2.51^a$	$4.33 \pm 0.98^b$
Duration of latency	14	$238.3 \pm 33.01$	$223.7 \pm 30.62$
	28	$57.82 \pm 11.65$	$73.94 \pm 15.77$
Number of vocalization	14	$177.5 \pm 30.25$	$154.5 \pm 31.66$
	28	$216.3 \pm 19.22^a$	$90.89 \pm 15.86^b$
Number of jumps	14	$0.71 \pm 0.34$	$0.84 \pm 0.26$
	28	$1.35 \pm 0.27^a$	$0.61 \pm 0.34^b$
Number of defecation	14	$0.77 \pm 0.18$	$0.53 \pm 0.16$
	28	$2.57 \pm 0.31^a$	$1.06 \pm 0.15^b$

<sup>ab</sup> Means for the same parameter having different superscript letters are significantly different.

### Tonic immobility duration in habituated broiler chickens

The effects of lycopene administration on TI duration in broiler chickens are presented in [Figure 1](#). The increased TI duration value of  $84.35 \pm 14.19$  sec recorded in the control group was higher than ( $P < 0.05$ ) the value of  $38.50 \pm 4.47$  sec obtained in the HB group. Following lycopene administration for 21 days, the TI duration obtained in the HB group was lower than ( $P < 0.05$ ) the value of  $65.35 \pm 7.65$  sec recorded in the HB+Lyco group.



**Figure 1** Tonic immobility duration in habituated broiler chickens administered lycopene for 21 days (n=20).

## DISCUSSION

Although lycopene administration for 14 days did not exert any significant effects, it affected behavioural responses in broiler chickens at day 28 of administration. This result suggests that lycopene exhibited dose-response effects on the behavioural responses of the birds subjected to the OF test. The finding agrees with the report of [Sahin et al. \(2016\)](#), who observed dose-dependent effects of lycopene in heat-stressed chickens. Lycopene significantly decreased ambulation and vocalisation in broiler chickens subjected to the OF test on day 28 of administration. This implies that it increased the level of fear in the chickens. The number of lines and central lines crossed, which represents the steps and area entered, is a good measure of ambulation ([Jones and Carmichael, 1997](#)). Decreased ambulation and vocalisation during the OF test are generally accepted to indicate higher fearfulness ([Jones, 1977](#); [Heiblum et al., 1998](#); [Katajamaa and Jensen, 2020](#)). [Jones \(1989\)](#) stated that the OF response represents a compromise between opposing tendencies to regain contact with the chick's companions and to minimise detection by potential predators. The response to OF reflects underlying fearfulness and social motivation.

The low number of escape attempts observed in broiler chickens administered lycopene may result from either the birds being very frightened or having low social reinstatement motivation ([Forkman et al., 2007](#)). Increased defecation has been considered to indicate a high level of fearfulness ([Walsh and Cummim, 1976](#); [Stanford, 2007](#)). However, the relationship between defecation and fearfulness in poultry subjected to the OF test is not always simple and direct. [Jones and Merry \(1988\)](#) showed that chicks tested individually in the OF test had significantly decreased activity, vocalisation, and defecation compared to when they were paired. This indicates that testing the birds in pairs reduces fear. In the present study, lycopene administration decreased the frequency of defecation in broiler chickens. Thus, the decreased ambulation, vocalisation, jumps, and defecation observed in broiler chickens administered lycopene imply that it may have undesirable effects on behavioural responses following 28 days of administration. Although this study focuses on the duration of lycopene administration, further investigation is recommended on the effects of variable dose concentrations of lycopene on the behaviours of broiler chickens.

The result of the present study indicates that habituated broiler chickens exhibit reduced TI duration when subjected to TI test. This finding is in agreement with an earlier report by [Nash and Gullap \(1976\)](#), who demonstrated that habituation reduces the TI response in broiler chickens. The TI test is a restraint test capable of activating the stress response mechanism in chickens ([Forkman et](#)

al., 2007). Repeated exposure to the test may lead to habituation, which is a basic “learning” or memory process where the organism naturally learns that a stimulus is not harmful (Patel et al., 2005; Rankin et al., 2009; Grissom and Bhatnagar, 2009; Herman, 2013; Patel et al., 2022). Thus, this finding suggests that habituation may be reducing the physiological burden on the animal stress mechanism. Lycopene administration for 21 days increased TI duration in broiler chickens. This indicates that lycopene did not improve habituation responses in the birds. Although the effects of lycopene on behaviour have been poorly studied, the present finding suggests that lycopene may be detrimental to the expression of habituation in chickens. Habituation is a natural behavioural coping strategy, and it occurs virtually in all animals, including domestic chickens (Kostal et al., 2020; Kremer et al., 2020). In broiler chicken production, the decreased ability to habituate to stimulus is an undesirable trait because the energy that the birds would have utilised for productive purposes will be redirected towards reversing the activation of the stress response mechanism. The decreased habituation in chickens administered lycopene is not desirable as it affects the expression of natural behaviour to fear-eliciting stimuli.

## CONCLUSIONS

In conclusion, lycopene administration increased fear responses in broiler chickens, as evidenced by decreased ambulation, vocalisation, jumps, and defecation during the OF test. This may be due to the decreased ability to habituate to fear-eliciting stimuli, which is shown by the increased TI duration in habituate broiler chickens administered lycopene compared to control. From an animal welfare perspective, lycopene administration in broiler chickens exhibited undesirable behavioural effects that may require further investigation.

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## AUTHOR CONTRIBUTIONS

Conceptualization, design, performed the experiment, formal data analyses, and writing (original draft, review and editing) by Tunde Ogundesi.

Conceptualization, design, supervision, and writing (review and editing) by Joseph Olusegun Ayo.

## CONFLICT OF INTEREST

None

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