A novel environmental enrichment device increased physical activity and walking distance in broilers

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ABSTRACT Modern broilers are selected for fast growth and a large proportion of breast tissue, contributing to a top-heavy phenotype, leg disorders, and inactivity as birds reach market weight. Therefore, the objective was to motivate broilers to move through environmental enrichment. A total of 1,200 Ross 308 broilers were housed in pens of 30 for 6 wk: 600 birds were exposed to a novel laser enrichment device (LASER) and 600 were control. Each device projected 2 randomly moving red laser dots onto the floor 4 times/day for 4-min "laser periods." Seven LASER and 7 control pens, with 5 focal birds/pen (n = 70), were randomly selected to be video-recorded day 0 to 8 and once weekly for the remainder of the trial. Videos were analyzed to measure broiler time-budget and behaviors such as latency to feed and distance walked during laser periods. Focal birds were gait scored weekly on-farm. A test of the human-approach paradigm was carried out on weeks 1 and 6 on all pens. LASER birds were more active on days 0, 1, 3, 4, 5, 7, and 8, moving 254% more on day 7 ($P \leq 0.05$). Time spent active was increased in LASER treatment by 114% on week 2; 157% on week 3; 90% on week 4; and 82% on week 5. LASER birds spent more time at the feeder on days 0, 1, 2, 5, 8, and on weeks 1 and 5, with 84% more time at feeder than control on day 5 ($P \leq 0.05$). LASER birds walked further during laser periods on day 0 to 8, reaching 646.5 cm greater (day 1), and on weeks 2, 3, 4, and 5, with an increase of 367.5 cm on week 2 ($P \leq 0.05$). Over week 1 to 6, 60.54 ± 7.4% of focal birds in the laser treatment were at the feeder during or within 5 min following laser periods. The laser enrichment device was successful in stimulating broiler physical activity and feeding, and did not negatively impact walking ability.

Key words: broiler, environmental enrichment, well-being, lameness, behavior

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INTRODUCTION

Today's commercial broiler is up to 5 times heavier than its 1950's predecessor at the same age due to genetic selection for 3-fold improved feed efficiency and a 300% increased growth rate, resulting in a bird reaching market weight in as little as 4 to 6 wk (Havenstein et al., 2003; Knowles et al., 2008; Zuidhof et al., 2014). However, this selection for increased growth rate has contributed to up to 30% of modern commercial broilers suffering from leg lameness or and reduced ability to move (Knowles et al., 2008; Bassler et al., 2013). Both increased age and lameness contribute to decreased time standing or walking. Sound birds spend around 76% of their time sitting or lying down, while lame birds spend up to 86% of their daily time budget inactive. At harvest weight (approximately 2 kg), healthy birds are reported to spend only 3.3% of their day walking vs. 1.5% in lame birds (Weeks et al., 2000). Weeks and others hypothesized that fast-growing, more feed efficient broilers are inherently more inactive. Inactivity increases litter contact and could result in a higher occurrence of breast blisters and contact dermatitis (Weeks et al., 2000; Bassler et al., 2013; Nääs et al., 2018), which are likely painful conditions caused by urea in the litter generating ammonia, creating chemical burns (Haslam et al., 2007). Hence, past broiler research has studied physical activity and methods to increase active behavior.

Reiter and Bessei (2009) used treadmill training to force broilers to exercise for sessions lasting 20 min or 100 m week 1 to 6 and saw improved locomotion. In an additional test from the same study, when distance was gradually increased over day 0 to 5 from 2 to 12 m between feed and water, locomotion was increased 3-fold compared to the control, where resources remained 2 m apart throughout. Similar methods have been successfully used to encourage broilers above the minimum range of movement by increasing distance or introducing barriers between feeders and waterers with-

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out compromising performance (Ventura et al., 2012; Ruiz-Feria et al., 2014), but other, non-resource-based methods have been less successful.

Prayitno and others (1997) used ambient red light to stimulate activity in broilers but saw birds with a mean final body weight 47–79 ± 12.4 g lighter than birds in a blue light treatment. However, broilers in the blue light were significantly less active. Bizeray et al. (2002a) tested different forms of enrichment by scattering wheat on the floor of the pen in one treatment, colored, moving spotlights in another, and barriers between feed and water in a third, but concluded that "forcing animals to exercise more…was more effective for increasing physical activity than was attempting to stimulate foraging activities." Shields and others (2005) hypothesized that broiler exercise would increase and leg lameness would decrease when provided sand bedding; however, birds rested and displayed more inactive behavior on the sand. Ventura et al. (2012) implemented barrier perches as environmental enrichment but saw no increase in walking. Straw bales as a form of "freedom food" were successful in increasing broiler activity, but performance or walking ability data were not reported. Performance may have been negatively impacted in the straw bale treatment due to increased fiber in the gut, indicated by the increased drinking behavior (Kells et al., 2001).

The National Chicken Council (**NCC**) guidelines for broilers (2017) have recognized leg lameness as a welfare concern and recommend gait scoring 100 birds/flock to evaluate leg health within 1 wk of slaughter using the U.S. Gait Scoring technique. This is a 3-point scoring system that has been validated in commercial broiler flocks for high reliability compared to a 6-point system (Webster et al., 2008). However, gait-scoring individual broilers in a research pen, rather than a commercial barn, may prove problematic due to limited space, feeders, and waterers in a confined area. Lameness hurts the industry economically, necessitating up to 2% culls in a $30 billion industry (USDA, 2017; Dunkley, 2007), and there is also considerable evidence that leg lameness is painful for the broiler. Birds with leg lameness eat more analgesic-containing feed than healthy birds, and birds fed an analgesic diet showed improved speed of walking, indicating relief from pain and discomfort caused by leg abnormalities (McGeown et al., 1999; Danbury et al., 2000).

Environmental enrichment has shown the ability to reduce fearfulness in broilers (Altan et al., 2013). The human-approach paradigm (**HAP**) is a validated measure of fearfulness in pigs that uses an unfamiliar human in the pen as a stimulus (Weimer et al., 2014). The HAP has not been studied thus far in broiler chickens, but is a potentially useful measure that takes into account both the movement and the orientation of the animal in relation to the human. The previously mentioned study by Bizeray et al. (2002a) is the only research that has measured the effects of environmental enrichment in the form of moving lights projected onto broiler pen floors. The authors implemented red, blue, green, and yellow spotlights but saw no change in broiler physical activity, and thus concluded that that the spotlights were too large and moved too quickly for broilers to follow. Moving light/visual enrichment successful in motivating broiler activity and improving well-being outcomes is absent in the literature. Furthermore, birds are visual feeders and prefer the color red (Ham and Osorio, 2007). Hence, the present work measured the effects of slowly moving, small particle-sized red laser dots projected onto broiler pen floors throughout the rearing period as a unique enrichment option. The objectives of this work were to stimulate broilers visually using a novel form of environmental enrichment to motivate physical movement, hence increasing walking distance and improving walking ability.

MATERIALS AND METHODS

All live bird procedures were approved by the Iowa State University Institutional Animal Care and Use Committee.

Animals

A total of 1,260 straight-run Ross 308 broiler chicks (day of hatch; BW 47.38 ± 0.14 g) were obtained from a commercial hatchery and transported to the Poultry Research and Teaching Unit at Iowa State University (International Poultry Breeders Hatchery, Bancroft, IA) for a 6-wk grow-out experiment in floor pens. A total of 1,200 were randomly assigned to treatments, and the remainder were culled following standard operating procedures of the farm. A total of 70 birds were randomly assigned upon arrival as focal birds (n = 5 birds/pen in 14 camera pens), identified with wing bands, and marked with unique animal-safe food coloring (red, blue, green, purple, and black; Wilton, Woodridge, IL). Food coloring was applied to a cotton ball, rubbed on the back of the chick's head and neck, and reapplied on an as-needed basis throughout the 6-wk trial.

Housing and Feeding

Birds were housed in forty 1.22 by 2.44 m pens of 30 across 2 rooms in the barn. One room contained 20 LASER pens (exposed to enrichment device), and the other contained 20 control pens, with an anteroom separating the 2 rooms so no crossover of enrichment device was possible; environmental conditions and management were kept the same across rooms. Approximately 10 cm deep fresh wood shavings provided bedding over the solid concrete floor, and PVC pipe dividers with mesh walls (1.22 m height) separated pens. High and low temperatures and humidity were monitored daily in the LASER and control rooms. Average temperatures are listed from the starter, grower, and finisher periods, respectively, from the LASER room of the barn: 85.47, 77.39, and 71.71°F, and the control room: 85.53, 77.46, and 71.5°F. Average relative humidity is listed from the

Table 1. Broiler bird home pen behavior ethogram; focal bird behavior was measured continuously during 4-min laser periods, 4 times daily at 05:30, 11:30, 17:30, and 23:30 for days 0 to 9, 16, 23, 30, and 37.

Measure[1]	Defined
Active	Bird legs were in a continuous forward motion (walking or running).
Inactive	Bird stood in one place or rested its abdomen on the litter, head rested or raised while any part of its body was or was not in contact with another bird.
At feeder	Bird head over feeder circle, bird in feeder or bird stood on feeder tray.
At drinker	Bird stood beneath drinker line.
Other	Dust-bathed, preened (head/beak twisted around in contact with feathers), or any behavior not otherwise identified.
Out of view[2]	Bird was obstructed or not observed due to being under the heat lamp or inside the feeder and could not be seen.

[1]All behaviors were collected as duration, defined as length of time behavior was exhibited in seconds.
[2]Behaviors categorized as "out of view" were so infrequent that the data could not be analyzed; relaxed convergence criteria was attempted to 10^{-4}.

starter, grower, and finisher periods, respectively, from the LASER room: 23.86, 27.21, and 33.93%, and the control room of the barn: 19.89, 23.93, 27.75%.

Birds were gradually adjusted from 24 h light on day 0, defined as day of arrival and placement (30 to 40 lux) to 20 h light (20 to 30 lux) from day 8 to 42. Chicks were brooded with 2-heat lamps/pen (22.9 cm reflectors with porcelain socket) using 125-W heat bulbs (Sylvania, Wilmington, MA) for the first week. Birds were fed an ad libitum diet formulated for Ross 308 commercial recommendations out of a hanging chicken feeder (BRHF151, Brower Equipment, Houghton, IA) gradually raised to accommodate bird height. Water was provided ad libitum from a hanging nipple water line (8 nipples/pen). Mortality throughout the trial was 3.5% in control birds and 3.33% in LASER birds.

Laser Enrichment Device

A total of 10 novel laser enrichment devices designed and built specifically for this research were affixed over 20 pens in 1 room of the barn. Each device was designed and calibrated to cover 2 adjoining pens. The enrichment device consisted of 2 independent red 650 nm lasers contained within a 20.5 by 20.5 cm metal box with a glass bottom mounted on a custom-designed structure made of 3 wooden beams (2.4 m height) raised above the pens. The lasers projected in a random pattern at a range of 7.6 to 30.5 cm/s onto the pen floor for 4-min "laser periods": 05:30 to 05:34, 11:30 to 11:34, 17:30 to 17:34, and 23:30 to 23:34 daily for the entirety of the trial.

Video Camera Set-Up and Training

A total of 70 focal birds (n = 5/pen) were randomly assigned to 14 randomly selected pens (7 LASER, 7 control) equipped with 1 Sony HDR-CX440 Handycam (Sony Corp. of America, New York, NY) each. Cameras were affixed above each pen using brackets adjusted to capture the entire pen. Filming occurred in real time (30 fps) for the first 10 D of the trial (day 0 to 9) and once weekly for the remainder. Video observers were trained by an individual with previous animal behavior observation experience to 90% agreeability using the 4-min laser period video clips from any day recorded (days 0 to 9, 16, 23, 30, and 37). All clips recorded were analyzed for the entirety of the enrichment period in LASER and control pens. Observers were not blinded to treatment; either the lasers or the supporting structure was visible in the videos/images.

Broiler Bird Home Pen Behavior

Trained observers watched the red-colored focal bird in each video-recorded pen (n = 14) during 4-min laser periods and categorized bird behavior continuously throughout the clips using a pre-determined behavior ethogram (Table 1) on days 0 to 9, 16, 23, 30, and 37. Frequency and duration (s) of each behavior were recorded; duration was then converted to percent of time spent on each behavior per 4-min period.

Latency to Feed

Latency to feed following laser turn-off was measured only in LASER pens on days 0 to 9, 16, 23, 30, and 37 due to necessity of laser turn-off and to determine if birds exposed to laser enrichment went to the feeder after the conclusion of laser exposure. At feeder behavior during laser periods was collected in both LASER and control pens as direct comparative measure. A student observer watched laser period video from the 7 LASER pens and identified the red-colored focal bird. At the end of the 4-min period, when the laser dots disappeared, the observer started a timer. The timer was stopped when the focal bird exhibited "at feeder behavior" (Table 1) or when 5 min had passed without the bird feeding. Latency to feed was recorded in seconds. Following data collection, latency to feed measures were categorized into 4 mutually exclusive categories, including (A) at feeder during laser period only (obtained from broiler home pen behavior data), (B) at feeder when laser turned off, (C) went to feeder <5 min following laser turn off, and (D) never went to feeder.

Walking Distance

The distance walked by the blue-colored focal bird in each video-recorded pen (n = 14) was measured over the 4-min laser periods (days 0 to 9, 16, 23, 30, and 37). The observer taped a clear sheet protector over the computer screen and watched each minute individually.

At the beginning of each minute, video was stopped and the observer drew a line at the bird's beak. Video was resumed, and if the bird moved the video was paused again and a line was drawn at the new position of the beak where the bird stopped. This was repeated each minute. Next, the observer used a ruler to draw a line connecting each stopping mark. After drawing the interconnecting line, the observer opened a pen template image in Adobe Photoshop (Adobe Systems Inc, San Jose, CA).

The observer then used a known length within the pen (58.4 cm between 2 segments of the water line, measured on-farm) to standardize the custom ruler tool on Photoshop, measured in pixels (58.4 cm = approximately 194 pixels). The tool would then equate x number of pixels to centimeters. The observer placed the clear sheet protector over the template image and used the custom ruler tool to measure the interconnecting lines drawn from video. This was repeated for each individual minute and then a sum of all line measurements, or the total distance walked each period, was calculated.

Walking Lameness

All focal birds (n = 70) were removed from their home pens once weekly and assessed for lameness. Two researchers conducted the lameness test, with 1 researcher assigning scores. Birds were placed on a custom-designed plywood runway 1.80 m long and 0.46 m wide, with 0.30 m tall walls on all sides. The runway had 0.15 m start and finish sections, a 1.5 m walking space, and delineations marking every 0.30 m and 2.5 cm. Birds were placed on the runway starting section. Birds either walked 1.5 m independently or were encouraged to walk by (1) a researcher slowly moving their hand back and forth directly behind the bird (2) a researcher gently tapping the bird on the vent region with a gloved hand or (3) a researcher both waving behind and gently tapping the bird with a ping-pong paddle. Individual birds were considered to have completed the task when both feet had crossed into the finish section. Scores were assigned using a 0 to 2 scale adapted from NCC guidelines where 0 indicated the ability to walk 1.5 m with no signs of lameness, 1 indicated the ability to walk 1.5 m but showed unevenness in steps or sat down at least once, and 2 indicated a bird that could not walk 1.5 m.

Human-Approach Paradigm

The HAP was completed once during week 1 and once during week 6 on all birds (n = 1,200) beginning at 09:00; pen order was kept the same each week (n = 40 pens). The barn was emptied of personnel apart from 2 researchers carrying out the HAP. Prior to the HAP, the researchers determined optimal bracket angle and camera location for each pen, so that 1 image captured an entire pen. Colored tape identified bracket location; locations ranged between 47.75 and 59.00 cm measuring out from the central PVC pipe. The HAP image was taken with a hand-held camera (Pentax Optio W90, Pentax Imaging Company, Golden, CO). The camera's focal length was 28 m with a 12.1-megapixel resolution.

Methods were based on swine nursery work completed by Weimer et al. (2014). Briefly, researcher A was defined as an unfamiliar human in the pen and researcher B placed the camera/bracket and took the image. Researcher A wore different colored coveralls than the rest of the research and farm crew, but the same boot covers. The researchers approached each pen quietly and recorded the number of birds per pen. Researcher B positioned the bracket on the pen's side in the pre-determined location and then researcher A stepped into the opposite side of the pen with a stopwatch in their right hand. Researcher A took one step towards the center of the pen opposite researcher B and crouched facing the camera with their body angled towards the birds and both arms held close to the body. Once in position, researcher A began the stopwatch, avoiding looking at the birds for 15 s. After 15 s, researcher A stopped the watch and looked up at the birds. Researcher B took an image at the precise moment researcher A looked up.

One student observer, trained using the same methods as video observers but with week 1 HAP images, reviewed the images. Within each digital image of individual pens, broilers were classified into 2 categories: interacting or not interacting. Interacting was defined as any bird in physical contact with or orientated directly towards the unfamiliar human. Birds classified as not interacting were further categorized into 3 mutually exclusive behaviors: feeder, drinker, or other (Table 2). Further, the pen images were split into fourths by tracing over PVC pipe supports every 0.6 m in the pen with a clear sheet and a marker. The number of birds present in each quadrant of the pen was counted, with quadrant 1 containing the unfamiliar human (Figure 1).

Statistical Analysis

In this experimental design, individual control pens (n = 20) were considered experimental units, but LASER pens were analyzed as a group of 2 pens with 1 shared laser device (n = 10). All data were analyzed using SAS software version 9.4 (SAS Institute Inc., 2016, Carey, NC). PROC UNIVARIATE was used to assess the distribution of data prior to analysis. Home pen behavior, walking distance, and HAP data were all abnormally distributed (Poisson distribution), and thus were analyzed using PROC GLIMMIX. GLIMMIX fits models to data with non-constant variability, correlations, or that are not normally distributed. Home pen behavior and walking distance data were analyzed by day (day 0 to 8) and by week (days 2, 9, 16, 23, 30, and 37), utilizing all laser periods within each day. Each model (behavior, walking distance, and HAP) included the fixed effect of treatment (enrichment vs. control),

Table 2. Broiler behavior classification using a digital image analysis upon conclusion of human-approach paradigm (HAP[1]); birds were first categorized as interacting or not interacting, then not interacting birds were separated into 3 mutually exclusive categories[2]: feeder, drinker, or other.

Measure	Definition
Classification at 15 s using digital image evaluation	
Interacting	Using a ruler and a clear sheet protector taped to the screen, a line was drawn from the midpoint of the bird's head to the pen edge. If the line intersected with researcher A, or if any part of the bird was physically contacting researcher A, the bird was classifed as interacting.
Not interacting	Birds not exhibiting the above 2 behavioral classifications.
Further classification of not interacting using digital image evaluation	
Feeder	Bird head over feeder circle, bird in feeder or bird stood on feeder tray.
Drinker	Bird stood beneath drinker line.
Other	Laying (rested its abdomen on the litter, head rested or raised[3]), preening (dust bathed or head/beak twisted around in contact with feathers), wings stretched out, piling (group of 3 or more birds pressed against each other and/or on top of each other, all bird heads facing away from the human in the pen and not performing any other discernible behavior[4]), or not visible.

[1]HAP was carried out on all pens once on week 1 and once on week 6. An unfamiliar human entered the pen and after 15 s, a photograph was taken to measure the birds' response.
[2]Ethogram adapted from Weimer et al. (2014).
[3]Kristensen et al. (2007).
[4]Campbell et al. (2016).

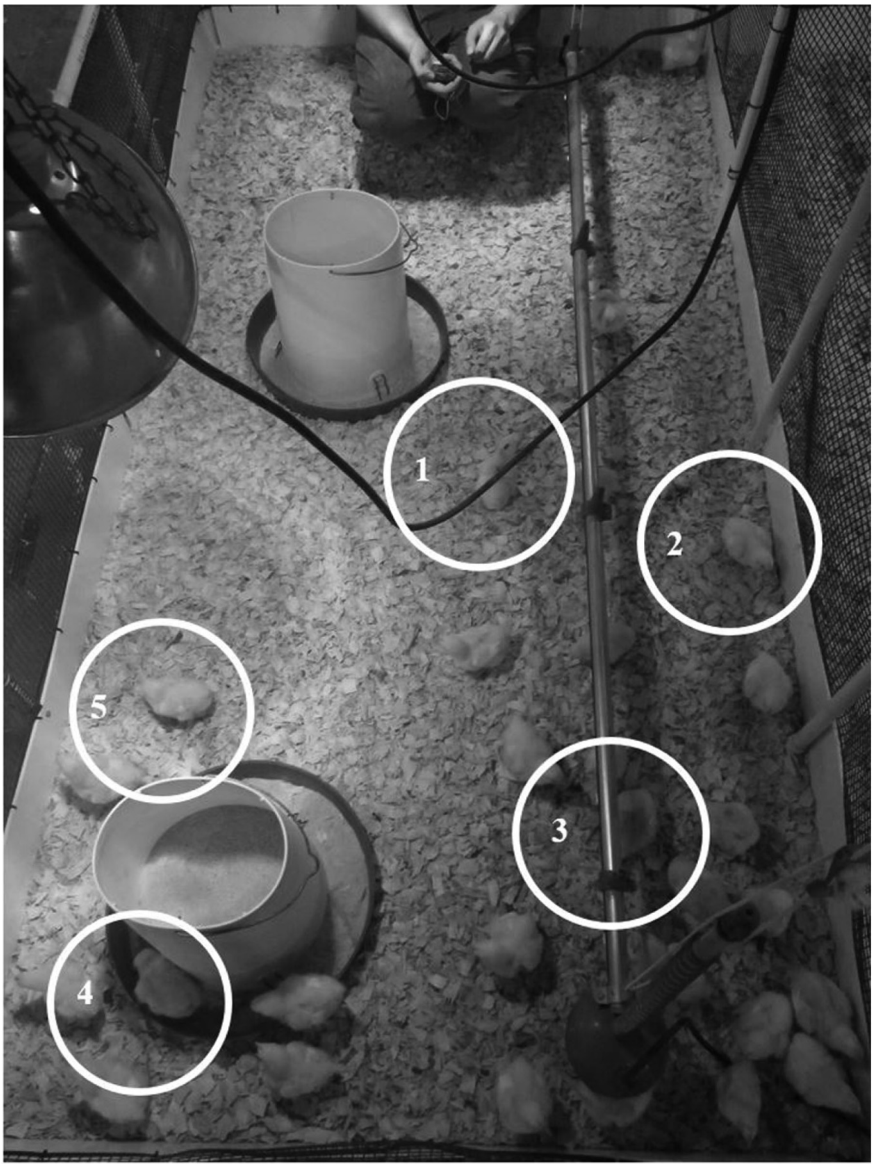

Figure 1. Digital human-approach paradigm (HAP) week 1 image used for evaluation.[1] [1]Bird 1: Interacting; Bird 2: Not interacting; Bird 3: At drinker; Bird 4: At feeder; Bird 5: Other

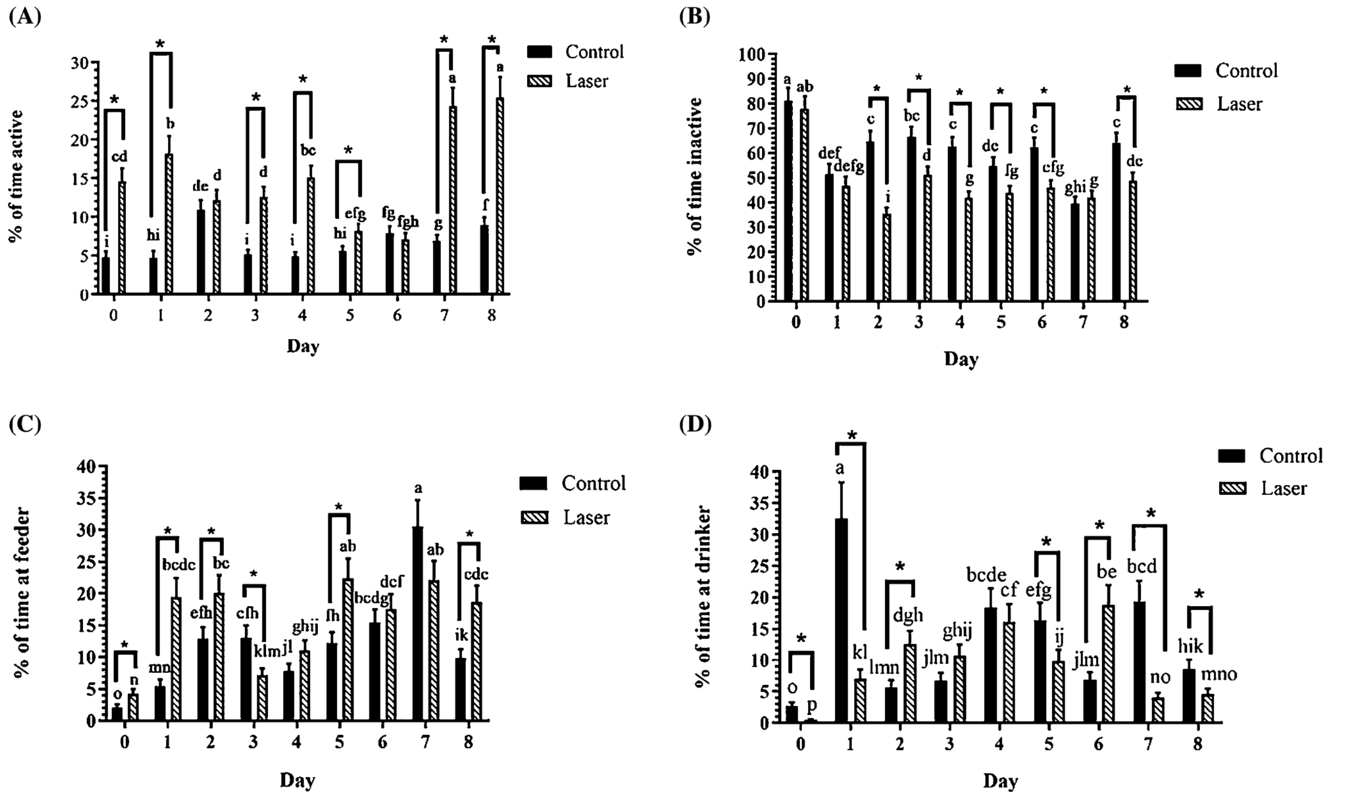

Figure 2. (A–D) Ross 308 broiler home pen behavior results of focal bird during 4-min laser periods: day x treatment LSMeans (±SEM)[1] percent of time spent: (A) active; (B) inactive; (C) at feeder[2]; and (D) at drinker; with day and treatment as main effects, day 0 to 8.[3] [1]Values lacking a common superscript are significantly different ($P \leq 0.05$). [2]At feeder convergence criteria relaxed to 10^{-6}. [3]Individual P-values from day 0 to 8, respectively: (A) <0.0001, <0.0001, 0.5052, <0.0001, <0.0001, 0.0172, 0.5317, <0.0001, <0.0001; (B) 0.6692, 0.3802, <0.0001, 0.0042, <0.0001, 0.0157, 0.001, 0.5467, 0.0041; (C) 0.0141, <0.0001, 0.0276, 0.0036, 0.0918, 0.0022, 0.5056, 0.0946, 0.002; and (D) 0.0002, <0.0001, 0.0023, 0.0702, 0.5939, 0.041, <0.0001, <0.0001, 0.0176.

week or day, and the treatment by week or day interaction, with the random effect of pen (or enriched pair of pens) within treatment, as birds were randomly assigned to pens.

Latency to feed and walking lameness categorical data were analyzed using PROC FREQUENCY and CHI SQUARE. The distribution of latency to feed data was observed by day and week; only LASER focal birds were analyzed. Thus, treatment was not included in the model. Lameness score distributions were observed by treatment and the association of score to treatment. For all measures, a value of $P \leq 0.05$ was considered significant and differences between means were detected using PDIFF.

RESULTS

Broiler Home Pen Behavior

The day x treatment interaction was significant for all behaviors measured: active, inactive, time at feeder, drinker, and other ($P < 0.01$). Birds out of view occurred so infrequently that data could not be analyzed: the frequency of out of view behavior on day 0 to 8, respectively, were 0.11 ± 0.31; 0.36 ± 1.08; 0.27 ± 0.59; 0.29 ± 0.53; 0.55 ± 1.01; 0.46 ± 0.77; 0.27 ± 0.66; 0.1 ± 0.36; and 0.05 ± 0.21. Out of view frequency for week 1 to 6, respectively, were 0.27 ± 0.59; 0.05 ± 0.21; 0, 0.04 ± 0.19; 0.05 ± 0.23; and 0.02 ± 0.13. LASER birds spent more time active (walking or running) on days 0, 1, 3, 4, 5, 7, and 8 compared to the control ($P \leq 0.05$, Figure 2A). The greatest increase in active behavior was observed on day 7, where LASER birds moved 17.4 ± 1.6% more, equal to a 253% increase, than their control counterparts. LASER birds were less inactive than the control on days 2, 3, 4, 5, 6, and 8, with a peak 29.3 ± 3.3% decrease on day 2 ($P \leq 0.05$, Figure 2B).

LASER birds spent more time at feeder on days 0, 1, 2, 5, and 8 than the control ($P \leq 0.05$, Figure 2C). On day 5, LASER birds were at the feeder 10.2 ± 2.4% more than control birds, equal to an 83.7% increase. Control birds spent a greater amount of time at the feeder on day 3. Control birds spent a greater percent of time at drinker on days 0, 1, 5, 7, and 8, but LASER birds spent more time at drinker on days 2 and 6 ($P \leq 0.05$, Figure 2D). Control birds displayed a greater percent of “other” behaviors on days 2, 5, and 8, while LASER birds showed a greater percent of this behavior on day 4 ($P \leq 0.05$). Other behavior did not contribute heavily to focal bird time budget, with a maximum percent of 4.01 ± 0.59% in LASER birds (day 3) and

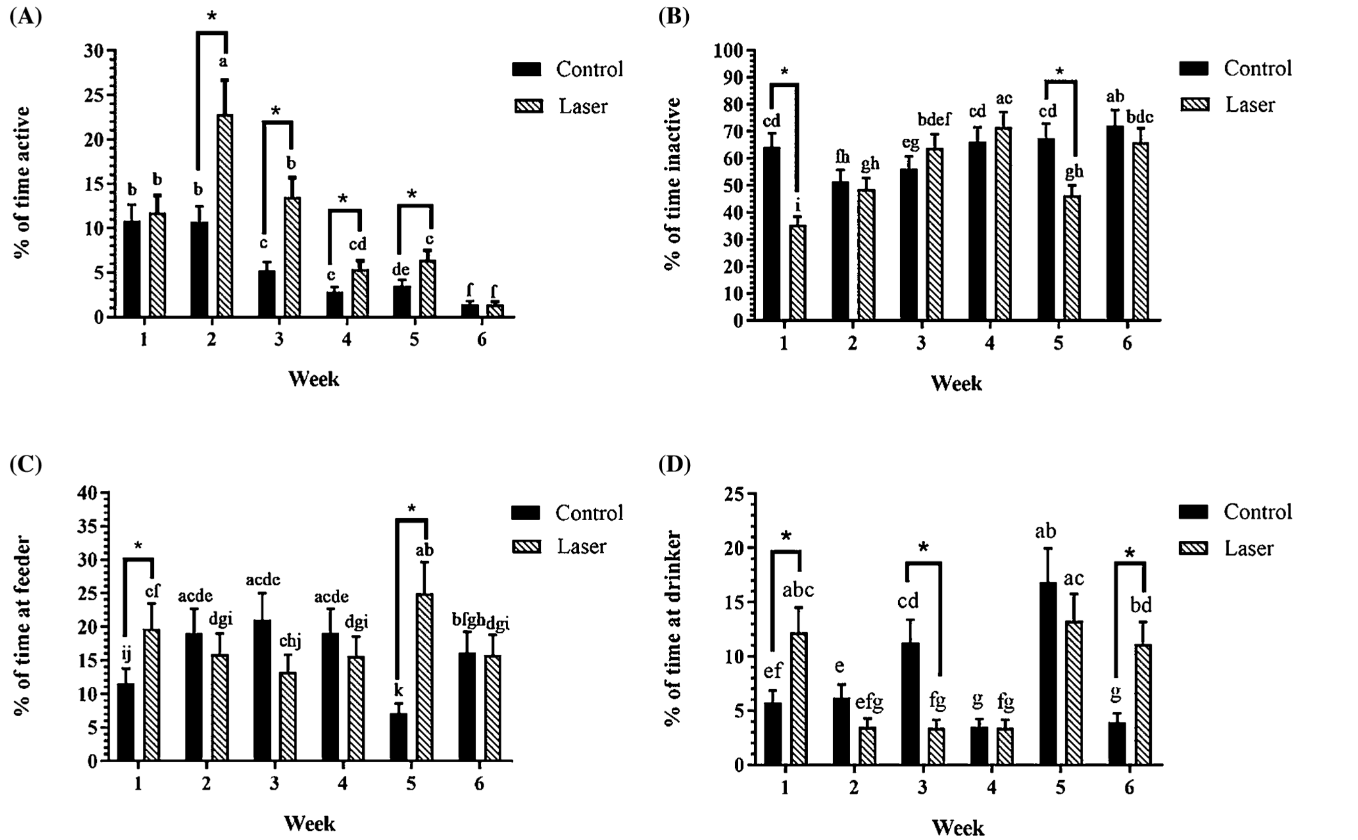

Figure 3. (A–D) Ross 308 broiler home pen behavior results of focal bird during 4-min laser periods: week by treatment LSMeans (±SEM)[1] percent of time spent: (A) active; (B) inactive; (C) at feeder; and (D) at drinker; with day and treatment as main effects, week 1 to 6.[2] [1]Values lacking a common superscript are significantly different ($P \leq 0.05$). [2]Individual P-values from week 1 to 6, respectively: (A) 0.7414, 0.0013, 0.0001, 0.014, 0.0182, 0.931; (B) <0.0001, 0.6187, 0.2516, 0.492, 0.0008, 0.4202; (C) 0.049, 0.5083, 0.0867, 0.4501, <0.0001, 0.9323; and (D) 0.006, 0.0519, <0.0001, 0.939, 0.3656, 0.0002.

4.02 ± 0.63% in control birds (day 2). The estimated mean time budgets of laser-enriched birds over day 0 to 8 were as follows: 14.1 ± 1.29% active, 47.09 ± 2.83% inactive, 14.05 ± 1.87% at feeder, 6.62 ± 1.13% at drinker, and 1.37 ± 0.19% engaged in other behaviors. The estimated time budgets of control birds over day 0 to 8 were as follows: 6.34 ± 0.60% active, 59.8 ± 3.57% inactive, 9.8 ± 1.32% at feeder, 10.17 ± 1.69% at drinker, and 1.66 ± 0.23% engaged in other behaviors.

When analyzing 1 d/wk (Thursday of each week), the week x treatment interaction was significant in all behavior categories ($P \leq 0.01$). LASER birds spent a greater percent of their time active than control birds on week 2, 12.2 ± 2.8% greater (114% increase); week 3, 8.2 ± 1.6% greater (157% increase); week 4, 2.5 ± 0.74% greater (90% increase); and week 5, 2.9 ± 0.87% greater (82% increase, $P \leq 0.05$, Figure 3A). LASER birds spent less time inactive than the control on week 1 and 5 ($P \leq 0.05$, Figure 3B). Time at feeder was increased in week 1 by 8.2 ± 3.0%, and in week 5 by 17.8 ± 3.0%, a 247% increase, in LASER birds ($P \leq 0.05$, Figure 3C). Time spent at drinker was increased in LASER birds by 6.5 ± 1.7% in week 1 and 7.2 ± 1.43% on week 6, and was 7.9 ± 1.4% higher in control birds on week 3 ($P \leq 0.05$, Figure 3D). Other behavior showed no differences by treatment within individual weeks. The estimated mean time budgets of laser-enriched birds over week 1 to 6 were as follows: 7.49 ± 1.2% active, 53.77 ± 4.07% inactive, 17.19 ± 3.17% at feeder, 6.49 ± 1.18% at drinker, and 0.83 ± 0.45% engaged in other behaviors. The estimated mean time budgets of control birds over week 1 to 6 were as follows: 4.55 ± 0.73% active, 62.64 ± 4.73% inactive, 14.75 ± 2.72% at feeder, 6.75 ± 1.22% at drinker, and 0.77 ± 0.42% engaged in other behaviors.

Latency to Feed

Latency to feed categorical distributions were affected by day (day 0 to 8, $P < 0.01$) and by week (week 1 to 6, $P = 0.03$). Over days 0 to 8, 15.34 ± 0.40% of LASER focal birds were at the feeder during laser periods (but not in 5 min following laser turn off), 33.33 ± 0.73% went to the feeder in <5 min following laser turn off, 22.22 ± 0.48% were already at the feeder when laser periods ended, and 29.1 ± 0.77% never went to the feeder during or in 5 min following laser period. Individual daily proportions are presented in Figure 4A. Over week 1 to 6, 5.44 ± 0.37% of birds were at the feeder during laser periods only, 28.57 ± 0.76% went

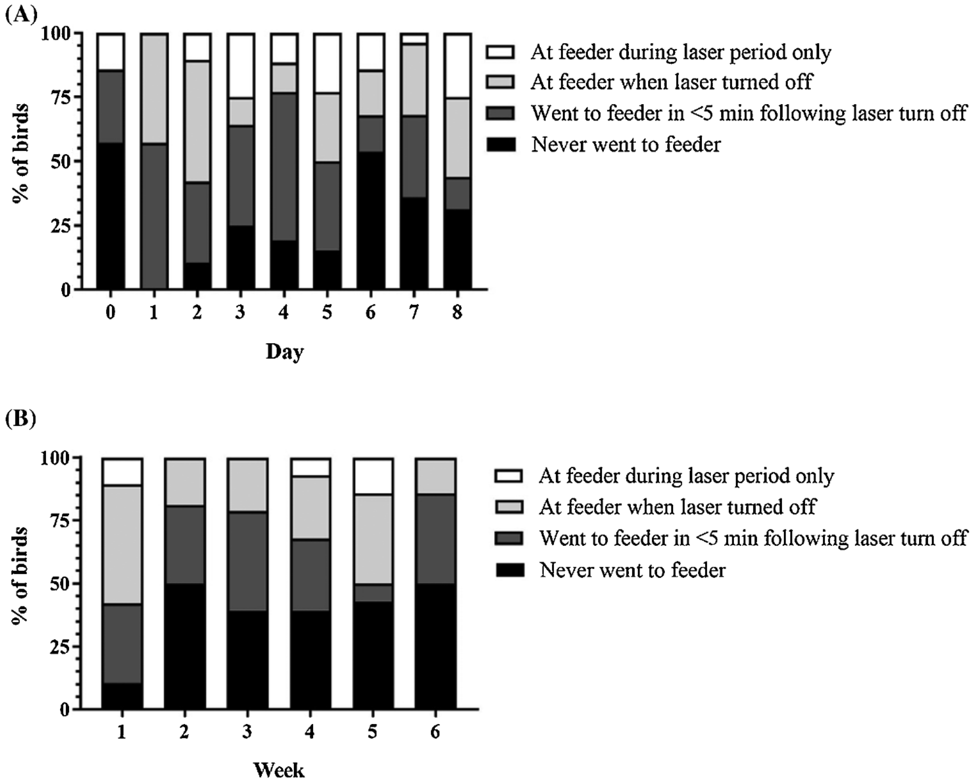

Figure 4. (A,B) Ross 308 broiler latency to feed of LASER focal birds (n = 7) by (A) day, $P = 0.0014$; and (B) week, $P = 0.03$. Latency was recorded in seconds using video recordings from laser periods and for 5 min following, then separated into 4 mutually exclusive categories.

to feeder within 5 min of laser turn off, 26.53 ± 0.62% were already at the feeder when the laser turned off, and 39.46 ± 0.96% were never at the feeder. Weekly breakdowns are presented in Figure 4B.

Walking Distance

For the first 9 D on trial, the day x treatment interaction was significant for each minute individually and total distance walked ($P \leq 0.01$). LASER birds walked further in the first minute on all days, with increases up to 151.1 ± 12.9 cm on day 1, a 452% increase, and 107.5 ± 11 cm on day 7, greater than a 228% increase ($P \leq 0.05$, Figure 5A). Likewise, LASER birds walked more on all days during the second minute, with an increase of 236.3 ± 30 cm, or a 237% increase, on day 1 ($P \leq 0.05$, Figure 5B). During minute 3, LASER birds walked more than control on days 0, 1, 3, 4, 5, 6, and 8, with a peak increase of 139.8 ± 21.2 cm, or a 270% increase, on day 1 ($P \leq 0.05$, Figure 5C). In minute 4, LASER birds walked greater distances on days 0, 1, 2, 4, 5, 6, 7, and 8, walking 150.9 ± 21 cm (287%) more on day 0 and 108.4 ± 19.2 cm (183%) more on day 1 ($P \leq 0.05$, Figure 5D). Over the total 4-min laser periods, LASER focal birds walked a greater distance than control on all days, with increases reaching up to 646.45 ± 64.6 cm, a 303% increase (day 1, $P \leq 0.05$, Figure 5E).

Analyzed on a weekly basis (1 d/wk), the week x treatment interaction was significant for each minute individually and total distance walked ($P \leq 0.01$). During minute 1 of laser periods, LASER birds walked more on weeks 1, 2, 3, 4, and 5, with increases up to 88.6 ± 15.7 cm (130%) on week 2 and 51.2 ± 7 cm (215%) on week 5 ($P \leq 0.05$, Figure 6A). During minute 2, LASER focal bird walking distance was higher on weeks 2 and 5 ($P \leq 0.05$, Figure 6B). In the third minute, the LASER birds walked more on weeks 2, 3, 4, and 5, with increases up to 80 ± 19.7 cm (108%) on week 2, $P \leq 0.05$, Figure 6C. Within minute 4 LASER focal birds walked further than the control on weeks 2 and 4, with an increase of 83.4 ± 19.1 cm (176%) on week 2 ($P \leq 0.05$, Figure 6D). Total distance walked during 4-min laser periods was increased on week 2 to 5 in LASER pens, with the greatest increase of 367.5 ± 61.9 cm, or 150%, on week 2 ($P \leq 0.05$, Figure 6E).

Walking Lameness

Out of the 420 lameness measures taken (70 focal birds/wk for 6 wk), 400 were scored 0 (no signs of lameness). There were 18 scores of 1 (bird showed unevenness in steps or sat down at least once), and only 2 scores of 2 (bird could not walk 1.5 m). In the control birds, 96.19% of scores were 0, 2.86% of scores were 1, and 0.95% of scores were 2. In the LASER birds, 94.29% of scores were 0, 5.71% were scores of 1, and no scores were of 2. The chi square relationship of score by treatment was not significant ($P = 0.13$).

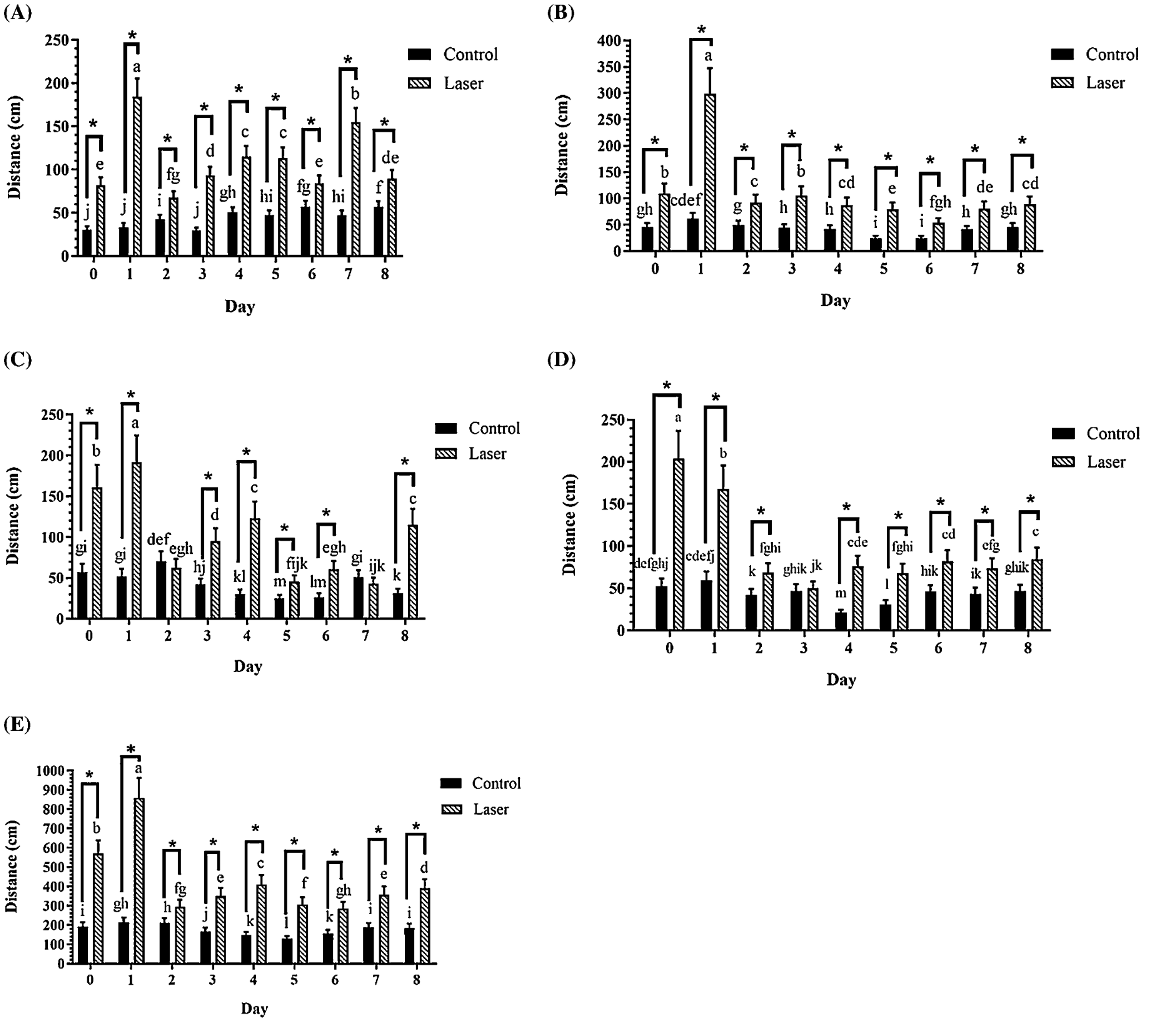

Figure 5. (A–E) Mean walking distance (cm) of focal bird during 4-min laser periods day x treatment LSMeans (±SEM)[1] during: (A) minute1; (B) minute 2; (C) minute 3; (D) minute 4; and (E) total distance walked[2] (4 min), with day and treatment as main effects, day 0 to 8.[3] [1]Values lacking a common superscript are significantly different ($P \leq 0.05$). [2] Total distance convergence criteria relaxed to 10^{-6}. [3]Individual P-values from day 0 to 8, respectively: (A) <0.0001, <0.0001, 0.0051, <0.0001, <0.0001, <0.0001, 0.015, <0.0001, 0.0046; (B) 0.0002, <0.0001, 0.0091, 0.0002, 0.0021, <0.0001, 0.0013, 0.0045, 0.0052; (C) <0.0001, <0.0001, 0.6176, 0.0008, <0.0001, 0.0154, 0.0008, 0.4844, <0.0001; (D) <0.0001, <0.0001, 0.0421, 0.7928, <0.0001, 0.0008, 0.0141, 0.0247, 0.0119; and E) <0.0001, <0.0001, 0.0469, <0.0001, <0.0001, <0.0001, 0.0004, 0.0002, <0.0001.

Human-Approach Paradigm

The week x treatment interaction was significant for percent of birds interacting ($P \leq 0.01$). During week 1, control birds interacted 2.2 ± 0.73% more ($P \leq 0.05$), but on week 6 there were no differences in birds interacting. Averaged over both treatments and weeks 1 and 6, 95.59 ± 2.19% of birds were not interacting and there was not a week x treatment interaction ($P = 0.35$, Table 3). In the not interacting further classified behavior categories, there were 3.7 ± 1.0% more control birds at the drinker on week 1 and 1.8 ± 0.59% more LASER birds at the drinker on week 6, with a week x treatment interaction ($P < 0.01$). There were no differences in percent of birds at the feeder week 1 or 6 (week x treatment interaction $P = 0.62$), and no differences in birds exhibiting other behaviors on week 1 or 6 (week x treatment $P = 0.22$, Table 3).

Regarding bird location in the home pen during the HAP, the main effect of week was significant for all quadrants of the pen and the week x treatment interaction was significant for the first and second quadrants ($P \leq 0.01$). There were no differences in percent of birds in the first quadrant on week 1, but there were 3.3 ± 1.07% greater LASER birds in this quadrant on week 6, more than double the percent of control birds

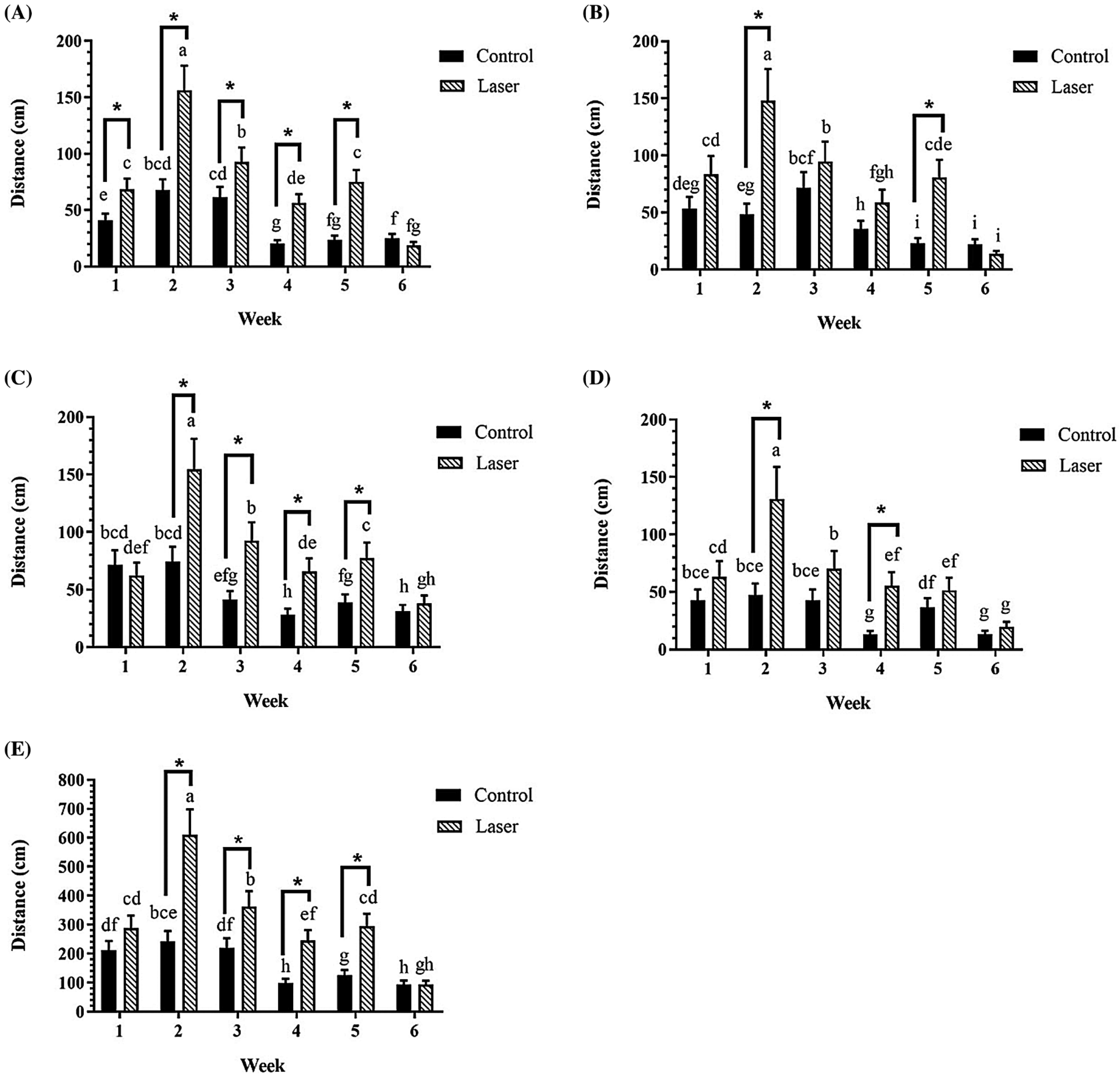

Figure 6. (A–E) Mean walking distance (cm) of focal bird during 4-min laser periods: week by treatment LSMeans (±SEM)[1] during: (A) minute 1; (B) minute 2[2]; (C) minute 3; (D) minute 4; and (E) total distance walked; with week and treatment as main effects, week 1 to 6.[3] [1]Values lacking a common superscript are significantly different ($P \leq 0.05$). [2] Min 2 convergence criteria relaxed to 10^{-5}. [3]Individual P-values from week 1 to 6, respectively: (A) 0.0125, <0.0001, 0.0426, <0.0001, <0.0001, 0.1839; (B) 0.0977, <0.0001, 0.3027, 0.0662, <0.0001, 0.0833; (C) 0.5823, 0.003, 0.0012, 0.0008, 0.0057, 0.4316; (D) 0.2079, 0.0009, 0.1009, <0.0001, 0.2708, 0.2253; and (E) 0.1363, <0.0001, 0.0165, <0.0001, <0.0001, 0.9931.

($P \leq 0.05$). There were 2.8 ± 0.89% more control birds in the second quadrant on week 1 ($P \leq 0.05$), but no differences in this quadrant on week 6. There were no differences due to enrichment in quadrant 3 or 4 (Table 4).

DISCUSSION

Motivation is understood to be a process driven by both external/environmental and internal/physiological factors resulting in goal-oriented behavior or action (Toates, 1986). Motivation in broiler birds, however, is not well understood. It has been shown that food and exploring novel objects motivates broilers (Newberry, 1999; Bokkers and Koene, 2002; Bokkers et al., 2004). A combination of these motivations may have driven the increase in active behavior seen every day in the first 9 D except days 2 and 6 (Figure 2A). Further, the performance of LASER birds was not compromised but was improved, with an overall decrease of 0.07 FCR points and total increased weight gain of 0.24 kg/bird (Meyer et al., 2019). Thus, the success of the novel laser device tested here in motivating, not forcing, an increase in active behavior and walking distance, is among the first to accomplish

Table 3. Human-approach paradigm (HAP[1]) results; percent of Ross 308 broilers interacting vs not Interacting and not interacting behavior further classified using digital image evaluation.

	Week 1 (%)			Week 6 (%)			*P*-value	
Behavior	Control	Laser	Pooled SEM	Control	Laser	Pooled SEM	Wk	Wk*Trt
Interacting	5.46	3.22	0.74	2.80	4.31	0.63	0.09	<0.01
Not interacting	94.00	96.33	2.18	96.93	95.09	2.19	0.70	0.35
			Not interacting further classification					
Feeder	4.55	4.67	0.87	3.81	3.53	3.67	0.03	0.62
Drinker	10.62	6.94	1.03	3.35	5.13	0.59	<0.01	<0.01
Other	83.62	86.93	2.07	92.11	90.24	2.14	<0.01	0.22

Values presented as week x treatment LSMeans (pooled SEM) on weeks 1 and 6. N = 80 observations.
[1]HAP was carried out on all pens once on week 1 and once on week 6. An unfamiliar human entered the pen and after 15 s, a photograph was taken to analyze the birds' response.
Data were collected in bird counts, converted to percent of birds in the pen exhibiting each behavior.

Table 4. Human-approach paradigm (HAP[1]) results; percent of Ross 308 broilers present in each quadrant of the pen.

	Week 1 (%)			Week 6 (%)			*P*-value	
Quadrant	Control	Laser	Pooled SEM	Control	Laser	Pooled SEM	Wk	Wk*Trt
1	6.86	5.57	1.41	3.00	6.34	1.07	<0.01	<0.01
2	8.95	6.18	0.89	20.44	22.18	2.10	<0.01	<0.01
3	26.00	26.62	1.46	38.38	36.07	1.86	<0.01	0.29
4	58.68	62.08	4.03	36.98	36.83	2.60	<0.01	0.36

Values presented as week x treatment LSMeans (pooled SEM) on weeks 1 and 6. N = 80 observations.
[1]HAP was carried out on all pens once on week 1 and once on week 6. An unfamiliar human entered the pen and after 15 s, a photograph was taken to analyze the birds' response.
Data were collected in bird counts, converted to percent of birds in the pen exhibiting each behavior.

increased movement in combination with improved performance.

We hypothesize that this success may be due in part to the laser dots stimulating pecking behavior, a documented response to small particles in broilers (Hogan, 1973), or to visual-based foraging and predatory behavior natural to the chicken's junglefowl ancestors (Fernandez-Juricic et al., 2004). Junglefowl continuously moves while foraging (Arshad et al., 2000), a behavior that may have been replicated in broilers choosing to follow lasers around the pen in this study. However, during week 6, there were no differences in proportion of active/inactive behavior due to enrichment. This is likely a result of maximal body weight overriding motivation to move as birds neared the end of the grow-out, rather than habituation to the lasers, as a similar pattern is observed in declined use of perches after week 5 in broilers (Bokkers and Koene, 2003). Further, work in laying hens when exposed to environmental enrichment in the form of strings for limited daily time periods (10 min), rather than constant exposure, maintained interest in pecking at the strings for 14 wk (Jones et al., 2000).

An interesting behavior pattern was detected in the weekly walking distance analysis where minute 3 more closely followed minute 1 than minute 2 in terms of increased distance walked, and LASER birds walked more than the control week 2 to 5 during this minute (Figure 6C). Hence, it appears that recording the distance walked during each minute of the 4-min laser period is necessary, as over time birds moved more in minutes 1 and 3 than in minutes 2 and 4. This likely contributed to the increased total distance walked during laser periods week 2 to 5 by LASER focal birds (Figure 6E), resulting in a 215% increase in walking distance on week 5, a notable outcome in birds nearing-market weight.

As the device was entirely novel, a 4-min laser period was used with the intention to determine if this length of time was successful in stimulating bird activity and walking distance. Our data indicate that within the first 9 D, a 4-min period was effective in promoting walking up to and including minute 4. When viewing the entire 6-wk grow-out, following week 4 the increase in walking distance and active behavior declined after minute 3. Thus, it is possible that a 3-min laser period would suffice for broilers older than 4 wk, but for birds 4 wk or younger, 4-min periods were effective. Although no difference in activity was seen during week 6 (day 35 to 42), weight gain was increased by 0.22 kg and FCR was decreased by 0.18 points in the critical finisher period (Meyer et al., 2019). The increased weight gain of 0.24 kg/bird overall could be translated to the range of $0.71 to $1.39 more saleable breast meat/bird, using current prices (USDA, 2019). Additionally, the inclusion of this device would not require altered management or human labor to increase bird activity, and could be easily cleaned and reused over multiple flocks. Birds cannot physically interact with the device; hence, no changes to biosecurity/cleaning are needed. This is practical compared to other forms of enrichment designed to stimulate activity that must be cleaned or replaced within and between flocks, such as straw bales (Kells et al., 2001), pecking strings (Bailie and O' Connell, 2015), or other novel objects (Altan et al., 2013). Additionally, the laser does not promote object-guarding behavior within the bird hierarchy.

The increased distance walked in proportion to the increase in activity (walking or running) makes it likely that LASER birds were moving at an increased speed to account for this increased distance. Future studies using this device that incorporate a walking speed measure could validate this hypothesis. In a work by Dawkins et al. (2009), an increased walking speed, along with increased time spent walking, resulted in decreased lameness. However, in our research conditions, a score of 2 was rare and no LASER birds received a 2; hence, we did not detect a difference due to laser enrichment. Taking into account the increased weight gain and improved FCR in LASER birds (Meyer et al., 2019), no detection of lameness is a positive outcome. These data indicate that the paradigm postulated by Weeks et al. (2000) that selection for improved FCR leads to less active animals, and thus more lameness, may have been counteracted by the increased exercise stimulated by the laser device.

The increase in feeder behavior seen in LASER birds on proportionally more days/weeks is logical, as LASER birds had 0.02 kg greater feed intake in the starter period, 0.05 kg greater in the grower period, and 0.14 kg greater in the finisher period (Meyer et al., 2019). Further, feeding latency showed that on day 0 to 8, approximately 71% of LASER focal birds were at the feeder at least once either during or within 5 min following laser periods. Over week 1 to 6, 60.5% of LASER focal birds were at the feeder either during or shortly following laser periods. This is a positive result, along with the increased time spent at feeder in LASER birds, indicating that the device increased bird movement but may have also encouraged feeding, perhaps by stimulating natural foraging or predatory behavior. In 3- to 4-min timepoints selected for behavior and walking distance analysis that were not during scheduled laser periods (06:30, 18:30, and 22:30), LASER birds walked further than control birds on day 1 and week 6, spent more time active on days 1 and 3, and were at the feeder more on weeks 4 and 5 ($P < 0.05$, unpublished data). These data indicate a maintenance of activity and feeder behavior outside of the laser periods.

The HAP was used here to measure fearfulness in the flock, a measure validated in swine by Weimer et al. (2014), based on methods used in pigs and cattle by Hemsworth et al. (1996). Results in these species have shown that animals with positive, regular interactions with humans were quicker to approach, indicative of decreased fear. Environmental enrichment has been shown to decrease fear responses in poultry, for example reducing freezing, avoiding novel objects, and latency to enter an unfamiliar environment (Jones and Waddington, 1992). Classical music (a form of sensory enrichment) played to layer-type chicks decreased tonic immobility duration and heterophil: lymphocyte ratio (Dávila et al., 2011). However, in a work by Bizeray et al. (2002b) a barrier treatment increased tonic immobility (a measure of fearfulness in birds). The "touch test" and "avoidance distance test" have been validated in laying hens, where response to humans was positively influenced by more than minimal human contact (Graml et al., 2008). Within this study, the overall proportion of control birds interacting with the human decreased from week 1 to 6, while the proportion of LASER birds interacting increased.

A greater number of LASER birds were counted in quadrant 1, closest to the unfamiliar human, during week 6 than control birds. Importantly, there was no evidence of piling, a negative behavior associated with fear in poultry (Campbell et al., 2016). However, this method requires further research in broilers and is likely to be more applicable in poultry species that naturally tend to approach people, such as commercial turkeys. Other stress markers, including measuring corticosterone concentrations from the serum or feathers, have been validated in broilers and may be an alternative methodology for determining stress (Weimer et al., 2018).

In summary, these data have provided strong evidence that this novel environmental enrichment device positively increased broiler bird physical activity without impacting lameness. This unique tool motivated broilers to move of their own volition by stimulating them visually, encompassing physical, occupational, sensory, and nutritional enrichment. The laser device is practical and applicable to commercial barns without changing grow-out procedures or negatively impacting bird welfare or performance.

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