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Do flickering lights impact turkey hen behavior, stress, and fear?

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ABSTRACT Little is known about the effect of light-flicker frequency on poultry, particularly on turkeys. This experiment examined the impact of light-flicker frequency on the behavior, stress, and fear response of Nicholas Select turkey hens reared to 11 wk. The experiment was a randomized complete block design (2 trials), with a one-way factorial analysis evaluating 3 light-flicker frequencies (30, 90, or 195 Hertz; Hz). Birds (n = 3,276 per trial) were housed in 9 individual environmentally controlled rooms (3 replicates per treatment per trial). Data collected included: behavior (4, 8, and 10 wk), incidence of aggressive damage, heterophil-to-lymphocyte ratio, and novel object test (daily d 1−7 and at 4, 8, and 11 wk). Data were analyzed using Proc Mixed (SAS 9.4), with significance declared at $P \leq 0.05$. Behavior data are presented as the percentage of time spent performing the behavior. At 4 wk, gentle feather pecking and exploratory behaviors were higher under 195 Hz compared to 30 Hz ($P = 0.04$ and $P = 0.05$, respectively). Preening was higher under 90 Hz compared to 30 Hz ($P = 0.05$). At 8 wk, wing flapping was lowest under 195 Hz ($P < 0.01$). Gentle feather pecking was higher under 90 and 195 Hz compared to 30 Hz ($P = 0.02$). Fighting ($P = 0.05$), aggressive pecking ($P = 0.02$), and aggressive behaviors ($P = 0.01$) were lower under 30 Hz compared to 90 Hz. At 10 wk, preening was decreased under 30 Hz ($P = 0.03$). Incidences of aggressive damage were reduced under 30 Hz compared to 90 Hz (0 d−4 wk; $P = 0.01$) and under 30 compared to both 90 and 195 Hz (4−8 wk; $P = 0.01$). At 11 wk, heterophil-to-lymphocyte ratios were lowest under 30 Hz ($P = 0.04$). The novel object test was unaffected by flicker treatment. In conclusion, many behaviors and the stress and fear responses were unaffected by either visible or non-visible flicker. However, visible flicker (30 Hz) reduced some comfort and exploratory behaviors early in life, and the impact on preening continued to older ages, suggesting minor negative impacts of flicker, particularly early in life.

Key words: flicker frequency, welfare, aggression, aggressive damage

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INTRODUCTION

Light flicker is an aspect of light that has been explored to a limited degree, with little known about its effect on poultry species. Not all flicker is visible to the viewer (humans or birds), with visible flicker present at frequencies below the flicker fusion frequency (**FFF**), which is the point at which the viewer sees flicker as a continuous stream of light. Flicker can also be perceived unconsciously (Lisney et al., 2012) at frequencies below the critical flicker fusion frequency (**CFF**) which is the point at which the light flicker is past a viewer's unconscious perception, meaning the brain no longer detects flicker. Several studies have examined the FFF of birds and reported conscious perception of flicker for laying hens between 90 and 105 Hertz (**Hz**; Nuboer et al., 1992; Jarvis et al., 2002; Lisney et al., 2012). Lisney et al. (2012) further examined unconscious perception in laying hens concluding that hens have a physiological response (**CFF**) to flicker up to 118 to 119 Hz.

There is little information on the behavior of birds raised with exposure to light flicker. High flicker-frequencies (26,000 Hz) have been associated with increases in activity levels in broilers compared to lower flicker-frequencies (100 Hz; Boshouwers and Nicaise, 1992). One study in laying hens demonstrated increases in aggression and cannibalism associated with lower flicker frequencies (120 Hz; Kavtarashvili and Gladin, 2022). Conversely, Widowski and Duncan (1996) found no effect of flicker frequency (120 Hz vs 20,000−60,000 Hz) on laying hen behavior, with hens demonstrating no flicker frequency preference. To the authors' knowledge, there are no current behavioral studies published concerning the effects of light flicker on turkeys.

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Flicker exposure in humans has shown detrimental effects on health and well-being including headaches, eye fatigue, anxiety, and seizures (Wilkins et al., 1989; Veitch and McColl, 1995; Inger et al., 2014; SCHEER, 2018; Batra et al., 2019). Direct examination of stress hormones, especially corticosterone (**CORT**), is often used to determine if birds are stressed. The presence of stress hormones such as CORT, norepinephrine, and epinephrine can alter the behavior of birds, bird fearfulness, and result in changes in the number of circulating white blood cells (Dohms and Metz, 1991; Shini et al., 2010; Borah et al., 2022). A recent study by Raabe et al. (2024) found no effect of light-flicker frequency (165, 500, or 16,000 Hz) on turkey tom feather corticosterone concentration. However, the frequencies evaluated were well above the CFF of poultry species (Lisney et al., 2012) and therefore were likely undetected by the birds. Changes in fearfulness can be noted through fear tests such as a novel object test (Forkman et al., 2007). Alterations to circulating white blood cells can be indicative of stress and can be evaluated through changes to the heterophil-to-lymphocyte (**H**/**L**) ratio (Gross and Siegel, 1983; Weimer et al., 2020; Hofman et al., 2021; Nasar et al., 2021; Tetel et al., 2022; Lee et al., 2022). Differences in these parameters may be indicative of the affective state of birds and their stress level.

The objectives of this study were to examine the effects of light-flicker frequency on turkey hen behavior, stress, and fear response when reared to 11 wk of age. Three flicker frequencies were selected, 30 Hz (visible flicker, within the conscious perception of birds), 90 Hz (on the cusp of the visible flicker range for poultry, likely part of the unconscious perception), and 195 Hz (above the CFF of poultry and therefore not perceived as flicker).

MATERIALS AND METHODS

All birds were cared for in accordance with the Guide to the Care and Use of Experimental Animals published by the Canadian Council of Animal Care (2009) and all procedures were approved by the University of Saskatchewan Animal Care Committee under the animal use protocol number 20210090.

Housing and Management

This experiment took place over 2 replicated trials, where 3 light-flicker frequency treatments (30, 90, or 195 Hz) were evaluated. Each trial consisted of 3,276 Nicholas Select turkey hens that were randomly allocated to 1 of 3 flicker treatments and housed in 9 light-tight environmentally controlled rooms (6.7 × 10.0 m, 364 birds per room), allowing for 6 room replicates per treatment. The hens were provided with ad libitum access to feed (aluminum tube feeders, diameter of 36 cm from 0-27 d, then 44 cm) and water (4 Lubing Easy Line drinkers; Lubing, Cleveland, TN). Birds were fed a 5-phase commercial diet, fed in specific quantities per bird (1.4 kg Starter 1 (28% CP), 1.8 kg Starter 2 (26% CP), 2.8 kg Grower 1 (24% CP), 3.8 kg Grower 2 (21.5% CP), ad libitum Finisher (19% CP). Pine wood shavings were used for bedding (depth 7−10 cm). The birds were initially placed into brooder rings (3 per room; approximately 5.0 × 7.0 m each) from d 1 to 9, with supplemental feeders and drinkers. Portable humidifiers were placed in each room from d 1 to 7 to maintain humidity between 50% and 60%. Initial brooding temperature was 34°C and temperature was gradually decreased to reach 16°C by 11 wk (Aviagen Turkeys, 2015).

Each room was equipped with ten 11-watt white LED lamps (AgriLamp 11W ES26/27; Greengage Lighting Ltd., Edinburgh, UK). Flicker was created using purpose-built electronics (Greengage Lighting Ltd. Edinburgh, UK) for the 30 and 90-Hz treatments. The 195 Hz treatment was created using a Symmetry Dimmer (Greengage Lighting Ltd., Edinburgh, UK) and the natural fluctuations in the barn power source. To ensure treatment conditions were maintained, light-flicker frequencies were verified weekly. The 30 and 90-Hz treatments were verified using a spectrometer (Lighting Passport Spectrometer, Asensetek, New Taipei City, Taiwan), and oscilloscope (TDS 210 Digital Real-Time Oscilloscope, Tektronix, Beaverton, OR), while a Lichtflimmer (LiFli; Messgerat LiFli, Fauser Elektrotechnik, München, Germany) was utilized for the 195-Hz treatment. The initial photoperiod program was 23L:1D and was gradually decreased to reach 18L:6D on d 9 (Aviagen Turkeys, 2015). Light intensity at placement was 40 lux and was gradually decreased to 10 lux on d 8. To ensure light intensity was maintained throughout the trial, light intensity was measured (Lighting Passport Spectrometer, Asensetek, New Taipei City, Taiwan) and adjusted weekly, if necessary.

Data Collection

Behavior. Bird behavior was recorded over a 24-h period at 4, 8, and 10 wk using infrared ceiling-mounted cameras (Bosch WZ45 Integrated IR Dome; Bosch Security Systems, Inc., Fairport, NY). The cameras were placed so that the field of view contained one drinker line, 4 feeders, and an edge of the room along the wall (Torrey et al., 2013; Beaulac and Schwean-Lardner, 2018). Videos were reviewed (Genetec Omnicast Software, Genetec Inc., Montreal, Canada) during the photophase and analyzed using a 20-min scan sampling technique, where the number of birds within the field of view were categorized according to their behaviors every 20 min. Behaviors observed are defined in the ethogram in Table 1. Behaviors were grouped into categories: nutritive, active, aggressive, comfort, exploratory, disturbances, other, and unknown.

Incidence of Aggressive Damage. Birds were monitored twice daily for signs of aggressive damage, including pecking damage, scratches, and general trauma to the body. All injured birds were caught, and pine tar was applied to the damaged area to reduce the chance of repeated aggression towards the area (Barnes and Greive, 2017; Beaulac and Schwean-Lardner, 2018). The

Table 1. Behavioral ethogram for turkey hens.

Nutritive behaviors	
At the feeder	Bird is standing or sitting at the feeder with its head in the feeder.
At the drinker	Bird is standing or sitting at the drinker with its head in the drinker cup.
Active behaviors	
Resting	Bird is lying down, not performing any other behavior. The bird may or may not be sleeping.
Standing	Bird is in upright position on both feet, not performing another behavior.
Walking	The bird takes 2 or more consecutive steps at a walking or running pace.
Jumping	Leaping with both feet off the ground.
Pacing	Bird opens their wings and crouches then erratically and rapidly runs back and forth.
Frolicking	Spontaneous, rapid running and/or jumping and wing-flapping with no obvious intention, often with rapid direction changes. Running without wing-flapping is not classified as frolicking.
Aggressive behaviors	
Fighting	Two or more birds, where one is posturing with its head back and breast thrust forward. The actions may also include one individual running and/or jumping at the other.
Aggressive Pecking	One bird forcefully pecks at a pen mate's head, body, or snood while standing or resting. Pen mate will typically move away.
Posturing	Two birds stand staring at each other for >2s with necks upstretched and breast pushed out.
Comfort behaviors	
Preening	The bird manipulates its own feathers with its beak while standing or resting.
Stretching	The bird extends its wing(s) and/or leg(s).
Wing flapping	The bird flaps both wings.
Dust bathing	Fluttering movement of the bird while in a lying position, shaking the feathers of the wings and body.
Feather ruffle	The bird shakes its whole body while standing or resting.
Head scratching	Head and base of the beak are scratched with the bird's foot.
Perching	Sitting or standing off the ground, typically on a structure.
Head rubbing	With one quick sweep, the side of the head is rubbed over the plumage of the body.
Exploratory behaviors	
Environmental pecking	Pecking walls, drinker line, or litter while standing or resting.
Feather pecking	One bird pecks at pen mate's feather while standing or resting. The pen mate does not typically move away.
Litter scratching	Scratching at the ground while standing or walking.
Disturbances	
Moderate disturbance	Bird in a laying posture opens its eyes, lifts its head, or moves its body as a result of another bird walking in front, on top, touching, or flapping near it.
Severe disturbance	A bird in a laying posture stands up as a result of another bird walking near it, on top of it, or flapping near it.
Other	Behaviors that occur extremely infrequently or were not occurring naturally.

Adapted from Guhl (1958), Kruijt (1964), Martrenchar (1999), Estevez et al. (2002), Javid et al. (2016) Mohammed et al. (2016), Vermette et al. (2016), Beaulac and Schwean-Lardner (2018), and Baxter et al. (2019).

location of the damage, date, and recurrence were noted (tail, wing, back, neck, head, snood, or skin tear). If the damage was severe, birds were removed from the trial and either moved to a recovery pen located in an adjacent building for the remainder of the trial or euthanized. Aggressive damage was further broken into time periods (0−4 wk, 4−8 wk, or 8−11 wk) to evaluate aggression at varying ages throughout the rearing period.

Heterophil-to-Lymphocyte Ratio. To assess chronic stress, H/L ratio was evaluated at 4, 8, and 11 wk. A subsample of 20 birds per room replicate were randomly selected. Approximately 2 mL of blood was drawn from the brachial vein into ethylenediamine tetraacetic acid (**EDTA**) vacutainer tubes. Blood smear slides were made immediately after and stained the following week (PROTOCOL Hema 3 manual staining system; Fischer Scientific; Ottawa, Canada). The H/L ratio was determined by counting the number of heterophils and lymphocytes until a total of 100 cells were identified using 100 x oil magnification (B-290TB; Optika; Bergamo, Italy). The number of heterophils was then divided by the number of lymphocytes to calculate the H/L ratio.

Novel Object Test. Novel object tests were performed to evaluate the fearfulness of birds. They were conducted daily for the first 7 d and then at 4, 8, and 11 wk. A new object (no previous exposure) was selected daily for the first 7 d to ensure the object remained novel (Figure 1), then 3 objects were randomly selected to be used again at wk 4, 8 and 11. During the first wk, 2 observers entered each room with 1 observer at the brooder ring in the rear left of the room and the other at the front left brooder ring. For wk 4, 8, and 11, 2 observers entered each room, 1 stationed in the center at the back of the room and the other in the center at the front of the room. The observers used the same object on the same day and placed the object on the litter, at the same time, in a set location and took a large step away from the object. The observer then recorded the latency for 3 individual birds to make intentional contact with the object. Intentional contact was considered a peck or a bird using its foot (intentionally) to make contact with the object. The maximum time allowed per room was 10 min. If one observer had 3 birds make contact prior to the other, their object was quietly removed to not be a distraction.

Statistical Analyses

Two trials (blocks) were conducted in a randomized complete block design with a 1-way factorial

Figure 1. The novel objects used for fear tests performed on d 1-7 and wk 4, 8, and 11.

arrangement for light-flicker treatment. Individual rooms were the experimental unit, allowing for 6 replications per flicker treatment. Data were checked for normality (Proc Univariate; SAS 9.4, Cary, NC) and log transformed (log+1) when necessary. Data were tested for block significance, with block removed as a random factor when $P \geq 0.05$. An analysis of variance (Proc Mixed; SAS 9.4, Cary, NC) was used to test for differences between group means and a Tukey's range test was completed for mean separation. Differences were considered significant when $P \leq 0.05$.

RESULTS

Behavior

Behaviors have been grouped into larger categories and only individual behaviors that demonstrated significant differences are presented. Individual behaviors that were unaffected by light flicker are as follows: feeding, drinking, walking, frolicking, pacing, jumping, standing, stretching, dust bathing, head scratching, feather ruffling, perching, head rubbing, litter scratching, environmental pecking, moderate disturbances, severe disturbances, and posturing. At 4 wk, turkey hens reared under 90 Hz spent a greater percentage of time preening than those under 30 Hz, with the 195-Hz treatment showing an intermediate response ($P = 0.05$; Table 2). Birds reared under 195 Hz spent a greater percentage of time gentle feather pecking compared to the 30-Hz treatment, while the 90-Hz treatment was not different from either ($P = 0.04$; Table 2). Exploratory behaviors demonstrated a similar response, with hens under 195 Hz spending a greater percentage of time in these behaviors compared to 30 Hz, with 90 Hz being intermediate ($P = 0.05$; Table 2).

At 8 wk of age, hens spent a greater percentage of time gentle feather pecking in the 90 and 195-Hz treatments compared to the 30-Hz treatment ($P = 0.02$; Table 3). Hens reared under 30 and 90 Hz spent a greater percentage of time wing flapping compared to hens reared under 195 Hz ($P < 0.01$; Table 3), although incidence was very low in all treatments. Both fighting and aggressive pecking were highest (percentage of time) in hens reared under 90 Hz compared to 30 Hz, with 195 Hz being intermediate ($P = 0.05$ and $P = 0.02$, respectively; Table 3). As a result, total aggressive behaviors demonstrated the

Table 2. Percentage (%) of time spent performing various behaviors by 4-wk-old turkey hens within the field of view during the photoperiod (18 h; 20-min scan sampling intervals), when reared under light-flicker frequencies of 30, 90, 195 Hz.

	Light-flicker frequency (Hz)				
Behaviors	30	90	195	SEM[1]	*P*-value
Individual behaviors					
Walking	20.94	21.21	19.58	0.930	0.60
Standing	12.33	14.19	13.80	0.507	0.09
Feeding	13.91	8.86	9.11	0.872	0.09
Drinking	2.10	2.39	2.27	0.251	0.70
Resting	15.29	14.81	14.84	0.803	0.99
Preening	6.76[b]	7.90[a]	7.11[ab]	0.322	0.05
Gentle feather pecking[2]	4.06[b]	4.28[ab]	5.82[a]	0.386	0.04
Wing flapping	0.07	0.04	0.07	0.016	0.73
Fighting	0.30	0.64	0.78	0.177	0.41
Aggressive pecking	1.25	1.08	0.92	0.093	0.44
Combined behaviors					
Nutritive	16.01	11.25	11.38	0.778	0.09
Active	33.75	35.77	33.83	0.717	0.57
Aggressive	1.25	1.85	1.88	0.251	0.31
Comfort	7.93	9.31	8.50	0.336	0.31
Exploratory	9.68[b]	10.20[ab]	12.65[a]	0.503	0.05
Disturbances[2]	1.40	1.49	1.52	0.153	0.43
Other	0.93	0.85	0.87	0.070	0.94
Unknown[2]	14.66	15.32	15.41	1.164	0.96

[a,b]Values with different letters within the same row differ significantly ($P \leq 0.05$).
[1]Standard error of mean.
[2]Block differed significantly: included as a random factor ($P \leq 0.05$).

Table 3. Percentage (%) of time spent performing various behaviors by 8-wk-old turkey hens within the field of view during the photoperiod (18 h; 20-min scan sampling intervals), when reared under light-flicker frequencies of 30, 90, 195 Hz.

	Light-flicker frequency (Hz)				
Behaviors	30	90	195	SEM[1]	*P*-value
Individual behaviors					
Walking	12.41	10.81	10.54	0.367	0.09
Standing[2]	11.83	13.84	12.96	0.578	0.182
Feeding	11.01	10.00	6.88	1.085	0.28
Drinking	1.92	1.91	2.07	0.280	0.90
Resting	27.68	22.08	24.77	1.096	0.15
Preening	8.14	8.00	8.53	0.370	0.11
Gentle feather pecking[2]	4.26[b]	5.61[a]	6.38[a]	0.493	0.02
Wing flapping[2]	0.18[a]	0.11[a]	0.07[b]	0.030	<0.01
Fighting	0.06[b]	0.17[a]	0.11[ab]	0.022	0.05
Aggressive pecking	0.68[b]	1.20[a]	1.07[ab]	0.094	0.02
Combined behaviors					
Nutritive	12.93	11.91	8.95	1.036	0.28
Active	24.48	24.79	23.63	0.611	0.65
Aggressive	0.80[b]	1.50[a]	1.24[ab]	0.112	0.01
Comfort[2]	9.41	9.05	9.64	0.360	0.73
Exploratory	9.92	11.15	12.03	0.762	0.18
Disturbances[2]	2.72	2.52	2.49	0.216	0.88
Other	0.59	0.51	0.63	0.037	0.43
Unknown[2]	15.83	16.97	17.22	1.695	0.88

[a,b]Values with different letters within the same row differ significantly ($P \leq 0.05$).
[1]Standard error of mean.
[2]Block differed significantly: included as a random factor ($P \leq 0.05$).

same results, with the rate of occurrence under 90 Hz being higher compared with 30 Hz, and the 195-Hz treatment showing an intermediate response ($P = 0.01$; Table 3).

At 10 wk, turkey hens spent a greater percentage of time preening when reared under 90 and 195 Hz, compared to 30 Hz ($P = 0.03$; Table 4). The percentage of time hens spent performing "other" behaviors was highest under 195 Hz compared to 30 Hz, with no differences under 90 Hz ($P = 0.01$; Table 4).

Aggressive Damage

Light-flicker frequency had no impact on the overall incidence of aggressive damage (0−11 wk) on the various body locations or on total damage (data not shown). However, when the data were evaluated for specific rearing periods (0−4 wk, 4−8 wk, or 8−11 wk) some treatment effects were found (Table 5).

From 0 to 4 wk, there was a higher percentage of aggressive damage to the tail in birds reared under

Table 4. Percentage (%) of time spent performing various behaviors by 10-wk-old turkey hens within the field of view during the photoperiod (18 h; 20-min scan sampling intervals), when reared under light-flicker frequencies of 30, 90, 195 Hz.

	Light-flicker frequency (Hz)				
Behaviors	30	90	195	SEM[1]	*P*-value
Individual behaviors					
Walking	8.38	8.23	8.77	0.346	0.68
Standing[2]	12.22	15.86	11.69	0.927	0.07
Feeding	9.41	6.53	7.17	0.735	0.50
Drinking	1.99	1.78	2.34	0.141	0.30
Resting[2]	25.86	23.71	23.19	0.573	0.27
Preening[2]	8.29[b]	9.17[a]	9.31[a]	0.520	0.03
Gentle feather pecking[2]	4.63	5.61	6.42	0.582	0.07
Wing flapping	0.09	0.09	0.12	0.023	0.83
Fighting	0.10	0.07	0.08	0.018	0.99
Aggressive pecking	0.50	0.66	1.04	0.120	0.20
Combined behaviors					
Nutritive	11.40	8.31	9.51	0.721	0.38
Active[2]	20.67	24.15	20.54	0.912	0.09
Aggressive	0.77	0.84	1.23	0.128	0.28
Comfort[2]	9.15	9.85	10.29	0.546	0.44
Exploratory[2]	11.72	10.63	14.29	1.400	0.37
Disturbances	2.16	2.03	2.47	0.185	0.57
Other	0.21[b]	0.27[ab]	0.36[a]	0.025	0.01
Unknown[2]	18.24	20.43	18.47	1.231	0.57

[a,b]Values with different letters within the same row differ significantly ($P \leq 0.05$).
[1]Standard error of mean.
[2]Block differed significantly: included as a random factor ($P \leq 0.05$).

Table 5. Effect of LED light-flicker frequency (30, 90, 195 Hz) on incidences of aggressive damage and skin tears as a percentage (%) of birds placed by location of damage in turkey hens from 0 to 4 wk, 4 to 8 wk, and 8 to 11 wk of age.

% of birds by location of damage	Light-flicker frequency (Hz)			SEM[1]	*P*-value
	30	90	195		
0–4 wk					
Tail[2]	0.00[b]	0.55[a]	0.55[ab]	0.142	0.05
Wing[2]	0.00	0.14	0.05	0.031	0.06
Back	0.00	0.00	0.00	-	-
Neck	0.00	0.00	0.00	-	-
Head	0.00	0.00	0.00	-	-
Snood	0.00	0.09	0.00	0.023	0.17
Skin tear	0.00	0.00	0.00	-	-
Total	0.00[b]	0.78[a]	0.60[ab]	0.149	0.01
4–8 wk					
Tail[2]	0.99[b]	5.63[a]	4.18[a]	0.942	<0.01
Wing	0.33	0.87	0.71	0.123	0.12
Back[2]	0.00	0.00	0.00	-	-
Neck[2]	1.76	1.42	1.32	0.337	0.13
Head	0.22	0.37	0.66	0.106	0.31
Snood[2]	2.42	2.93	2.20	0.472	0.78
Skin tear	0.11	0.00	0.00	0.023	0.09
Total[2]	5.82[b]	11.22[a]	9.07[a]	1.597	0.01
8–11 wk					
Tail[2]	0.22	1.01	0.99	0.239	0.29
Wing[2]	0.16[b]	0.78[a]	0.16[b]	0.130	<0.01
Back	0.00	0.00	0.00	-	-
Neck[2]	1.54[a]	0.50[b]	0.82[b]	0.210	<0.01
Head	0.16	0.05	0.16	0.061	0.69
Snood[2]	0.93[b]	1.88[ab]	2.03[a]	0.335	0.05
Skin tear[2]	0.16	0.18	0.27	0.069	0.42
Total[*]	3.19	4.40	4.45	0.791	0.39

[a,b]Values with different letters within the same row differ significantly ($P \leq 0.05$).
[1]Standard error of the mean.
[2]Block differed significantly: included as a random factor ($P \leq 0.05$).

90 Hz compared to 30 Hz, with 195 Hz being intermediate ($P = 0.05$). As a result, the total aggressive damage for that period followed the same pattern ($P = 0.01$).

During the 4 to 8-wk period, aggressive damage remained higher under both the 90 and 195-Hz treatments ($P = 0.01$). The majority of this damage occurred to the tail ($P < 0.01$), and no other areas were impacted.

During the 8 to 11 wk period, the percentage of birds with damage to the wing area was greater under 90 Hz compared to both 30 and 195 Hz ($P < 0.01$). Of the areas examined, the majority of pecking damage was found on the neck and snood of the birds. In this time period, birds under 30 Hz displayed the most neck damage compared to both 90 and 195 Hz ($P < 0.01$), and birds under 195 Hz displayed more snood damage compared to 30 Hz ($P = 0.05$).

Heterophil-to-Lymphocyte Ratio

Light-flicker frequency did not affect the H/L ratio of turkey hens at 4 or 8 wk ($P = 0.38$ and $P = 0.94$, respectively; Table 6). At 11 wk, the H/L ratio was higher in turkey hens reared under 195 Hz compared to 30 Hz, with 90 Hz being intermediate ($P = 0.04$; Table 6).

Novel Object Test

There was no effect of light-flicker frequency on the latency to make intentional contact with a novel object

Table 6. Effect of LED light-flicker frequency (30, 90, 195 Hz) on the heterophil-to-lymphocyte ratio of turkey hens at 4, 8, and 11 wk of age.

Age (wk)	Light-flicker frequency (Hz)			SEM[1]	*P*-value
	30	90	195		
4	0.69	0.60	0.67	0.016	0.38
8[2]	0.69	0.67	0.69	0.020	0.94
11[2]	0.94[b]	1.03[ab]	1.07[a]	0.022	0.04

[a,b]Values with different letters within the same row differ significantly ($P \leq 0.05$).
[1]Standard error of the mean.
[2]Block differed significantly: included as a random factor ($P \leq 0.05$).

Table 7. Effect of LED light-flicker frequency (30, 90, 195 Hz) on the average time (s) for 3 turkey hens to make intentional contact with a novel object at d 1−7 and 4, 8, and 11 wk of age.

	Light-flicker frequency (Hz)				
Age	30	90	195	SEM[1]	*P*-value
1 d	46	89	85	13.6	0.50
2 d[2]	21	18	34	5.9	0.63
3 d[2]	76	49	108	21.7	0.51
4 d	95	28	60	19.8	0.35
5 d	23	20	22	3.6	0.53
6 d	39	58	40	14.1	0.98
7 d[2]	8	6	6	0.7	0.53
4 wk	486	333	422	41.7	0.33
8 wk	9	8	23	4.8	0.70
11 wk[2]	11	12	20	3.7	0.49

[a,b]Values with different letters within the same row differ significantly ($P \leq 0.05$)
[1]Standard error of the mean
[2]Block differed significantly: included as a random factor ($P \leq 0.05$)

during the first wk (d 1−7) of life or later in the rearing cycle (4, 8, or 11 wk; Table 7).

DISCUSSION

With limited previous information regarding the effects of light-flicker frequency on turkeys, the objective of the overall study was to evaluate the effect of flicker on a wide range of parameters relating to the performance, health, and well-being of turkey hens. This paper focuses on behavior, stress, and fear responses, while performance and health data relating to mobility, foot health, feather condition, and ocular parameters are reported elsewhere (Hammond et al., 2024).

The current study found minimal effects on bird behavior, particularly at older ages. Hens reared under the lower flicker frequency appeared to participate less in aggressive behaviors and as a result there were fewer incidences of aggressive damage. This is contrary to the effects documented in turkey toms and laying hens. Turkey toms reared under frequencies of 165, 500, or 16,000 Hz showed no difference in the incidence of injuries (Raabe et al., 2024). However, the flicker frequencies evaluated in that study were much higher than those in the current one. The study evaluating behavior of laying hens found that hens were more aggressive under low flicker frequency (120 Hz; Kavtarashvili and Gladin, 2022). Gentle feather pecking also occurred less frequently in the low-frequency treatments. While other studies have not noted the differences in aggression and feather pecking, it can be hypothesized that light flicker may result in alterations to the bird's vision (Hammond et al., 2024) and therefore influence their behavior. As turkeys are generally curious, and may peck at objects (debris, dirt, or feather damage) on the plumage, it could be that the birds under the flicker treatment are not seeing these differences on the plumage. This is further supported by the decrease in exploratory behavior at 4 wk. Changes in vision could also relate to the decrease in aggression, as pecking behavior was lower overall in the lower frequency treatments. Additionally, staff noted the young poults appeared to be less active in the lower frequency treatments which may have contributed to lower aggression. Other behavioral differences were found in relation to preening behavior with birds spending a higher percentage of time preening under 90 Hz. While there is no clear explanation for these results, previous literature suggests that preening may be performed as a displacement behavior when birds are stressed (Fraser, 2008; Kozak et al., 2019). This explanation could be related to the increased levels of aggression as well as the increase in H/L ratio observed at wk 11.

It was hypothesized that low flicker frequencies would create additional stress for the birds, however this was not observed as the H/L ratio was greater under the higher flicker-frequency treatments. While this was not expected, it is possible that the increase in aggression observed in the higher flicker-frequency treatments may have contributed to the stress levels of the birds. When evaluating the effect of flicker frequency on turkey toms, Raabe et al. (2024) found no effect on feather corticosterone at 17 wk, which is similar to the results for the 4 and 8 wk measures in the current study. It is possible that birds become accustomed to the flicker over time and therefore examining more parameters early in life could be of value.

The novel object test was used in the current study in order to compare fear levels under different light-flicker treatments. The turkeys' response towards a novel object indicated that the test may not have functioned as intended. In some cases, the hens picked up the novel objects and ran with them, similar to what is known as the play behaviour "worm running" (Cloutier et al., 2004). As turkeys are naturally inquisitive, the novel object test may not be an accurate way of examining their fearfulness. In addition, it was noted by staff that the poults in the current study placed under 30 Hz exhibited avoidance behaviors on the day of placement, gathering in the shadows of the feeders and brooder rings. However, we did not collect data at this early age to support this observation.

Overall, visible light flicker had minor effects on turkey hen behavior, with differences noted in aggressive, comfort (preening), and gentle feather pecking behaviors. The 90-Hz treatment appeared to have the most

negative influence on bird welfare through increased aggression. While the current study was unable to accurately evaluate the fear response of turkeys early in life, future studies could focus on this period, using different methodologies. Further consideration should also be given to evaluating the effects of flicker frequencies within the visible or conscious perception range of turkey toms, as tom behavior differs from that of hens.

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