



Play behaviour reduced by environmental enrichment in fast-growing broiler chickens

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ABSTRACT

The objective of this study was to evaluate effects of environmental enrichment on play behaviour in fast-growing broiler chickens. Chicks (19 of each sex) were randomly assigned to 6 non-enriched (NE) pens and 6 pens enriched (E) with a raised platform, hanging weighing scale, peck stone, and suet feeder filled with wood shavings which was refilled every other day. Three worm running tests (on days 10, 24, and 38), and three free-space tests (on days 8, 21, and 35) were performed to stimulate play behaviour. From video recordings, occurrences of worm exchange, worm pecking, worm chasing, and worm running in the worm running tests, and running, frolicking (i.e. running with wing use), wing flapping (while stationary), and sparring in the free-space tests, were quantified. Video recordings were also made on days 23, 30, and 37 to observe spontaneous play. Generalized linear mixed models were used to investigate effects of enrichment, age and their interaction on occurrence of the different forms of play behaviour per bird per 5 min in the worm running and free-space tests, and per hour in spontaneous play observations. In worm running tests, chickens in NE pens performed more worm exchange ($P = 0.034$), worm chasing ($P < 0.001$), and worm running ($P = 0.035$) than those in E pens. In free-space tests, running ($P < 0.001$), frolicking ($P = 0.016$), and all play behaviour combined (sum of running, frolicking, wing flapping, and sparring; $P < 0.001$), were more frequent in NE than E pens. In the test contexts, worm chasing declined with age in both NE and E pens ($P < 0.001$), and running, frolicking and all play behaviour combined declined with age in NE pens while remaining low and unchanged in E pens. In contrast, occurrence of worm pecking ($P = 0.004$) and wing flapping ($P < 0.001$), both performed while stationary, increased with age in both NE and E pens. Total spontaneous play behaviour (sum of running, frolicking, wing flapping, and sparring) did not differ between treatments ($P = 0.644$) but did decline with age ($P = 0.013$). In conclusion, while NE birds did not differ from E birds in the performance of spontaneous play, they were more responsive than E birds during tests intended to stimulate play behaviour in broilers. This was possibly because of the larger contrast between their relatively unstimulating environment and the test conditions, resulting in them being more easily aroused. The findings also show that the worm running and free-space tests were effective in stimulating play.

1. Introduction

Play behaviour is common in young homeothermic animals (Špinková et al., 2001; Diamond & Bond, 2003). Although functions of play are debated, there are several common characteristics of play across species: 1) play is most common during infancy and juvenile periods; 2) play is not critical to present survival; 3) play may occur repeatedly but not stereotypically, and 4) play typically occurs when stress is low (Burghardt, 2005). Play behaviour can be categorized as locomotor play (eg.

running and jumping), object play (i.e. interacting with inanimate objects), and social play (involving more than one animal; Špinková et al., 2001; Diamond & Bond, 2003; Held & Špinková, 2011).

Limited research has been conducted on play behaviour in domestic chickens. Behaviour patterns suggested to meet criteria for play in chickens include frolicking (Duncan, 1998; Appleby et al., 2004; Nicol, 2015; Baxter et al., 2019), sparring (Duncan, 1998; Mench, 1988; Nicol, 2015) and worm running (Cloutier et al., 2004; also referred to as food running, Baxter et al., 2019). These behaviour patterns occur mainly

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when young and seem to be less common and less varied than forms of play behaviour described in some mammalian species (Appleby et al., 2004; Nicol, 2015).

Worm running occurs when a bird picks up a food item or other object and runs with it while other birds chase after the running bird and attempt to grab the item (Kruijt, 1964; Cloutier et al., 2004). It was first described as feeding behaviour (i.e. 'food running') and was proposed to function as a way to attract other birds, resulting in tearing up a relatively large food item such as a worm or caterpillar into smaller pieces for consumption (Kruijt, 1964). However, Cloutier et al. (2004) suggested that worm running with inedible objects under conditions when food is abundant (e.g. when fed *ad libitum*) fits criteria for play behaviour, including locomotion, object and social play, because it is not associated with social dominance and because it has no immediate survival value when the objects are non-nutritive.

Sparring, also known as play fighting, is reported in some birds, including domestic chickens (Dawson and Siegel, 1967; Baxter et al., 2019; Vasdal et al., 2019). Birds performing sparring display some elements used in adult fighting such as jumping and physical contact, but without aggressive pecking and injuries (Diamond & Bond, 2003). Using factor analysis to describe the ontogeny of social behaviour in chicks, Rushen (1982) found that sparring was closely associated with running during the first 6 weeks of life, and that sparring and running were replaced by aggressive behaviour in older birds. Mench (1988) also found that sparring performed in juveniles was replaced by the aggressive and potentially harmful form used in adult fighting. Moreover, because youthful sparring was significantly less frequent when birds were feed deprived, Mench (1988) suggested that sparring shares characteristics of mammalian play behaviour.

Frolicking involves spontaneous running with raised or flapping wings and is socially contagious in groups (Dawson and Siegel, 1967). It is performed in a repeated but non-stereotypical fashion (Baxter et al., 2019) and may lead to sparring (Wood-Gush, 1971). Wing flapping can also occur when stationary. Wing flapping involves rapid bilateral, usually repeated, wing movements, unlike slow and unilateral wing stretching. It is performed by adult males as a display of vigour in the context of reproduction (Millman et al., 2000; McGary et al., 2003). However, like sparring, wing flapping appears playful in youngsters. It occurs in association with frolicking and sparring and has, thus, been included in studies on play in young chickens (e.g. Keeling and Zimmerman, 2009; Vasdal et al., 2019). In broilers, frolicking and sparring were reported to disappear by 10 weeks of age (Dawson and Siegel, 1967).

Some conditions are reported to stimulate play behaviour in young domestic fowl. A 'worm running test' provides chickens with an opportunity to perform object play (Cloutier et al., 2004). During the test, a worm-like object (e.g. pipe cleaner or piece of twisted paper) is thrown into the home pen. Chickens will peck at the "worm", run with it protruding from their beak, or chase after the worm running bird. Keeling and Zimmerman (2009) observed increased locomotor play in broiler chickens when objects (small plastic toothpicks, a ball and a cardboard box) were introduced into their pen for 30 min. Another method for stimulating play involves displacing chickens by walking through their housing. The birds then perform social and locomotory forms of play behaviour when re-entering the empty space behind the walking person (Newberry et al., 2018; Baxter et al., 2019). A further method involves providing repeated temporary access to additional space outside the home enclosure. Newberry (1999) observed that, despite low stocking density in the home pen (11.6 kg/m²), opening a gate to give short-term daily access to an empty pen next to the home pen led to broilers running into the open space, often with wings flapping. She suggested that this was possibly related to boredom in the home pen. Because spontaneous play may take up only a small proportion of the behavioural time budget, the use of tests designed to stimulate play could be efficient for studying factors affecting play in chickens.

Play behaviour is thought to indicate primarily positive physical and

affective states because it can serve as a reward in a learning paradigm (e.g. Humphreys & Eimon, 1981; Calcagnetti & Schechter, 1992), and because its frequency is often reduced when an animal is exposed to a biological challenge (Held & Špinka, 2011; Lawrence et al. 2018). For example, castration of domestic lambs led to depressed play for at least 3 days (Thornton & Waterman-Pearson, 2002). Similarly, physical or mental illness has been associated with reduced play in people (McGrath et al., 1990; Coplan et al., 2004). Such findings suggest that, especially during the age range when play is most common, animals free from diseases, injuries, and chronic negative affective states will play more, indicating better welfare. However, play has also been associated with negative affective states (Ahloy-Dallaire et al., 2018). For example, following early separation from their mothers, kittens performed more object play than controls that remained with their mothers (Bateson et al., 1981), and rats engaged in more rough-and-tumble play after being injected with a moderate dose of a stress hormone (ACTH; Arellis, 2006). Possible non-mutually exclusive explanations for these findings are that: 1) because play is pleasurable, its performance alleviates stress (Špinka et al., 2001), and 2) if play enables animals to better prepare for challenging situations, it may be performed at higher levels by individuals that have experienced adversity as, for example, in the kittens' case (Ahloy-Dallaire et al., 2018).

Environmental enrichment is practiced with the goal of improving animal welfare. Depending on the types provided and overall environmental complexity, environmental enrichment has been associated with some welfare benefits in fast-growing broiler chickens, including lower mortality (BenSassi et al., 2019), improved leg health (Kaukonen et al., 2017; Pedersen & Forkman, 2019) and increased exploratory and comfort behaviours (Vasdal et al., 2019). However, Baxter et al. (2019) detected no specific association between environmental enrichment and play behaviour in broiler chickens and, while Vasdal et al. (2019) observed increased wing flapping in enriched housing, other playful activities including spontaneous running, worm running and play fighting, were unaffected. Nevertheless, relatively little research has been conducted on environmental enrichment for broiler chickens and many questions remain (Riber et al., 2018); for example, if and/or how play behaviour in broilers is affected by enrichment types, the context of play ("play test" conditions or spontaneous play), the strain and age of the chickens, and the methods used to quantify play.

The objective of this study was to investigate the effects of environmental enrichment on play behaviour during worm running and free-space tests, as well as on spontaneous play, in fast-growing broilers at different ages. We hypothesized that broilers reared in an enriched environment have better welfare and, therefore, would play more than broilers in a non-enriched (control) environment, especially at older ages when they would otherwise be more susceptible to health problems. This was part of a larger study on effects of environmental enrichment on health and welfare of broiler chickens (Liu, 2019).

2. Methods

2.1. Animals, housing, and management

The study was conducted in an experimental room at the Arkell Poultry Research Station, Guelph, Canada, and all procedures were approved by the Animal Care Committee at the University of Guelph (Animal Utilization Protocol #3746). A flock of 456 feather-sexed Ross 708 broiler chicks was obtained from a commercial hatchery at 1 day of age and housed in 12 floor pens (160 cm wide × 238 cm long; 38 birds/pen, expected mean stocking density at end of production = 29 kg/m², 19 females and 19 males randomly assigned to each pen). All pens were supplied with one round hanging feeder (diameter: 33.8 cm), a line of nipple drinkers (5 nipples per pen) and fresh pinewood shavings (approximately 6 cm in depth). The pens were divided by solid white plastic walls. All chicks were vaccinated at the hatchery with Bronchitis vaccine (mass type, live virus; Merial, Athens, GA, USA; method: spray),

Newcastle-Bronchitis vaccine (B1 type, B1 strain, Mass & Conn types; Merial, Athens, GA, USA; method: spray), and Marek's disease vaccine (Ceva, Lenexa, KS, USA; method: subcutaneous injection). Female chicks were marked with a small amount of animal-safe pink paint (KONK Livestock Markers - Red, Viceroy Distributors, Winnipeg, MB, Canada) on their heads for another experimental purpose.

Birds were given *ad libitum* access to water and antibiotic-free, plant-based feed that was milled on-site. They were fed a starter diet from day 1 to day 14, a grower diet from day 15 to day 28, and a finisher diet after day 28. The experimental room was climate-controlled at approximately 32 °C on day 1, 31 °C on day 5, 29 °C in week 2, 27 °C in week 3, 24 °C in week 4, and 21 °C in week 5. The light:dark schedule was 23L:1D from day 1 to day 4. After day 4, the lights were turned on at 06:15 and turned off at 22:15 (16L:8D), with a dawn/dusk period (30 min each). The light intensity was approximately 56 lux from day 1 to day 4, 20 lux from day 5 to day 28, and 7 lux from day 29 until the end of the trial. The weekly mortality rate was 0.19% for the first, second, and third week, and

increased to 1.74% in the fourth week. Mortality was similar across pens. Necrotic enteritis was diagnosed when the birds were 28 days of age, and the condition was controlled by treatment of all birds with antibiotics via drinking water (Pot-Pen, Vetoquinol, Lavaltrie, QC, Canada) from day 29 to 34. The mortality rate decreased to 0.39% in week 5, and there was no further mortality from day 33 until the end of the trial. Birds were checked twice daily for health status and no moribund birds were found. All chickens were processed on day 43.

2.2. Experimental design

Half of the pens were non-enriched (NE) while the other half were enriched (E) with the following: a raised platform (Red Rooster Mark 2 Black Slats, Clark Ag Systems, Caledonia, ON, Canada, 58 × 39 cm, length × width) with a 25° ramp of the same material (79 × 39 cm, length × width), placed at the back of each pen, a weighing scale platform (Clark Ag Systems Ltd., Caledonia, ON, Canada; diameter: 51 cm)



Fig. 1. Enriched pen, showing the enrichments. a) an elevated platform with angled ramp, b) a hanging weighing scale, c) a peck stone, and d) a suet feeder filled with wood shavings). Non-enriched pens were the same except lacking enrichment. The feeder has been temporarily removed from its chain to reveal the enrichment behind it. Brown paper offering additional feed was present only during the first week in all pens.

hanging above the floor (1 cm high in week 1 and another 1 cm added each week), ¼ of a mineral peck stone (PECKstone (hard), Protekta, Lucknow, ON, Canada, full size: 12 × 19 × 11 cm, length × width × height) against the left pen wall, and a suet cage feeder (Scotts, Hagersville, ON, Canada, 13 × 13 × 5 cm) filled with pinewood shavings, suspended above the floor and adjusted regularly to around shoulder-height of the birds (Fig. 1). The suet feeder was refilled with fresh shavings every other day (on days when no video recordings were being made; see below). The birds pecked at the shavings in the suet feeders frequently and typically emptied the feeders within one day. The same amount of fresh shavings was also added to the litter in NE pens on the same days when the suet feeders in E pens were refilled to standardize the potential effect of added shavings on litter quality.

Proximity of pens to the outside wall of the room, and to heaters and air inlets, resulted in a slight temperature gradient in the room. To control for any potential temperature effect, the pens were assigned to 6 blocks comprising one NE pen and one E pen, with treatments balanced on each side of the room across blocks. Within the room, there was also one extra NE pen and one extra E pen. If a bird died, another bird of the same sex, similar body weight, and reared under the same treatment condition (NE or E) was added to maintain an equivalent stocking density across all pens. A total of 6 NE birds and 8 E birds were replaced over the course of the experiment. These replacement birds were added on days 6, 13, 15, 22, 23, 24, 25, 29, and 33. On two occasions (days 23 and 24), behavioural observations were conducted prior to the addition of replacement birds. In general, young, fast-growing broilers are non-cannibalistic, and considerably less aggressive than other domestic fowl (Mench, 1988; Pettit-Riley et al., 2002; Bokkers & Koene, 2003). No cannibalism or aggression was directed towards the replacement birds and these birds appeared to adapt to their new pens almost immediately.

2.3. Worm running tests

Three 5-min worm running tests were performed in each pen, on days 10, 24, and 38, respectively, between approximately 10:00-11:00. The procedure was adapted from Cloutier et al. (2004; Table 1). The worms were made from twisted white tissue paper and were increased in length as the birds grew, from 5 cm long on day 10 to 6 cm long on day 24 and 7 cm long on day 38. The diameter of the paper worms was 3 mm. Before conducting the test, a researcher stood in front of a pen for approximately one minute to allow the birds to habituate to the researcher's presence. Once the birds returned to their undisturbed behaviour, a worm was gently thrown into the pen. The entire test was observed and, if a worm was destroyed, eaten, or lost, a new worm was

Table 1

Ethogram for worm running tests. Worm pecking, worm running, and worm chasing were mutually exclusive, and each bird could only be assigned to one behaviour per 10-s scan. Worm exchanges were recorded as a total number per 5 min. Definitions adapted from Cloutier et al. (2004).

Behaviour	Description
Worm pecking	Bird pecks at worm on the ground. The worm is usually seen to move when impacted by the beak, and may be lifted off the ground but not carried.
Worm running	While walking or running, bird carries a worm projecting from its beak. The bird typically makes rapid changes of direction, and typically attracts other birds to follow. If the bird also performs worm pecking or worm chasing in the same scan, it is recorded as worm running.
Worm chasing	While walking or running, a bird follows or moves along with a worm running bird and may attempt to grab the worm from its beak. If this bird also performs worm pecking in the same scan, it is recorded as worm chasing.
Worm exchange	A worm is transferred from one bird's beak to another, when grabbed from a worm running bird by a worm chasing bird or grabbed from a stationary bird holding a worm with at least one end off the ground.

supplied to replace it. Two digital video cameras (Sony Digital High Definition Video Camera; HDR-CX405 and DCR-SR68 models, Tokyo, Japan) mounted on monopods (Digiant MP-3606 Professional Video Monopod 70", Zhejiang, China) were attached to the front wall of the pen (one at each corner, angled down) to record the test. Due to high bird activity, it was not always possible to keep track of the worm in real-time; therefore, a 10-min recording was made. Pens within the same block were tested concurrently by different researchers, and worms were removed after the test.

For ease of scoring, the videos were analyzed in 10-s segments, starting from the moment the worm landed on the pen floor. Any segment during which the worm was not present was not used for analysis. The first 30 segments where the worm was present in the videos were scored (5 min in total). For each segment, all occurrences of birds performing worm pecking, worm running, and worm chasing were recorded (Table 1). For each segment, the total number of times a worm exchange occurred was also recorded (Table 1). Occasionally, a missing worm reappeared during the observation period after it had been replaced by another worm and, when this occurred, only behaviour directed towards the replacement worm was recorded. One observer (ZL) with previous experience collecting behavioural data from chickens and who was not blind to treatment or hypotheses performed all observations in this study. The observer re-reviewed a sample of video recordings at the start and end of data collection to determine intra-observer reliability. The Kappa coefficient for the worm running test was 0.92.

2.4. Free-space tests

Three free-space tests were performed in all pens, on days 8, 21 and 35, respectively, between approximately 10:00 - 11:00. The procedure and ethogram (Table 2) were adapted from Newberry et al. (2018) and Baxter et al. (2019). To perform the test, a researcher walked into a pen with a pen divider panel and gently pushed all birds back to the drinker line. Meanwhile, a second researcher removed the feeder, opening up a standard amount of 'new' free space in the pen. E pens still retained the enrichment, so the total amount of unfurnished space differed between treatments. After approximately 10 s, the divider was removed and both researchers quietly moved to the next non-adjacent pen, where the procedure was repeated. Four non-adjacent pens were video recorded simultaneously for 5 min, using two digital video cameras on monopods as described for the worm running tests. Feeders were then returned to the pens and tests commenced in the next round of four non-adjacent pens, followed by a third round. A pre-determined, systematic test order was followed, avoiding disturbance to the birds during recording and ensuring that each round included two NE and two E pens.

From the videos, the observer used continuous all-occurrences sampling of the behavioural events listed in Table 2 to record every occurrence of running, frolicking, wing flapping, and sparring over the

Table 2

Ethogram for free-space tests and spontaneous play. All behaviour patterns were mutually exclusive. The start of one behaviour indicated the end of the previous behaviour. Definitions adapted from Dawson and Siegel (1966).

Behaviour	Description
Running	Forward movement, often including rapid direction change, at least 2-3 times normal walking speed. No wing flapping involved.
Frolicking	Forward movement, at least 2-3 times normal walking speed, with wings extended to each side or flapping, often includes sudden direction change.
Wing flapping	Rapid bilateral up and down movements of wings while standing still or walking up to 2 steps. Excludes wing flaps performed by a bird to balance itself.
Sparring	Two birds interact face to face as in fighting. May include hopping or chest bumping but no physical contact necessary. Brief, with no aggressive pecking. Each interaction between two birds was counted once.

whole observation period (5 min). The Kappa coefficient for intra-observer reliability in the free-space test was 0.91.

2.5. Spontaneous play

Video recordings were made of all pens concurrently on days 23, 30, and 37 between 11:00 and 15:00 to observe spontaneous play behaviour in a non-test context. One digital video camera on a monopod (described in section 2.3) was mounted at the centre of the front wall of each pen for video recording. Continuous all-occurrences sampling was used to record every occurrence of running, frolicking, wing flapping and sparring during 1 h of observation (11:00 to 11:30 and 13:00 to 13:30) using the same ethogram as for free-space tests (Table 2).

2.6. Statistical analyses

Prior to analysis, data from each pen were standardized as occurrences per bird per 5 min (by dividing all frequencies by the number of birds in the pen) for worm running and free-space tests, and occurrences per bird per hour for spontaneous play. For the free-space test and spontaneous play observations, an aggregated “all play behaviour” variable (sum of running, frolicking, wing flapping, and sparring) was also calculated. There were two pens with missing data due to video recording failure (one E pen in the worm-running test at 39 days and spontaneous play in one E pen at 23 days).

Statistical analyses were computed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Pen was the experimental unit, and the sample size was 6 pens per treatment. Generalized linear mixed models included treatment, age, and their interactions as fixed effects. To account for temperature variation in the room, block was included as a random effect, with pen as subject included in the random statement. Since repeated measurements were made on each pen at different ages, a compound symmetry covariance structure was specified to account for within-subject correlated errors. Least squares means were calculated, and pairwise means comparisons were adjusted for multiple comparisons using the Tukey option. Residuals were generated using the output statement and assessed for normality in Proc Univariate based on the Shapiro-Wilk statistic. Most of the dependent variables adhered to a Gaussian distribution, and their least squares means, and standard errors are reported in results. Worm pecking, frolicking in free-space tests, and running during spontaneous play observations were log-transformed to normalize the data by specifying modelling of the lognormal distribution in Proc Glimmix, and back transformed least squares means and standard errors are presented. All residuals were re-checked with

Shapiro-Wilk to confirm the distribution. Log transformation resulted in exclusion of eight 0 values from the worm pecking analysis.

3. Results

3.1. Worm running tests

There were overall treatment effects on worm exchange ($F_{(1,19)} = 5.26$, $P = 0.034$), worm chasing ($F_{(1,19)} = 32.15$, $P < 0.001$), and worm running ($F_{(1,19)} = 5.15$, $P = 0.035$), with NE birds performing more of these behaviours than E birds (Fig. 2). There was no effect of treatment on worm pecking ($F_{(1,11)} = 1.61$, $P = 0.231$).

There was a decrease in the occurrence of worm chasing as the birds aged ($F_{(2,19)} = 44.16$, $P < 0.001$), and an increase in the occurrence of worm pecking on day 38 ($F_{(2,11)} = 9.52$, $P = 0.004$; Fig. 3). The occurrence of worm exchange ($F_{(2,19)} = 6.56$, $P = 0.007$) and worm running ($F_{(2,19)} = 8.72$, $P = 0.002$) peaked on day 24 (Fig. 3). There was no treatment \times age interaction on worm exchange ($F_{(2,19)} = 0.70$, $P = 0.507$), worm pecking ($F_{(2,11)} = 1.73$, $P = 0.222$), worm chasing ($F_{(2,19)} = 2.27$, $P = 0.130$), or worm running ($F_{(2,19)} = 0.44$, $P = 0.651$).

3.2. Free-space tests

During the free-space tests, there were effects of treatment, age, and a treatment \times age interaction on all play behaviour ($F_{(1,20)} = 24.71$, $P < 0.001$; $F_{(2,20)} = 30.96$, $P < 0.001$; $F_{(2,20)} = 19.00$, $P < 0.001$, respectively), running ($F_{(1,20)} = 33.55$, $P < 0.001$; $F_{(2,20)} = 39.71$, $P < 0.001$; $F_{(2,20)} = 16.74$, $P < 0.001$, respectively), and frolicking ($F_{(1,20)} = 6.99$, $P = 0.016$; $F_{(2,20)} = 12.10$, $P < 0.001$; $F_{(2,20)} = 8.20$, $P = 0.003$, respectively; Fig. 4). NE birds performed more running than E birds on days 8 ($P < 0.001$) and 21 ($P = 0.048$), but not on day 35 ($P = 0.997$). The occurrence of running decreased at each age tested for NE birds but not for E birds, which had a lower running frequency at all three ages observed. NE birds performed more frolicking than E birds only on day 8 ($P = 0.006$), after which the frequency of this behaviour declined considerably in NE birds. The frequency of frolicking was low in E birds at all ages. The occurrence of all play behaviour was higher in NE than E birds on day 8 ($P < 0.001$) whereas the difference was smaller on day 21 ($P = 0.062$) and gone on day 35 ($P = 1.000$). The occurrence of all play behaviour declined at each age tested for NE birds but not for E birds.

There was no treatment effect ($F_{(1,20)} = 1.40$, $P = 0.251$) or treatment \times age interaction ($F_{(2,20)} = 2.51$; $P = 0.107$) on frequency of wing flapping. Wing flapping was affected by age ($F_{(2,20)} = 11.75$, $P < 0.001$), occurring less often on day 8 than on day 21 or 35 (0.05 ± 0.020 , $0.19 \pm$

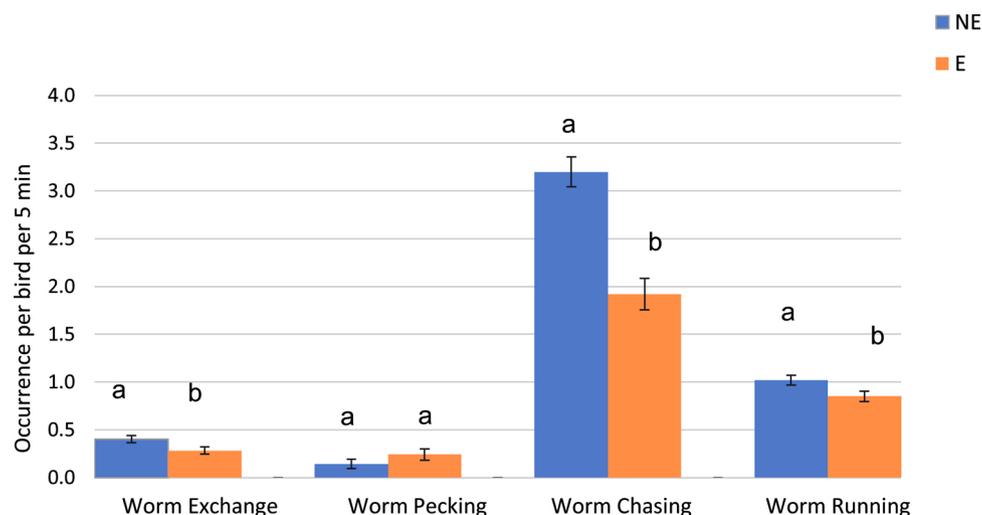


Fig. 2. Least squares mean occurrence (\pm SE) of worm exchange, worm pecking, worm chasing, and worm running per bird per 5 min during worm running tests in non-enriched (NE) and enriched (E) pens on days 10, 24, and 38. Means with the same letters within each behaviour are not different ($P \geq 0.05$).

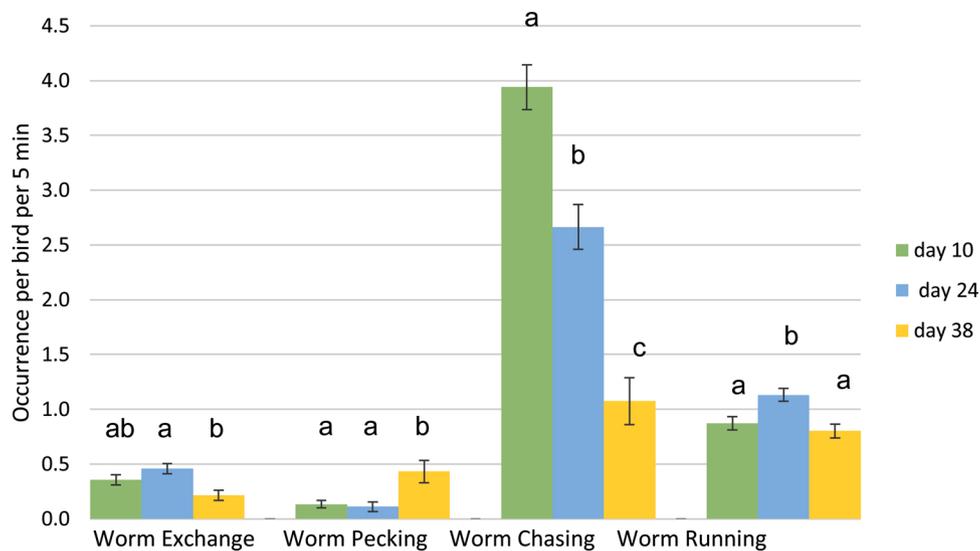


Fig. 3. Least squares mean occurrence (\pm SE) of worm exchange, worm pecking, worm chasing, and worm running per bird per 5 min during worm running tests on days 10, 24, and 38. Means with the same letters within each behaviour are not different ($P \geq 0.05$).

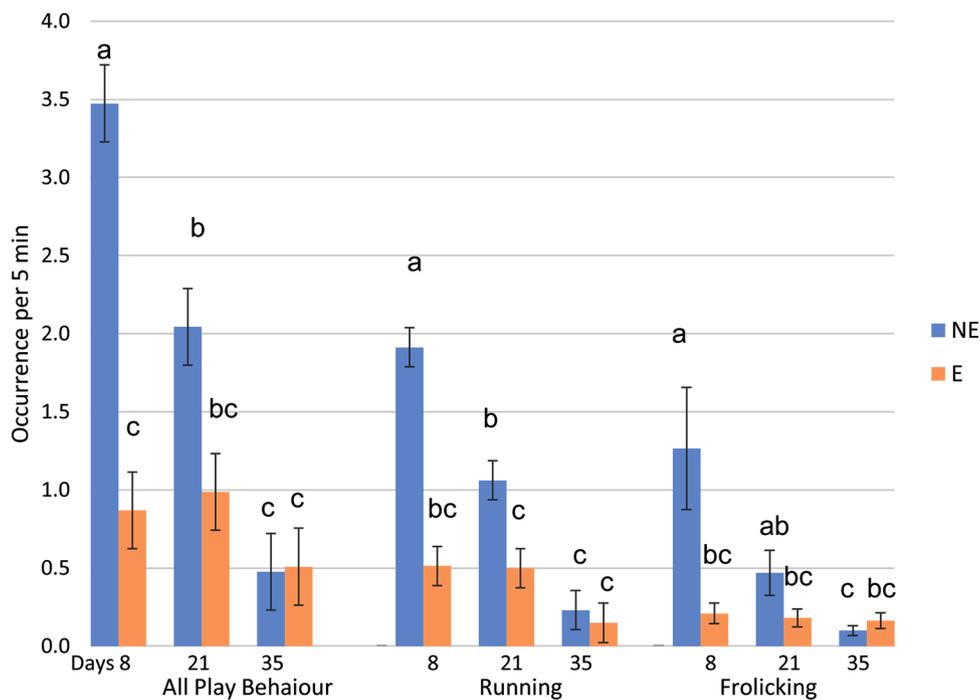


Fig. 4. Least squares mean occurrence per bird per 5 min (\pm SE) of all play behaviour (sum of running, frolicking, wing flapping, and sparring), running, and frolicking in non-enriched (NE) and enriched (E) pens during free-space tests on days 8, 21, and 35. Treatment and age interacted for all behaviours. Means with the same letters within each behaviour are not different ($P \geq 0.05$).

0.020, and 0.12 ± 0.020 occurrences per bird per 5 min, respectively; day 8 vs 21, $P < 0.001$; day 8 vs 35, $P = 0.047$). The frequency of sparring was very low so it was not analyzed.

3.3. Spontaneous play

There was no treatment effect ($F_{(1,19)} = 0.22$, $P = 0.644$; $F_{(1,19)} = 0.00$, $P = 0.967$; $F_{(1,19)} = 0.52$, $P = 0.481$; $F_{(1,19)} = 1.43$, $P = 0.247$) or treatment by age interaction ($F_{(2,19)} = 1.53$, $P = 0.242$; $F_{(2,19)} = 1.95$, $P = 0.170$; $F_{(2,19)} = 1.91$, $P = 0.176$; $F_{(2,19)} = 1.93$, $P = 0.173$) on all play behaviour, running, frolicking, or wing flapping, respectively, during spontaneous play. Sparring was too rare for statistical analysis.

Occurrences per bird per hour in the NE and E pens, respectively, were 1.08 ± 0.057 and 1.04 ± 0.059 for all play behaviour, 0.31 ± 0.028 and 0.30 ± 0.029 for running, 0.29 ± 0.029 and 0.32 ± 0.029 for frolicking, and 0.37 ± 0.030 and 0.32 ± 0.030 for wing flapping. The frequencies of all play behaviour ($F_{(2,19)} = 5.53$, $P = 0.013$), running ($F_{(2,19)} = 15.00$, $P < 0.001$), and frolicking ($F_{(2,19)} = 4.07$, $P = 0.034$) decreased with age, particularly from 23 to 30 days, whereas there was no age effect on wing flapping ($F_{(2,19)} = 2.24$, $P = 0.134$; Fig. 5).

4. Discussion

Contrary to our hypothesis, the results show that NE birds played

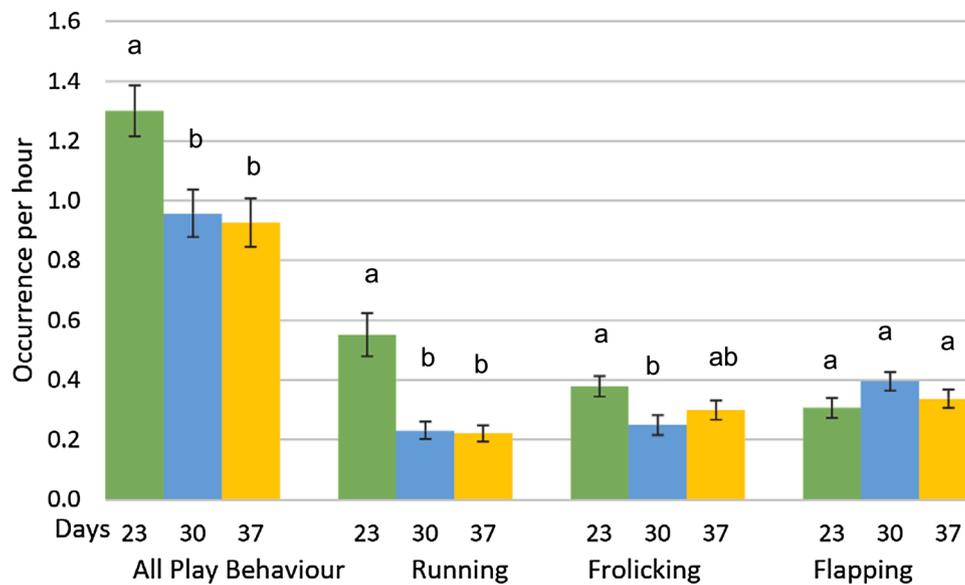


Fig. 5. Least squares mean occurrence per bird per hour (\pm SE) of all play behaviour (sum of running, frolicking, wing flapping, and sparring), running, frolicking, and wing flapping on days 23, 30, and 37 during spontaneous play observations. Means with the same letters within each behaviour are not different ($P \geq 0.05$).

more than E birds in test contexts, and no difference was found in spontaneous play. We hypothesized that E birds would play more because animals in enriched environments are generally expected to have fewer negative, and perhaps, more positive affective experiences. Several studies have indicated that animals such as mink (Vinke et al., 2005) and rats (Morley-Fletcher et al., 2003) living in an enriched environment play more than those living in barren environments. Another reason for our hypothesis of more play in E birds was that before conducting the experiments, we expected E birds to have better mobility and thus to be more physically able to play. However, the results from Liu (2019) indicated that this was not the case; there was no difference in mobility between E and NE birds based on a latency-to-leave test and an obstacle test.

There are several possible explanations for why NE birds engaged in more play than E birds during worm running and free-space tests, which are not mutually exclusive. Firstly, the NE birds may have experienced fewer opportunities for play than the E birds, resulting in them being more likely to show a rebound in play behaviour when given temporary opportunities during testing. Rebounds in play have previously been observed whereby calves subjected to spatial restriction performed more play than calves housed in more spacious pens when both groups tested in a novel and spacious environment (Jensen & Kyhn, 2000; Rushen & de Passillé, 2014). This explanation is countered by the observation that NE birds performed similar rather than lower levels of spontaneous play under baseline conditions.

Secondly, the larger contrast between the NE environment before and during the tests (i.e. opening up space and offering “worms”) compared to that in the E pens may have led the NE birds to be more easily stimulated to play during the tests. According to this interpretation, the higher play behaviour in the NE birds reflected transiently higher responsiveness in the test context rather than reflecting an underlying state of greater positive welfare when kept in a NE environment.

A third explanation is that NE birds could have been experiencing boredom (Burn, 2017), thus being more responsive during the tests. There is some evidence supporting the idea that animals suffer from boredom when living in unstimulating barren environment, making them more easily aroused when offered stimuli not usually available. For example, Meagher and Mason (2012) found that NE mink showed increased interest in all types of stimuli (aversive, ambiguous, and rewarding) in this context, and Stolba and Wood-Gush (1980) reported

that the more barren the environment, the stronger the interest that pigs exhibited towards a stimulus. While we did not detect differences in spontaneous play between the E and NE birds, the E birds may nevertheless have experienced better welfare associated with the additional behavioural opportunities offered in the E pens, including resting in “safe” elevated locations, gentle swaying when on the hanging scale, and foraging from the pecking stone and suet box. Although not observed systematically, E birds were seen resting on top and around the platform, pecking stone and scale, and they did peck at the pecking stones and remove the wood shavings from the suet boxes each time they were refilled.

Fourthly, the NE birds may have simply had greater net play opportunities than E birds during the tests. The enrichment items may have served as obstacles that impeded rapid playful locomotion and interaction stimulated during the tests. This explanation may explain why E birds showed consistently low running, frolicking, and all play combined in the free-space tests with increasing age. It was only the NE birds that showed elevated levels of play when young, with a decline in play with increasing age. If the lower play in E than NE pens resulted from a reduction in useable space due to the introduction of enrichment structures, this finding suggests that, when practicing environmental enrichment, a reduction in stocking density is needed to reap the full benefit of the enrichment.

Regarding age effects, worm chasing declined with age in the worm running tests. There were also declines with age in running, frolicking, and all play combined during the free-space and spontaneous play observations. Other studies on fast-growing broilers have indicated that time spent in walking, running, and other active behaviours declined as they grew older (Newberry et al., 1986; Weeks et al., 2000; Tickle et al., 2018). Although Baxter et al. (2019) did not detect differences in the levels of frolicking and sparring between 3 and 5 weeks of age, Vasdal et al. (2019) found a decrease in running, worm running, and play fighting from day 16 to day 30, and Dawson & Siegel (1967) reported peaks in frolicking and sparring at around 28 days and 32 days, respectively, followed by a decline to negligible levels by 10 weeks of age.

It may be argued that a decline in play behaviour is a normal developmental change as an animal becomes mature (Spear, 2004). However, broilers are typically slaughtered prior to sexual maturity and, therefore, play could be expected to persist until slaughter (Yamada, 1999). Moreover, laying pullets performed worm running behaviour at

similar rates at 8 to 12, and 68 to 70, days of age (Cloutier et al., 2004), contrary to the decline in worm chasing, and increase in stationary worm pecking observed in this study. These changes with age were, therefore, likely related to the broilers' increasing body weights, and associated increased stocking density and reduced mobility (Bokkers & Koene, 2003; Liu, 2019), rather than their age alone. The relatively small size of the experimental pens may have exacerbated these effects compared to observations of broilers in commercial housing. In addition, necrotic enteritis was diagnosed at 29 d of age, although rapidly controlled by antibiotics. Play behaviour is known to be decreased when animals are ill (Ahloy-Dallaire et al. 2018). Therefore, it is possible that illness suppressed play in some birds during the spontaneous play observation on day 30. The reduction in light intensity to 7 lux on day 29 may have also contributed to reduced spontaneous play at this age (Newberry et al., 1988; Blatchford et al., 2009). Space restriction may have been the greatest limiting factor on play at higher ages given that stationary forms of play behaviour including worm pecking and wing flapping increased with age.

Play was recorded during 5-min observations in the free-space tests and for a total of 1 hour in the spontaneous play context using the same methods. The level of all play behaviour was 5 to 34 times (in NE pens) and 7 to 9 times (in E pens) higher (depending on test dates) in the tests than during spontaneous conditions. The level of worm chasing in the worm running tests was of a similar magnitude to all play behaviour in the free-space tests. These results indicate that the two tests were effective in stimulating play as predicted, as well as in detecting treatment differences. Therefore, these tests can be valuable for use in future research on responses in these contexts. Nevertheless, it is interesting that none of the results revealed higher levels of play in the E than the NE treatment, contrary to the prediction that the enriched environment would stimulate more play. As described earlier, studies have indicated suppression of play under adverse conditions that could be perceived as life-threatening. This was not the case in the current study, as chickens grew at similar rates in both treatments (Liu, 2019) and in accordance with expected growth according to the breeder manual (Aviagen, 2019). In addition, mortality rates were comparable and within rates typical for the breed (Liu, 2019). The findings show that both the context under which play is observed and the timing of observations relative to stimulation of activity are critical to the interpretation of play data.

5. Conclusions

NE birds performed more play behaviour than E birds under worm running and free-space test conditions. The NE birds might have been more responsive as a result of a higher contrast between their relatively unstimulating normal environment and the test conditions (the presence of "worms" and extra space), possibly exacerbated by boredom. Birds performed less energetic play behaviour and became more sedentary as they aged, likely because higher body weights and stocking densities reduced their mobility.

Declaration of Competing Interest

The authors declare there was no conflict of interest in the conduct of the present study.

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