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

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9

10   **Abstract**

11   The objective of this study was to evaluate effects of environmental enrichment on play

12   behaviour in fast-growing broiler chickens. Chicks (19 of each sex) were randomly assigned to 6

13   non-enriched (NE) pens and 6 pens enriched (E) with a raised platform, hanging weighing scale,

14   peck stone, and suet feeder filled with wood shavings which was refilled every other day. Three

15   worm running tests (on days 10, 24, and 38), and three free-space tests (on days 8, 21, and 35)

16   were performed to stimulate play behaviour. From video recordings, occurrences of worm

17   exchange, worm pecking, worm chasing, and worm running in the worm running tests, and

18   running, frolicking (i.e. running with wing use), wing flapping (while stationary), and sparring in

19   the free-space tests, were quantified. Video recordings were also made on days 23, 30, and 37 to

20   observe spontaneous play. Generalized linear mixed models were used to investigate effects of

21   enrichment, age and their interaction on occurrence of the different forms of play behaviour per

22   bird per 5 min in the worm running and free-space tests, and per hour in spontaneous play

23   observations. In worm running tests, chickens in NE pens performed more worm exchange

24   (P=0.034), worm chasing (P<0.001), and worm running (P=0.035) than those in E pens. In free-

25   space tests, running (P<0.001), frolicking (P=0.016), and all play behaviour combined (sum of

26 running, frolicking, wing flapping, and sparring; P<0.001), were more frequent in NE than E

27 pens. In the test contexts, worm chasing declined with age in both NE and E pens (P<0.001), and

28 running, frolicking and all play behaviour combined declined with age in NE pens while

29 remaining low and unchanged in E pens. In contrast, occurrence of worm pecking (P=0.004) and

30 wing flapping (P<0.001), both performed while stationary, increased with age in both NE and E

31 pens. Total spontaneous play behaviour (sum of running, frolicking, wing flapping, and sparring)

32 did not differ between treatments (P=0.644) but did decline with age (P=0.013). In conclusion,

33 while NE birds did not differ from E birds in the performance of spontaneous play, they were

34 more responsive than E birds during tests intended to stimulate play behaviour in broilers. This

35 was possibly because of the larger contrast between their relatively unstimulating environment

36 and the test conditions, resulting in them being more easily aroused. The findings also show that

37 the worm running and free-space tests were effective in stimulating play.

38

39 **Keywords:**

40 Broiler chickens, Environmental enrichment, Play behaviour, Animal welfare, Behavioural

41 development

42

43 **Highlights:**

44 • Standardised worm running and free-space tests were used to stimulate play
45 • In tests, less play behaviour occurred in enriched than non-enriched pens
46 • Spontaneous play behaviour was also quantified outside the test contexts
47 • Similar levels of spontaneous play occurred in enriched and non-enriched pens
48 • Play behaviour became less energetic with increasing age of broilers
49

50   ## 1. Introduction

51   Play behaviour is common in young homeothermic animals (Špinka et al., 2001;

52   Diamond & Bond, 2003). Although functions of play are debated, there are several common

53   characteristics of play across species: 1) play is most common during infancy and juvenile

54   periods; 2) play is not critical to present survival; 3) play may occur repeatedly but not

55   stereotypically, and 4) play typically occurs when stress is low (Burghardt, 2005). Play

56   behaviour can be categorized as locomotor play (eg. running and jumping), object play (i.e.

57   interacting with inanimate objects), and social play (involving more than one animal; Špinka et

58   al., 2001; Diamond & Bond, 2003; Held & Špinka, 2011).

59   Limited research has been conducted on play behaviour in domestic chickens. Behaviour

60   patterns suggested to meet criteria for play in chickens include frolicking (Duncan, 1998;

61   Appleby et al., 2004; Nicol, 2015; Baxter et al., 2019), sparring (Duncan, 1998; Mench, 1988;

62   Nicol, 2015) and worm running (Cloutier et al., 2004; also referred to as food running, Baxter et

63   al., 2019). These behaviour patterns occur mainly when young and seem to be less common and

64   less varied than forms of play behaviour described in some mammalian species (Appleby et al.,

65   2004; Nicol, 2015).

66   Worm running occurs when a bird picks up a food item or other object and runs with it

67   while other birds chase after the running bird and attempt to grab the item (Kruijt, 1964; Cloutier

68   et al., 2004). It was first described as feeding behaviour (i.e. 'food running') and was proposed to

69   function as a way to attract other birds, resulting in tearing up a relatively large food item such as

70   a worm or caterpillar into smaller pieces for consumption (Kruijt, 1964). However, Cloutier et al.

71   (2004) suggested that worm running with inedible objects under conditions when food is

72   abundant (e.g. when fed *ad libitum*) fits criteria for play behaviour, including locomotion, object

73   and social play, because it is not associated with social dominance and because it has no

74   immediate survival value when the objects are non-nutritive.

75   Sparring, also known as play fighting, is reported in some birds, including domestic

76   chickens (Dawson and Siegel, 1967; Baxter et al., 2019; Vasdal et al., 2019). Birds performing

77   sparring display some elements used in adult fighting such as jumping and physical contact, but

78   without aggressive pecking and injuries (Diamond & Bond, 2003). Using factor analysis to

79   describe the ontogeny of social behaviour in chicks, Rushen (1982) found that sparring was

80   closely associated with running during the first 6 weeks of life, and that sparring and running

81   were replaced by aggressive behaviour in older birds. Mench (1988) also found that sparring

82   performed in juveniles was replaced by the aggressive and potentially harmful form used in adult

83   fighting. Moreover, because youthful sparring was significantly less frequent when birds were

84   feed deprived, Mench (1988) suggested that sparring shares characteristics of mammalian play

85   behaviour.

86   Frolicking involves spontaneous running with raised or flapping wings and is socially

87   contagious in groups (Dawson and Siegel, 1967). It is performed in a repeated but non-

88   stereotypical fashion (Baxter et al., 2019) and may lead to sparring (Wood-Gush, 1971). Wing

89   flapping can also occur when stationary. Wing flapping involves rapid bilateral, usually repeated,

90   wing movements, unlike slow and unilateral wing stretching. It is performed by adult males as a

91   display of vigour in the context of reproduction (Millman et al., 2000; McGary et al., 2003).

92   However, like sparring, it appears playful in youngsters, when not being performed in the adult,

93   serious context. It occurs in association with frolicking and sparring has, thus, been included in

94   studies on play in chickens (e.g. Keeling and Zimmerman, 2009; Vasdal et al., 2019). In broilers,

95   frolicking and sparring were reported to disappear by 10 weeks of age (Dawson and Siegel,

96   1967).

97   Some conditions are reported to stimulate play behaviour in young domestic fowl. A

98   'worm running test' provides chickens with an opportunity to perform object play (Cloutier et

99   al., 2004). During the test, a worm-like object (e.g. pipe cleaner or piece of twisted paper) is

100   thrown into the home pen. Chickens will peck at the "worm", run with it protruding from their

101   beak, or chase after the worm running bird. Keeling and Zimmerman (2009) observed increased

102   locomotor play when providing objects for 30 min (small plastic toothpicks, a ball and a

103   cardboard box) than when these objects were not present. Another method for stimulating play

104   involves displacing chickens by walking through their housing. The birds then perform social

105   and locomotory forms of play behaviour when re-entering the empty space behind the walking

106   person (Newberry et al., 2018; Baxter et al., 2019). A further method involves providing

107   repeated temporary access to additional space outside the home enclosure. Newberry (1999)

108   observed that, despite low stocking density in the home pen ($11.6 \, \text{kg/m}^2$), opening a gate to give

109   short-term daily access to an empty pen next to the home pen led to broilers running into the

110   open space, often with wings flapping. She suggested that this was possibly related to boredom

111   in the home pen. Because spontaneous play may take up only a small proportion of the

112   behavioural time budget, the use of tests designed to stimulate play could be efficient for

113   studying factors affecting play in chickens.

114   Play behaviour is thought to indicate primarily positive, physical and affective states

115   because it can serve as a reward in a learning paradigm (e.g. Humphreys & Einon, 1981;

116   Calcagnetti & Schechter, 1992), and because its frequency is often reduced when an animal is

117   exposed to a biological challenge (Held & Špinka, 2011; Lawrence et al. 2018). For example,

118   castration of domestic lambs led to depressed play for at least 3 days (Thornton & Waterman-

119   Pearson, 2002). Similarly, physical or mental illness has been associated with reduced play in

120   people (McGrath et al., 1990; Coplan et al., 2004). Such findings suggest that, especially during

121   the age range when play is most common, animals free from diseases, injuries, and chronic

122   negative affective states will play more, indicating better welfare. However, play has also been

123   associated with negative affective states (Ahloy-Dallaire et al., 2018). For example, following

124   early separation from their mothers, kittens performed more object play behaviour than controls

125   that remained with their mothers (Bateson et al., 1981), and rats engaged in more rough-and-

126   tumble play after being injected with a moderate dose of a stress hormone (ACTH; Arelis, 2006).

127   Possible non-mutually exclusive explanations for these findings are that: 1) because play is

128   pleasurable, its performance alleviates stress (Špinka et al., 2001), and 2) if play enables animals

129   to better prepare for challenging situations, it may be performed at higher levels by individuals

130   that have experienced adversity as, for example, in the kittens' case (Ahloy-Dallaire et al., 2018).

131   Environmental enrichment is practiced with the goal of improving animal welfare.

132   Depending on the types provided and overall environmental complexity, environmental

133   enrichment has been associated with some welfare benefits in fast-growing broiler chickens,

134   including lower mortality (BenSassi et al., 2019), improved leg health (Kaukonen et al., 2017;

135   Pedersen & Forkman, 2019) and increased exploratory and comfort behaviours (Vasdal et al.,

136   2019). However, Baxter et al. (2019) detected no specific association between environmental

137   enrichment and play behaviour in broiler chickens and, while Vasdal et al. (2019) observed

138   increased wing flapping in enriched housing, other playful activities including spontaneous

139   running, worm running and play fighting, were unaffected. Nevertheless, relatively little research

140   has been conducted on environmental enrichment for broiler chickens and many questions

141   remain (Riber et al., 2018); for example, if and/or how play behaviour in broilers is affected by

142   enrichment types, the context of play ("play test" conditions or spontaneous play), the strain and

143   age of the chickens, and the methods used to quantify play.

144   The objective of this study was to investigate the effects of environmental enrichment on

145   play behaviour during worm running and free-space tests, as well as on spontaneous play, in fast-

146   growing broilers at different ages. We hypothesized that broilers reared in an enriched

147   environment have better welfare and, therefore, would play more than broilers in a non-enriched

148   (control) environment, especially at older ages when they would otherwise be more susceptible

149   to health problems. This was part of a larger study on effects of environmental enrichment on

150   health and welfare of broiler chickens (Liu, 2019).

151   **2.   Methods**

152   **2.1. Animals, housing, and management**

153   The study was conducted in an experimental room at the Arkell Poultry Research Station,

154   Guelph, Canada, and all procedures were approved by the Animal Care Committee at the

155   University of Guelph (Animal Utilization Protocol #3746). A flock of 456 feather-sexed Ross

156   708 broiler chicks was obtained from a commercial hatchery at 1 day of age and housed in 12

157   floor pens (160 cm wide × 238 cm long; 38 birds/pen, expected mean stocking density at end of

158   production=29 kg/m^2, 19 females and 19 males randomly assigned to each pen). All pens were

159   supplied with one round hanging feeder (diameter: 33.8 cm), a line of nipple drinkers (5 nipples

160   per pen) and fresh pinewood shavings (approximately 6 cm in depth). The pens were divided by

161   solid white plastic walls. All chicks were vaccinated at the hatchery with Bronchitis vaccine

162   (mass type, live virus; Merial, Athens, GA, USA; method: spray), Newcastle-Bronchitis vaccine

163   (B1 type, B1 strain, Mass & Conn types; Merial, Athens, GA, USA; method: spray), and

164   Marek's disease vaccine (Ceva, Lenexa, KS, USA; method: subcutaneous injection). Female

165   chicks were marked with a small amount of animal-safe pink paint (KONK Livestock Markers -

166   Red, Viceroy Distributors, Winnipeg, MB, Canada) on their heads for another experimental

167   purpose.

168   Birds were given *ad libitum* access to water and antibiotic-free, plant-based feed that was

169   milled on-site. They were fed a starter diet from day 1 to day 14, a grower diet from day 15 to

170   day 28, and a finisher diet after day 28. The experimental room was climate-controlled at

171   approximately 32 ℃ on day 1, 31 ℃ on day 5, 29 ℃ in week 2, 27 ℃ in week 3, 24 ℃ in week

172   4, and 21 ℃ in week 5. The light:dark schedule was 23L:1D from day 1 to day 4. After day 4,

173   the lights were turned on at 06:15 and turned off at 22:15 (16L:8D), with a dawn/dusk period (30

174   min each). The light intensity was approximately 56 lux from day 1 to day 4, 20 lux from day 5

175   to day 28, and 7 lux from day 29 until the end of the trial. The weekly mortality rate was 0.19%

176   for the first, second, and third week, and increased to 1.74% in the fourth week. Mortality was

177   similar across pens. Necrotic enteritis was diagnosed when the birds were 28 days of age, and the

178   condition was controlled by treatment of all birds with antibiotics via drinking water (Pot-Pen,

179   Vetoquinol, Lavaltrie, QC, Canada) from day 29 to 34. The mortality rate decreased to 0.39% in

180   week 5, and there was no further mortality from day 33 until the end of the trial. Birds were

181   checked twice daily for health status and no moribund birds were found. All chickens were

182   processed on day 43.

183   **2.2. Experimental design**

184 Half of the pens were non-enriched (NE) while the other half were enriched (E) with the

185 following: a raised platform (Red Rooster Mark 2 Black Slats, Clark Ag Systems, Caledonia,

186 ON, Canada, 58×39 cm, length×width) with a 25° ramp of the same material (79×39 cm,

187 length×width), placed at the back of each pen, a weighing scale platform (Clark Ag Systems

188 Ltd., Caledonia, ON, Canada; diameter: 51 cm) hanging above the floor (1 cm high in week 1

189 and another 1 cm added each week), ¼ of a mineral peck stone (PECKstone (hard), Protekta,

190 Lucknow, ON, Canada, full size: 12×19×11 cm, length×width×height) against the left pen wall,

191 and a suet cage feeder (Scotts, Hagersville, ON, Canada, 13×13x5 cm) filled with pinewood

192 shavings, suspended above the floor and adjusted regularly to around shoulder-height of the birds

193 (Figure 1). The suet feeder was refilled with fresh shavings every other day (on days when no

194 video recordings were being made; see below). The birds pecked at the shavings in the suet

195 feeders frequently and typically emptied the feeders within one day. The same amount of fresh

196 shavings was also added to the litter in NE pens on the same days when the suet feeders in E

197 pens were refilled to standardize the potential effect of added shavings on litter quality.

198 Proximity of pens to the outside wall of the room, and to heaters and air inlets, resulted in

199 a slight temperature gradient in the room. To control for any potential temperature effect, the

200 pens were assigned to 6 blocks comprising one NE pen and one E pen, with treatments balanced

201 on each side of the room across blocks. Within the room, there was also one extra NE pen and

202 one extra E pen. If a bird died, another bird of the same sex, similar body weight, and reared

203 under the same treatment condition (NE or E) was added to maintain an equivalent stocking

204 density across all pens. A total of 6 NE birds and 8 E birds were replaced over the course of the

205 experiment. These replacement birds were added on days 6, 13, 15, 22, 23, 24, 25, 29, and 33.

206 On two occasions (days 23 and 24), behavioural observations were conducted prior to the

207   addition of replacement birds. In general, young, fast-growing broilers are non-cannibalistic, and

208   considerably less aggressive than other domestic fowl (Mench, 1988; Petit-Riley et al., 2002;

209   Bokkers & Koene, 2003). No cannibalism or aggression was directed towards the replacement

210   birds and these birds appeared to adapt to their new pens almost immediately.

211   **2.3. Worm running tests**

212   Three 5-min worm running tests were performed in each pen, on days 10, 24, and 38,

213   respectively, between approximately 10:00-11:00. The procedure was adapted from Cloutier et

214   al. (2004; Table 1). "Worms" were made from twisted white tissue paper and were increased in

215   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

216   38. The diameter of the paper worms was 3 mm. Before conducting the test, a researcher stood in

217   front of a pen for approximately one minute to allow the birds to habituate to the researcher's

218   presence. Once the birds returned to their undisturbed behaviour, a "worm" was gently thrown

219   into the pen. The entire test was observed and, if a "worm" was destroyed, eaten, or lost, a new

220   "worm" was supplied to replace it. Two digital video cameras (Sony Digital High Definition

221   Video Camera; HDR-CX405 and DCR-SR68 models, Tokyo, Japan) mounted on monopods

222   (Digiant MP-3606 Professional Video Monopod 70", Zhejiang, China) were attached to the front

223   wall of the pen (one at each corner, angled down) to record the test. Due to high bird activity, it

224   was not always possible to keep track of the "worm" in real-time; therefore, a 10-min recording

225   was made. Pens within the same block were tested concurrently by different researchers, and

226   "worms" were removed after the test.

227   For ease of scoring, the videos were analyzed in 10-s segments, starting from the moment

228   the "worm" landed on the pen floor. Any segment during which the worm was not present was

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229   not used for analysis. The first 30 segments where the worm was present in the videos were

230   scored (5 min in total). For each segment, all occurrences of birds performing worm pecking,

231   worm running, and worm chasing were recorded (Table 1). For each segment, the total number

232   of times a worm exchange occurred was also recorded (Table 1). Occasionally, a missing

233   "worm" reappeared during the observation period after it had been replaced by another "worm"

234   and, when this occurred, only behaviour directed towards the replacement "worm" was recorded.

235   One observer (ZL) with previous experience collecting behavioural data from chickens and who

236   was not blind to treatment or hypotheses performed all observations in this study. The observer

237   re-reviewed a sample of video recordings at the start and end of data collection to determine

238   intra-observer reliability. The Kappa coefficient for the worm running test was 0.92.

239   **2.4. Free-space tests**

240   Three free-space tests were performed in all pens, on days 8, 21 and 35, respectively,

241   between approximately 10:00 - 11:00. The procedure and ethogram (Table 2) were adapted from

242   Newberry et al. (2018) and Baxter et al. (2019). To perform the test, a researcher walked into a

243   pen with a pen divider panel and gently pushed all birds back to the drinker line. Meanwhile, a

244   second researcher removed the feeder, opening up a standard amount of 'new' free space in the

245   pen. E pens still retained the enrichment, so the total amount of unfurnished space differed

246   between treatments. After approximately 10 s, the divider was removed and both researchers

247   quietly moved to the next non-adjacent pen, where the procedure was repeated. Four non-

248   adjacent pens were video recorded simultaneously for 5 min, using two digital video cameras on

249   monopods as described for the worm running tests. Feeders were then returned to the pens and

250   tests commenced in the next round of four non-adjacent pens, followed by a third round. A pre-

251 determined, systematic test order was followed, avoiding disturbance to the birds during

252 recording and ensuring that each round included two NE and two E pens.

253   From the videos, the observer used continuous all-occurrences sampling of the

254 behavioural events listed in Table 2 to record every occurrence of running, frolicking, wing

255 flapping, and sparring over the whole observation period (5 min). The Kappa coefficient for

256 intra-observer reliability in the free-space test was 0.91.

257   **2.5. Spontaneous play**

258   Video recordings were made of all pens concurrently on days 23, 30, and 37 between

259 11:00 and 15:00 to observe spontaneous play behaviour in a non-test context. One digital video

260 camera on a monopod (described in section 2.3) was mounted at the centre of the front wall of

261 each pen for video recording. Continuous all-occurrences sampling was used to record every

262 occurrence of running, frolicking, wing flapping and sparring during 1 h of observation (11:00 to

263 11:30 and 13:00 to 13:30) using the same ethogram as for free-space tests (Table 2).

264   **2.6. Statistical analyses**

265   Prior to analysis, data from each pen were standardized as occurrences per bird per 5 min

266 (by dividing all frequencies by the number of birds in the pen) for worm running and free-space

267 tests, and occurrences per bird per hour for spontaneous play. For the free-space test and

268 spontaneous play observations, an aggregated "all play behaviour" variable (sum of running,

269 frolicking, wing flapping, and sparring) was also calculated. There were two pens with missing

270 data due to video recording failure (one E pen in the worm-running test at 39 days and

271 spontaneous play in one E pen at 23 days).

272   Statistical analyses were computed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Pen

273   was the experimental unit, and the sample size was 6 pens per treatment. Generalized linear

274   mixed models included treatment, age, and their interactions as fixed effects. To account for

275   temperature variation in the room, block was included as a random effect, with pen as subject

276   included in the random statement. Since repeated measurements were made on each pen at

277   different ages, a compound symmetry covariance structure was specified to account for within-

278   subject correlated errors. Least squares means were calculated, and pairwise means comparisons

279   were adjusted for multiple comparisons using the Tukey option. Residuals were generated using

280   the output statement and assessed for normality in Proc Univariate based on the Shapiro-Wilk

281   statistic. Most of the dependent variables adhered to a Gaussian distribution, and their least

282   squares means, and standard errors are reported in results. Worm pecking, frolicking in free-

283   space tests, and running during spontaneous play observations were log-transformed to

284   normalize the data by specifying modelling of the lognormal distribution in Proc Glimmix, and

285   back transformed least squares means and standard errors are presented. All residuals were re-

286   checked with Shapiro-Wilk to confirm the distribution. Log transformation resulted in exclusion

287   of eight 0 values from the worm pecking analysis.

288   **Results**

289   **3.1. Worm running tests**

290   There were overall treatment effects on worm exchange ($F_{(1,19)}$=5.26, P=0.034), worm

291   chasing ($F_{(1,19)}$=32.15, P<0.001), and worm running ($F_{(1,19)}$=5.15, P=0.035), with NE birds

292   performing more of these behaviours than E birds (Figure 2). There was no effect of treatment on

293   worm pecking ($F_{(1,11)}$=1.61, P=0.231).

294   There was a decrease in the occurrence of worm chasing as the birds aged ($F_{(2,19)}$=44.16,

295   P<0.001), and an increase in the occurrence of worm pecking ($F_{(2,11)}$=9.52, P=0.004; Figure 3).

296   The occurrence of worm exchange ($F_{(2,19)}$=6.56, P=0.007) and worm running ($F_{(2,19)}$=8.72,

297   P=0.002) varied inconsistently across days of observation (Figure 3). There was no treatment $\times$

298   age interaction on worm exchange ($F_{(2,19)}$=0.70, P=0.507), worm pecking ($F_{(2,11)}$=1.73, P=0.222),

299   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

301   During the free-space tests, there were effects of treatment, age, and a treatment $\times$ age

302   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,

303   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,

304   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,

305   P=0.003, respectively; Figure 4). NE birds performed more running than E birds on days 8

306   (P<0.001) and 21 (P=0.048), but not on day 35 (P=0.997). The occurrence of running decreased

307   at each age tested for NE birds but not for E birds, which had a lower running frequency at all

308   three ages observed. NE birds performed more frolicking than E birds only on day 8 (P=0.006),

309   after which the frequency of this behaviour declined considerably in NE birds. The frequency of

310   frolicking was low in E birds at all ages. The occurrence of all play behaviour was higher in NE

311   than E birds on day 8 (P<0.001) whereas the difference was smaller on day 21 (P=0.062) and

312   gone on day 35 (P=1.000). The occurrence of all play behaviour declined at each age tested for

313   NE birds but not for E birds.

314   There was no treatment effect ($F_{(1,20)}$=1.40, P=0.251) or treatment $\times$ age interaction

315   ($F_{(2,20)}$=2.51; P=0.107) on frequency of wing flapping. Wing flapping was affected by age

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316   ($F_{(2,20)}$=11.75, P<0.001), occurring less often on day 8 than on day 21 or 35 (0.05±0.020,

317   0.19±0.020, and 0.12±0.020 occurrences per bird per 5 min, respectively; day 8 vs 21, P<0.001;

318   day 8 vs 35, P=0.047). The frequency of sparring was very low so it was not analyzed.

319   **3.3. Spontaneous play**

320   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,

321   P=0.481; $F_{(1,19)}$=1.43, P=0.247) or treatment by age interaction ($F_{(2,19)}$=1.53, P=0.242;

322    $F_{(2,19)}$=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,

323   running, frolicking, or wing flapping, respectively, during spontaneous play. Sparring was too

324   rare for statistical analysis. Occurrences per bird per hour in the NE and E pens, respectively,

325   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,

326   0.29±0.029 and 0.32±0.029 for frolicking, and 0.37±0.030 and 0.32±0.030 for wing flapping.

327   The frequencies of all play behaviour ($F_{(2,19)}$=5.53, P=0.013), running ($F_{(2,19)}$=15.00, P<0.001),

328   and frolicking ($F_{(2,19)}$=4.07, P=0.034) decreased as the birds got older, whereas there was no age

329   effect on wing flapping ($F_{(2,19)}$=2.24, P=0.134; Figure 5).

330   **3. Discussion**

331   Contrary to our hypothesis, the results show that NE birds played more than E birds in test

332   contexts, and no difference was found in spontaneous play. We hypothesized that E birds would

333   play more because animals in enriched environments are generally expected to have fewer

334   negative, and perhaps, more positive affective experiences. Several studies have indicated that

335   animals such as mink (Vinke et al., 2005) and rats (Morley-Fletcher et al., 2003) living in an

336   enriched environment play more than those living in barren environments. Another reason for

337   our hypothesis of more play in E birds was that before conducting the experiments, we expected

338   E birds to have better mobility and thus to be more physically able to play. However, the results

339   from Liu (2019) indicated that this was not the case; there was no difference in mobility between

340   E and NE birds based on a latency-to-lie test and an obstacle test.

341   There are several possible explanations for why NE birds engaged in more play than E

342   birds during worm running and free-space tests, which are not mutually exclusive. Firstly, the

343   NE birds may have experienced fewer opportunities for play than the E birds, resulting in them

344   being more likely to show a rebound in play behaviour when given temporary opportunities

345   during testing. Rebounds in play have previously been observed whereby calves subjected to

346   spatial restriction performed more play than calves housed in more spacious pens when both

347   groups tested in a novel and spacious environment (Jensen & Kyhn, 2000; Rushen & de Passillé,

348   2014). This explanation is countered by the observation that NE birds performed similar rather

349   than lower levels of spontaneous play under baseline conditions.

350   Secondly, the larger contrast between the NE environment before and during the tests

351   (i.e. opening up space and offering "worms") compared to that in the E pens may have led the

352   NE birds to be more easily stimulated to play during the tests. According to this interpretation,

353   the higher play behaviour in the NE birds reflected transiently higher responsiveness in the test

354   context rather than reflecting an underlying state of greater positive welfare when kept in a NE

355   environment.

356   A third explanation is that NE birds could have been experiencing boredom (Burn, 2017),

357   thus being more responsive during the tests. There is some evidence supporting the idea that

358   animals suffer from boredom when living in unstimulating barren environment, making them

359   more easily aroused when offered stimuli not usually available. For example, Meagher and

360 Mason (2012) found that NE mink showed increased interest in all types of stimuli (aversive,

361 ambiguous, and rewarding) in this context, and Stolba and Wood-Gush (1980) reported that the

362 more barren the environment, the stronger the interest that pigs exhibited towards a stimulus.

363 While we did not detect differences in spontaneous play between the E and NE birds, the E birds

364 may nevertheless have experienced better welfare associated with the additional behavioural

365 opportunities offered in the E pens, including resting in "safe" elevated locations, gentle swaying

366 when on the hanging scale, and foraging from the pecking stone and suet box. Although not

367 observed systematically, E birds were seen resting on top and around the platform, pecking stone

368 and scale, and they did peck at the pecking stones and remove the wood shavings from the suet

369 boxes each time they were refilled.

370   Fourthly, the NE birds may have simply had greater net play opportunities than E birds

371 during the tests. ,The enrichment items may have served as obstacles that impeded rapid playful

372 locomotion and interaction stimulated during the tests. This explanation may explain why E birds

373 showed consistently low running, frolicking, and all play combined in the free-space tests with

374 increasing age. It was only the NE birds that showed elevated levels of play when young, with a

375 decline in play with increasing age. If the lower play in E than NE pens resulted from a reduction

376 in useable space due to the introduction of enrichment structures, this finding suggests that, when

377 practicing environmental enrichment, a reduction in stocking density is needed to reap the full

378 benefit of the enrichment.

379   Regarding age effects, worm chasing declined with age in the worm running tests. There

380 were also declines with age in running, frolicking, and all play combined during the free-space

381 and spontaneous play observations. Other studies on fast-growing broilers have indicated that

382 time spent in walking, running, and other active behaviours declined as they grew older

383   (Newberry et al., 1986; Weeks et al., 2000; Tickle et al., 2018). Although Baxter et al. (2019) did

384   not detect differences in the levels of frolicking and sparring between 3 and 5 weeks of age,

385   Vasdal et al. (2019) found a decrease in running, worm running, and play fighting from day 16 to

386   day 30, and Dawson & Siegel (1967) reported peaks in frolicking and sparring at around 28 days

387   and 32 days, respectively, followed by a decline to negligible levels above 9 weeks.

388   It may be argued that a decline in play behaviour is a normal developmental change as an

389   animal becomes mature (Spear, 2004). However, broilers are typically slaughtered prior to

390   sexual maturity and, therefore, play could be expected to persist until slaughter (Yamada, 1999).

391   Moreover, laying pullets performed worm running behaviour at similar rates at 8–12, and 68–70,

392   days of age (Cloutier et al., 2004), contrary to the decline in worm chasing, and increase in

393   stationary worm pecking observed in this study. These changes with age were, therefore, likely

394   related to the broilers' increasing body weights, and associated increased stocking density and

395   reduced mobility (Bokkers & Koene, 2003; Liu, 2019), rather than their age alone. The relatively

396   small size of the experimental pens may have exacerbated these effects compared to observations

397   of broilers in commercial housing. In addition, necrotic enteritis was diagnosed at 29 d of age,

398   although rapidly controlled by antibiotics. Play behaviour is known to be decreased when

399   animals are ill (Ahloy-Dallaire et al. 2018). Therefore, it is possible that illness suppressed play

400   in some birds during the spontaneous play observation on day 30. The reduction in light

401   intensity to 7 lux on day 29 may have also contributed to reduced spontaneous play at this age

402   (Newberry et al., 1988; Blatchford et al., 2009). Space restriction may have been the greatest

403   limiting factor on play at higher ages given that stationary forms of play behaviour including

404   worm pecking and wing flapping increased with age.

405   Play was recorded during 5-min observations in the free-space tests and for a total of 1

406   hour in the spontaneous play context using the same methods. The level of all play behaviour

407   was 5-34 times (in NE pens) and 7-9 times (in E pens) higher (depending on test dates) in the

408   tests than during spontaneous conditions. The level of worm chasing in the worm running tests

409   was of a similar magnitude to all play behaviour in the free-space tests. These results indicate

410   that the two tests were effective in stimulating play as predicted, as well as in detecting treatment

411   differences. Therefore, these tests can be valuable for use in future research on responses in these

412   contexts. Nevertheless, it is interesting that none of the results revealed higher levels of play in

413   the E than the NE treatment, contrary to the prediction that the enriched environment would

414   stimulate more play. As described earlier, studies have indicated suppression of play under

415   adverse conditions that could be perceived as life-threatening. This was not the case in the

416   current study, as chickens grew at similar rates in both treatments (Liu, 2019) and in accordance

417   with expected growth according to the breeder manual (Aviagen, 2019). In addition, mortality

418   rates were comparable and within rates typical for the breed (Liu, 2019). The findings show the

419   importance of considering the context under which play is observed and show that the timing of

420   observations relative to stimulation of activity is critical to the interpretation of play data.

421   **4. Conclusions**

422   NE birds performed more play behaviour than E birds under worm running and free-

423   space test conditions. The NE birds might have been more responsive as a result of a higher

424   contrast between their relatively unstimulating normal environment and the test conditions (the

425   presence of "worms" and extra space), possibly exacerbated by boredom. Birds performed less

426   energetic play behaviour and became more sedentary as they aged, likely because higher body

427   weights and stocking densities reduced their mobility.

428   **Conflict of interest**

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

430

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437   **References**

438   Ahloy-Dallaire, J., Espinosa, J., & Mason, G., 2018. Play and optimal welfare: does play indicate

439   the presence of positive affective states? *Behavioural Processes*, *156*, 3–15.

440   https://doi.org/10.1016/j.beproc.2017.11.011.

441   Arelis, C. L., 2006. *Stress and the Power of Play*. Unpublished M.Sc. thesis. Lethbridge, Alberta,

442   CA: University of Lethbridge. https://hdl.handle.net/10133/342.

443   Aviagen, 2019. *Ross 708 Broiler Performance Objectives 2019*. Retrieved July 11 2019 from

444   http://en.aviagen.com/tech-center/download/1340/Ross-708-BroilerPO2019-EN.pdf.

445   Appleby, M. C., Mench, J. A., & Hughes, B. O. (2004). *Poultry Behaviour and Welfare*.

446   Wallingford, UK: CABI.

447 Bateson, P., Martin, P., & Young, M., 1981. Effects of interrupting cat mothers' lactation with

448   bromocriptine on the subsequent play of their kittens. *Physiology & Behavior*, *27*(5),

449   841-845. https://doi.org/10.1016/0031-9384(81)90051-2.

450 Baxter, M., Bailie, C. L., & O'Connell, N. E., 2019. Play behaviour, fear responses and activity

451   levels in commercial broiler chickens provided with preferred environmental enrichment.

452   *Animal*, *13*(1), 171-179. https://doi.org/10.1017/S1751731118001118.

453 BenSassi, N., Vas, J., Vasdal, G., Averós, X., Estévez, I., & Newberry, R. C., 2019. On-farm

454   broiler chicken welfare assessment using transect sampling reflects environmental inputs

455   and production outcomes. *PLoS ONE, 14*(4).

456   https://doi.org/10.1371/journal.pone.0214070.

457 Blatchford, R. A., Klasing, K. C., Shivaprasad, H. L., Wakenell, P. S., Archer, G. S., Mench, J.

458   A., 2009. The effect of light intensity on the behavior, eye and leg health, and immune

459   function of broiler chickens. *Poultry Sci., 88*(1), 20-28. https://doi.org/10.3382/ps.2008-

460   00177.

461 Bokkers, E. A. M., & Koene, P., 2003. Behaviour of fast- and slow growing broilers to 12 weeks

462   of age and the physical consequences. *Applied Animal Behaviour Science*, *81*(1), 59–72.

463   https://doi.org/10.1016/S0168-1591(02)00251-4.

464 Burghardt, G. M., 2005. *The Genesis of Animal Play: Testing the Limits*. Cambridge, MA, USA:

465   MIT Press.

466   Burn, C. C., 2017. Bestial boredom: A biological perspective on animal boredom and

467   suggestions for its scientific investigation. *Animal Behaviour, 130,* 141-151.

468   https://doi.org/10.1016/j.anbehav.2017.06.006.

469   Calcagnetti, D. J., & Schechter, M. D., 1992. Place conditioning reveals the rewarding aspect of

470   social interaction in juvenile rats. *Physiology & Behavior, 51*, 667–672.

471   https://doi.org/10.1016/0031-9384(92)90101-7.

472   Cloutier, S., Newberry, R. C., & Honda, K., 2004. Comparison of social ranks based on worm

473   running and aggressive behaviour in young domestic fowl. *Behavioural Processes*, *65*(1),

474   79–86. https://doi.org/10.1016/j.beproc.2003.07.001.

475   Coplan, R. J., Prakash, K., O'Neil, K., & Armer, M., 2004. Do you "want" to play?

476   Distinguishing between conflicted shyness and social disinterest in early childhood.

477   *Developmental Psychology*, *40*(2), 244–258. https://doi.org/10.1037/0012-1649.40.2.244.

478   Dawson, J. S., & Siegel, P. B., 1967. Behavior patterns of chickens to ten weeks of age. *Poultry*

479   *Science, 46*, 615–622. https://doi.org/10.3382/ps.0460615.

480   Diamond, J., & Bond, A., 2003. A comparative analysis of social play in birds. *Behaviour*, *140*,

481   1091-1115. https://doi.org/10.1163/156853903322589650.

482   Duncan, I. J., 1998. Behavior and behavioral needs. *Poultry Science*, *77*(12), 1766–1772.

483   https://doi.org/10.1093/ps/77.12.1766.

484   Held, S. D. E., & Špinka, M., 2011. Animal play and animal welfare. *Animal Behaviour*, *81*(5),

485   891–899. https://doi.org/10.1016/j.anbehav.2011.01.007.

486   Humphreys, A. P., & Einon, D. F., 1981. Play as a reinforcer for maze-learning in juvenile rats.

487   *Animal Behaviour, 29*, 259-270. https://doi.org/10.1016/S0003-3472(81)80173-X.

488   Jensen, M. B., & Kyhn, R., 2000. Play behaviour in group-housed dairy calves, the effect of

489   space allowance. *Applied Animal Behaviour Science, 67*(1-2), 35-46.

490   https://doi.org/10.1016/S0168-1591(99)00113-6.

491   Kaukonen E, Norring M, & Valros A., 2017. Perches and elevated platforms in commercial

492   broiler farms: use and effect on walking ability, incidence of tibial dyschondroplasia and

493   bone mineral content. *Animal, 11,* 864–871.

494   https://doi.org/10.1017/S1751731116002160.

495   Keeling, L. and Zimmerman, P., 2009. Positive emotions in poultry. pp. 103-111 in Forkman, B.,

496   & Keeling, L. (Eds.), *Assessment of Animal Welfare Measures for Layers and Broilers.*

497   Welfare Quality Reports No. 9. Cardiff, UK: Cardiff University.

498   Kruijt, J.P., 1964. Ontogeny of social behaviour in Burmese red jungle fowl (*Gallus gallus*

499   *spadiceus*). *Behaviour* 12, 1–201.

500   Lawrence, A. B., Newberry, R. C., & Špinka, M., 2018. 15 - Positive welfare: what does it add to

501   the debate over pig welfare? pp. 415-444 in Špinka, M. (Ed.), *Advances in Pig Welfare.*

502   Duxford, UK: Elsevier Woodbridge Publishing. https://doi.org/10.1016/B978-0-08-

503   101012-9.00014-9.

504   Liu, Z., 2019. *The Effects of Environmental Enrichment on the Health, Behaviour, and Welfare*

505   *of Fast-Growing Broilers.* Unpublished M.Sc. thesis. Guelph, ON, CA: University of

506   Guelph. https://atrium.lib.uoguelph.ca/xmlui/handle/10214/16985.

507   Mench, J. A., 1988. The development of aggressive behavior in male broiler chicks: A

508   comparison with laying-type males and the effects of feed restriction. *Applied Animal*

509   *Behaviour Science*, 21(3), 233-242. https://doi.org/10.1016/0168-1591(88)90112-8.

510   McGrath, P. J., Beyer, J., Cleeland, C., Eland, J., McGrath, P. A., & Portenoy, R., 1990. Report

511   of the Subcommittee on Assessment and Methodologic Issues in the Management of Pain

512   in Childhood Cancer. *Pediatrics*, *86*(5), 814–817.

513   https://pediatrics.aappublications.org/content/86/5/814.

514   McGary, S., Estevez, I., & Russek-Cohen, E., 2003. Reproductive and aggressive behavior in

515   male broiler breeders with varying fertility levels. *Applied Animal Behaviour Science*,

516   *82*(1), 29–44. https://doi.org/10.1016/S0168-1591(03)00038-8.

517   Meagher, R. K., & Mason, G. J., 2012. Environmental enrichment reduces signs of boredom in

518   caged mink. *PLoS ONE*, *7*(11). https://doi.org/10.1371/journal.pone.0049180.

519   Millman, S. T., Duncan, I. J. & Widowski, T. M., 2000. Male broiler breeder fowl display high

520   levels of aggression toward females. *Poultry Science, 79*(9), 1233–1241.

521   https://dx.doi.org/10.1093/ps/79.9.1233.

522   Morley-Fletcher, S., Rea, M., Maccari, S., & Laviola, G., 2003. Environmental enrichment

523   during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis

524   reactivity in rats. *European Journal of Neuroscience, 18*, 3367–3374.

525   https://doi.org/10.1046/j.14609568.2003.03070.x.

526   Newberry, R. C., 1999. Exploratory behaviour of young domestic fowl. *Applied Animal*

527   *Behaviour Science, 63*(4), 311-321. https://doi.org/10.1016/S0168-1591(99)00016-7.

528   Newberry, R. C., Hunt, J. R., & Gardiner, E. E., 1986. Light intensity effects on performance,

529   activity, leg disorders, and sudden death syndrome of roaster chickens. *Poultry Science*,

530   *65*(12), 2232–2238. https://doi.org/10.3382/ps.0652232.

531   Newberry, R. C., Hunt, J. R., & Gardiner, E. E., 1988. Influence of light intensity on behavior

532   and performance of broiler chickens. *Poultry Science*, *67*(7), 1020–1025.

533   https://doi.org/10.3382/ps.0671020.

534   Newberry, R. C., Vas, J., BenSassi, N., Goold, C., Vasdal, G., Averós, X., & Estévez, I., 2018.

535   Chickens play in the wake of humans. P. 199 in Cochram, M., Tennessen, T., Bate, L.,

536   Bergeron, R., Cloutier, S., Fisher, A. D. & Hötzel, M. (Eds.), *ISAE 2018 Proceedings of*

537   *the 52nd Congress of the International Society for Applied Ethology.* Wageningen, NL:

538   Wageningen Academic Publishers. https://doi.org/10.3920/978-90-8686-870-4.

539   Nicol, C. J., 2015. *The Behavioural Biology of Chickens*. Wallingford, UK: CABI., p. 95.

540   Pettit-Riley, R., Estevez, I., & Russek-Cohen, E., 2002. Effects of crowding and access to

541   perches on aggressive behaviour in broilers. *Applied Animal Behaviour Science*, *79*(1),

542   11–25. https://doi.org/10.1016/S0168-1591(02)00116-8.

543 Pedersen, I. J., & Forkman, B., 2019. Improving leg health in broiler chickens: a systematic

544   review of the effect of environmental enrichment. *Animal Welfare, 28*(2), 215-230.

545   https://doi.org/10.7120/09627286.28.2.215.

546 Riber, A. B., van de Weerd, H. A., de Jong, I. C., & Steenfeldt, S., 2018. Review of

547   environmental enrichment for broiler chickens. *Poultry Science*, *97*(2), 378–396.

548   https://doi.org/10.3382/ps/pex344.

549 Rushen, J., 1982. Development of social behaviour in chickens: A factor analysis. *Behavioural*

550   *Processes*, *7*(4), 319–333. https://doi.org/10.1016/0376-6357(82)90004-3.

551 Rushen, J., & de Passillé, A. M., 2014. Locomotor play of veal calves in an arena: are effects of

552   feed level and spatial restriction mediated by responses to novelty? *Applied Animal*

553   *Behaviour Science*, *155*, 34–41. https://doi.org/10.1016/j.applanim.2014.03.009.

554 Spear, L. P., 2004. Adolescent brain development and animal models. *Annals of the New York*

555   *Academy of Sciences*, *1021*, 23–26. https://doi.org/10.1196/annals.1308.002

556 Špinka, M., Newberry, R. C., & Bekoff, M., 2001. Mammalian play: training for the unexpected.

557   *The Quarterly Review of Biology*, *76*(2), 141–168. https://doi.org/10.1086/393866.

558 Stolba, A., & Wood-Gush, D. G. M., 1980. Arousal and exploration in growing pigs in different

559   environments. *Applied Animal Ethology*, *6*(4), 382–383. https://doi.org/10.1016/0304-

560   3762(80)90140-6.

561 Thornton, P. D. & Waterman-Pearson, A. E., 2002. Behavioural responses to castration in lambs.

562   *Animal Welfare*, *11*, 203-212.

563   Tickle, P. G., Hutchinson, J. R., & Codd, J. R., 2018. Energy allocation and behaviour in the

564   growing broiler chicken. *Scientific Reports*, *8*(1), 4562. https://doi.org/10.1038/s41598-

565   018-22604-2.

566   Vasdal, G., Vas, J., Newberry, R. C., & Moe, R. O., 2019. Effects of environmental enrichment

567   on activity and lameness in commercial broiler production. *Journal of Applied Animal*

568   *Welfare Science*, *22*(2), 197-205. https://doi.org/10.1080/10888705.2018.1456339.

569   Vinke, C., van Leeuwen, J., & Spruijt, B., 2005. Juvenile farmed mink (*Mustela vison*) with

570   additional access to swimming water play more frequently than animals housed with a

571   cylinder and platform, but without swimming water. *Animal Welfare*, *14*(1), 53-50.

572   Weeks, C.A., Danbury, T.D., Davies, H.C., Hunt, P., & Kestin, S.C., 2000. The behaviour of

573   broiler chickens and its modification by lameness. *Applied Animal Behaviour Science*,

574   *67*(1–2), 111–125. https://doi.org/10.1016/S0168-1591(99)00102-1.

575   Wood-Gush, D. G. M., 1971. *The Behaviour of the Domestic Fowl*. London, UK: Heinemann

576   Educational Books LTD, p. 49.

577   Yamada, Y., 1999. Quantitative and qualitative differences between adult and juvenile agonistic

578   behavior. *Journal of Ethology*, *17*(2), 63–71. https://doi.org/10.1007/BF02769299.

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583 **Table 1.** Ethogram for worm running tests. Worm pecking, worm running, and worm chasing
584 were mutually exclusive, and each bird could only be assigned to one behaviour per 10-s scan.
585 Worm exchanges were recorded as a total number per 5 min. Definitions adapted from Cloutier
586 et al. (2004).

587

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.

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591 **Table 2.** Ethogram for free-space tests and spontaneous play. All behaviour patterns were
592 mutually exclusive. The start of one behaviour indicated the end of the previous behaviour.
593 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.

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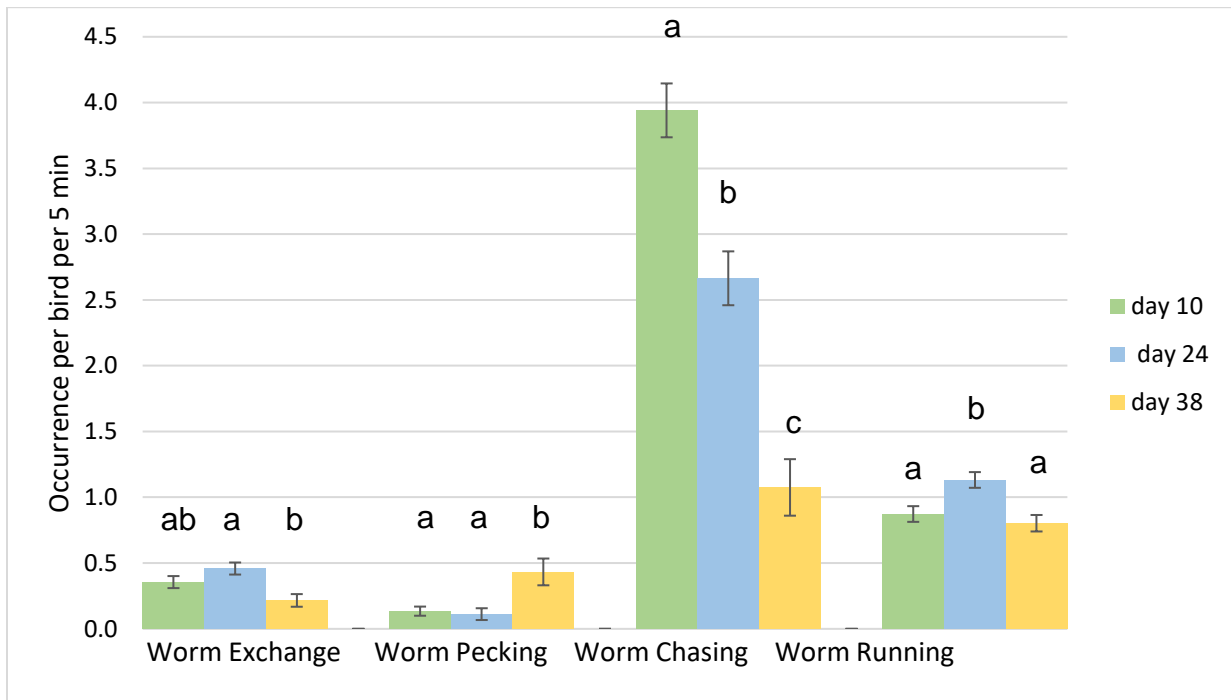601 was present only during the first week in all pens.

602
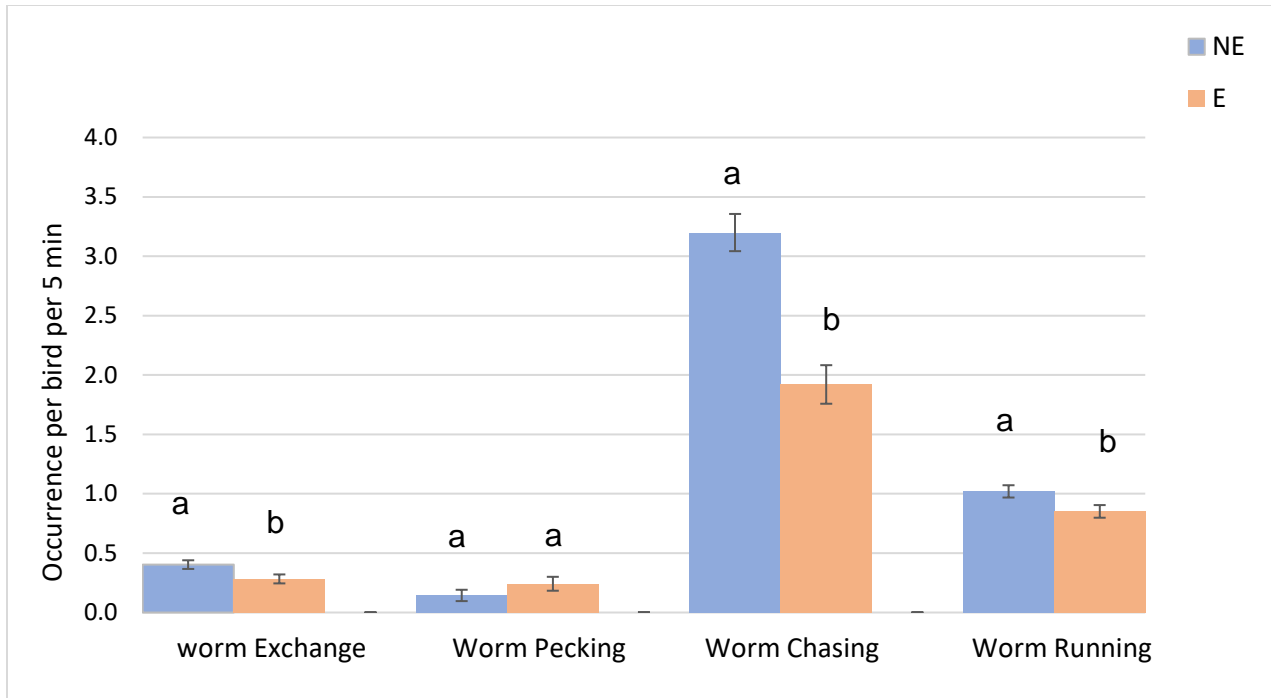
Figure 2. Least squares mean occurrence (±SE) of worm exchange, worm pecking, worm chasing, and worm running per bird per 5 min during worm running tests in non-enriched (NE) and enriched (E) pens on days 10, 24, and 38. Means with the same letters within each behaviour are not different (P≥0.05).

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Figure 3. Least squares mean occurrence (±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≥0.05).

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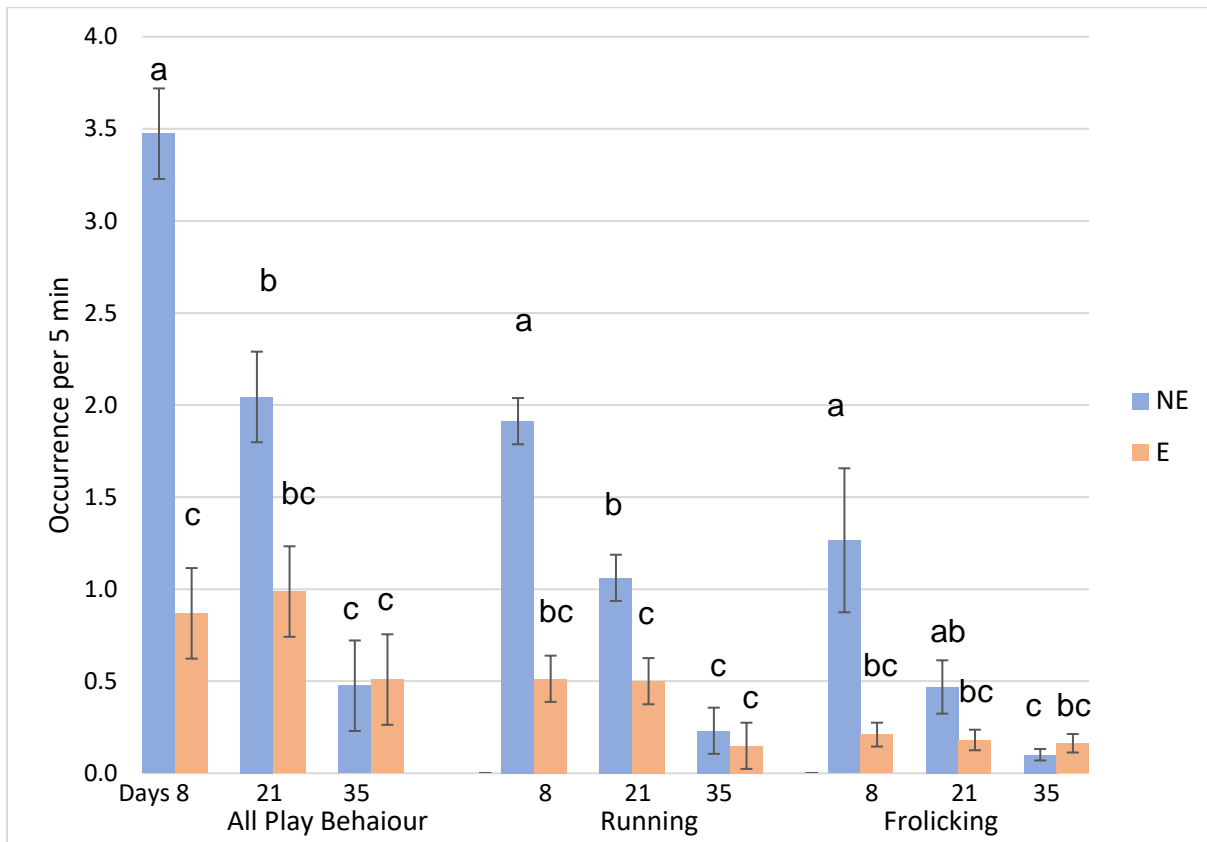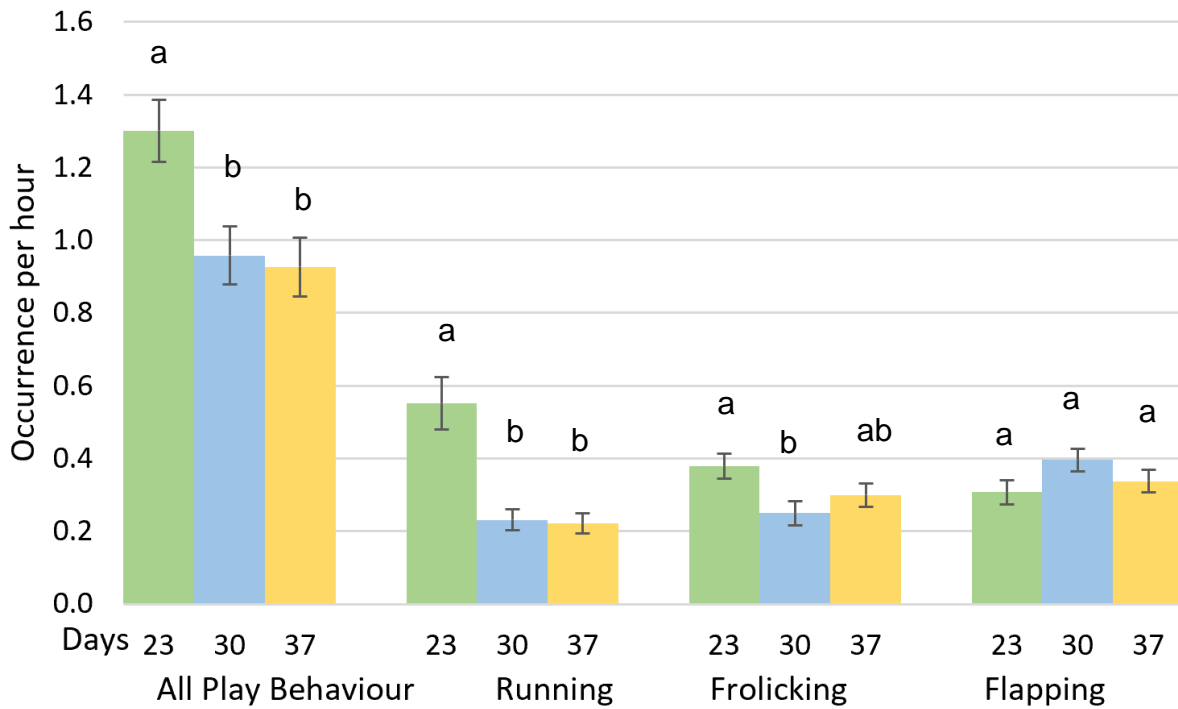618

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Figure 4. Least squares mean occurrence per bird per 5 min (±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≥0.05).

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629 **Figure 5.** Least squares mean occurrence per bird per hour (±SE) of all play behaviour (sum of
630 running, frolicking, wing flapping, and sparring), running, frolicking, and wing flapping on days
631 23, 30, and 37 during spontaneous play observations. Means with the same letters within each
632 behaviour are not different (P≥0.05).

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