Are changes in behavior of fast-growing broilers with slight gait impairment (GS0-2) related to pain?

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ABSTRACT Impaired walking ability in terms of slight or definite defects is more common in broiler production than lameness that obviously hinders movement, but it has received limited scientific attention. This study aimed to compare behavior of conventional broilers with impaired walking ability (assessed as gait score (**GS**) 2) with those walking normally (GS0) and those with only a slight gait defect (GS1). Behavior in the home environment was registered, and an analgesic intervention to quantify changes in time budgets indicating pain relief was applied. The study included 192 Ross 308 broilers. On day 27 of age, the birds were distributed as evenly as possible into birds of GS0 and GS2 of each sex based on obtained gait score. Following this, each experimental bird was housed with 3 companion birds. On days 30 and 32 of age, the behavior in the home pens was recorded. All experimental birds were injected with the NSAID carprofen on one of the 2 d and saline on the other. The statistical analyses used the GS scored on the day of recording as explanatory factor. Compared to GS0 birds, GS2 birds tended to be more inactive (mean (CI): 4,193 (3,971–4416) vs. 4,005 (3,753–4,257) s; $P = 0.074$), spent more time sitting while feeding (306 (266–353) vs. 213 (180–251) s; $P = 0.026$), were less likely to perch (probability: 0.78 (0.69–0.85) vs. 0.91 (0.85–0.95); $P = 0.012$), and spent less time performing comfort behavior (749 (689–814) s vs. 875 (792–967) s; $P = 0.043$). Compared to GS1 birds, GS2 birds spent more time inactive (GS1: 4,022 (3,818–4225) s; $P = 0.027$), less time foraging (289 (253–329) vs. 347 (309–388) s; $P = 0.047$), and were less likely to perch (GS1: 0.90 (0.86–0.93); $P = 0.001$). For some of these behavioral variables, administration of carprofen led to behavioral changes across the GSs, which may suggest that the behavioral expression of the broilers was limited by pain. These findings are of relevance to animal welfare, but the underlying causes are still not fully clarified.

Key words: behavior, broiler, gait, pain, walking impairment

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INTRODUCTION

Impaired walking ability, varying from slight changes in gait to obvious lameness or even lack of mobility, is common and constitutes a significant welfare challenge in broiler production (e.g., Knowles et al., 2008; Kittelsen et al., 2017; Louton et al., 2018). A recent survey of Danish conventional broiler production showed a prevalence of 77% of birds showing signs of impaired walking ability, mainly due to a high proportion of birds with gait scores (**GS**) 1 and 2 (Tahamtani et al., 2018). The gait of birds assessed with GS1 and GS2 has a slight or definite defect, respectively, but does not appear to hinder movement (Kestin et al., 1992). In the present study, these birds are referred to as having impaired walking abilities, whereas those with GS3-5 are considered lame.

Potential links between GS and different aspects of broiler welfare have been studied. However, most experiments have compared lame broilers (GS $\geq$ 3) with broilers assessed as GS0 (e.g., McGeown et al., 1999; Hothersall et al., 2016; Aydin, 2017) and thus often excluded birds of GS1 and GS2 as being

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intermediate scores. As an exception, Skinner-Noble and Teeter (2009) compared different aspects of the welfare of broilers assessed as GS2 vs. GS3, but to our knowledge, no studies have examined and compared characteristics of birds specifically with GS0–GS2.

Broiler behavior is an aspect of animal welfare that has been studied across GS categories. Weeks et al. (2000) included birds of GS0–GS3 and observed their behavior during the last week before slaughter (around 49 d of age at that time). The aforementioned authors did not perform pairwise comparisons between GSs, but found that higher GS led to increased time spent lying, more time lying with one leg stretched, and less time preening (which is part of comfort behavior). No differences were found for time spent feeding, but increasing GS was associated with a larger proportion of sitting while feeding rather than standing. The authors concluded that while some of the alterations in the time budgets may have been directly linked to intensive genetic selection for faster growth rate and improved feed conversion efficiency, others were more likely to be a consequence of altered physiology and morphology rather than altered motivation. Recently, Norring et al. (2018) confirmed the finding of birds with higher GS to have longer lying time.

Previous studies have shown a relationship between GS and indicators of pain. In a test of locomotor ability, broilers assessed as GS3 took longer to transverse an obstacle course compared to GS0 broilers, but this difference disappeared if the lame birds were administered carprofen, a nonsteroidal anti-inflammatory drug (**NSAID**) (McGeown et al., 1999). Furthermore, another study comparing broilers assessed as GS2–GS4 vs. GS0–GS1 found that the former group showed decreased latency to lie in a motivational test and that administration of NSAIDs (carprofen and meloxicam) increased this latency (Hothersall et al., 2016). These results suggest that pain can be a factor in the impaired walking ability: impaired birds experienced pain relief from the drugs, which improved their performance in the mobility tests. This commonly used experimental setup to assess the involvement of pain (see more in Weary et al. (2017)) has until now not been used to investigate the possible relationship between impaired walking ability and pain in broilers when kept in their home environment.

The aim of the present study was to examine potential links between GS $\leq$ 2 and behavior of conventional Ross 308 broilers in their home environment. Behavior was registered and an analgesic intervention (administration of carprofen) to quantify changes in time budgets indicating pain relief was applied to facilitate inference about possible pain as a potential underlying affective state. This study was part of a larger experiment, where potential relations between GS $\leq$ 2 and indicators of locomotor ability and pain (Tahamtani et al., 2021) as well as morphology and pathology (Riber et al., unpublished data) were also investigated.

MATERIALS AND METHODS

Ethical statement

Following the Danish legislation, all experimental procedures were approved by the Danish Animal Experiments Inspectorate (Permit No. 2018-15-0201-01,434). The study was conducted in accordance to Danish legislation BEK No. 1047 from 13/08/2018 and the EU Directive 2010/63/EU.

Animals and housing

The study was performed in 4 blocks during the period from April to October 2018 and involved 3 main factors: GS (0–2), sex (male or female), and injection solution (carprofen or saline). Each of the 4 blocks consisted of 300 male and 300 female Ross 308 broilers acquired as day-old chicks from a commercial hatchery (DanHatch A/S, Sønderborg, DK) and wing-tagged with unique IDs on arrival to the experimental facilities at Aarhus University, AU-Foulum, Tjele, Denmark. On days 0 and 1 of age, the light schedule was programmed for 24 h of light. Subsequently, every day, 2 h of darkness were added until 18 h of light and 6 h of darkness (light period: 4.30–22.30) was reached on day 5 of age and maintained until the end of the experiment. The light intensity was 29.5 lx. The feed and feeding program used were recommended by the commercial feed company DLG (Tjele, DK).

Within each block, all chickens were reared together until day 27 of age in a pen measuring 4 m $\times$ 9 m. Commercial conditions were simulated by keeping the stocking density at an estimated 40 kg/m^2 on day 34 of age. Feed was available for *ad libitum* intake from round feeders (1.8 cm of feeder space per bird) and *ad libitum* access to water was provided with 10 birds per drinking nipple. Three wooden perches were present from day 0. The perches measured 3.8 cm $\times$ 5.7 cm $\times$ 400 cm (H $\times$ W $\times$ L) and were mounted 10.5 cm above the floor bedding.

At 27 d of age, all 600 birds within one block were individually weighed and gait scored according to the Bristol scale (Kestin et al., 1992) by 2 experienced observers. In this scale, walking ability is scored as one of 6 categories (0–5), from completely normal to immobile. Chickens are scored as 0, 1, or 2 if the gait (manner of walking) has no, a slight, or definite defect, respectively, but movement does not seem to be hindered. Score 3 is given when the gait defect affects the maneuvering and acceleration ability of the birds. Birds that only walk a couple of steps when encouraged by the assessor, and those unable to stand or walk at all, are scored as 4 and 5, respectively. Gait scoring was performed in the home pen. During scoring, the perches were removed and the observer encouraged each bird to walk by approaching the bird and, if necessary, with a gentle touch by use of a stick, if the bird showed unwillingness to walk when reached by the observer. Prior to commencement of the study, the 2 observers had gait scored more than 4,000 broilers each. Furthermore, they had refreshed their gait scoring skills by observing videos of birds with known GSs and by gait scoring 100 broilers together, discussing the gait of each bird for agreement to be attained. Each of the 36 video examples was scored on 3 separate occasions by each observer, approximately a week apart. Based on this, the observers had substantial levels of interobserver agreement (kappa value 0.70) and from substantial to almost perfect levels of intraobserver agreement (kappa values 0.77 and 0.90) (Landis and Koch, 1977).

Based on the gait scoring, 24 GS0 and 24 GS2 birds were selected in the following manner. The broilers of each GS were separated into males and females using the wing tag ID attached as day-old chicks. The 10% lightest and 10% heaviest birds of each group were excluded from selection, to reduce variation in body weight to avoid the inclusion of extreme individuals. From the remaining birds in each group, 12 experimental birds were randomly selected. This procedure could only be followed if the group consisted of more than 12 birds when the selection started; if a group consisted of only 12 or fewer birds, then all of them were included as experimental birds. Any group with fewer than 12 individuals was supplemented with birds having the same GS, but the opposite sex. Using this approach, 48 experimental birds were selected in each of the 4 blocks with an equal number of GS0 and GS2 and of each sex, when possible. Each experimental bird, marked on the back with blue coloring spray for identification, was housed with 3 companion GS1 birds. The GS1 companion birds to go into each pen were selected by convenience, however, ensuring that the sex ratio in each experimental pen was balanced, so that each pen always housed 2 female and 2 male birds.

The experimental pens used from day 27 were placed in a room adjacent to the room of rearing. The room contained 48 permanent experimental pens, predefining the limit to 48 experimental birds per block. Each pen measured 1 m × 1.65 m, provided 4 drinking nipples and one round feeder of 38 cm in diameter. Feed and water were available for *ad libitum* intake. A perch of 3.8 cm × 5.7 cm × 100 cm (H × W × L) was present in each pen and was mounted 10.5 cm above the floor bedding. All experimental and companion birds were housed in these experimental pens until they were humanely killed by CO_2 gassing at day 38 of age.

Data collection

Observation of behavior in the home environment was performed on days 30 and 32 of age. Between 8.00 and 9.15 in the morning on each of these days, all experimental birds were gait-scored in the home pen, as described previously, and weighed. Then, half of them were administered carprofen (Norodyl Vet., Scanvet A/S, Fredensborg, Denmark) at a dosage of 25 mg/kg, subcutaneously in the neck, and the other half with a corresponding volume of saline (NaCl, 0.9%, B. Braun, Melsungen, Germany). The allocation of carprofen or saline was balanced across GSs as assessed at 27 days of age (GS0 or GS2) and across sex without taking into account the GS on the days of observation (Figure 1). Birds that were administered carprofen on day 30 then received saline on day 32 and vice versa. The exact time of administration of the injection solution was noted for each experimental bird. The dosage of 25 mg carprofen/kg was chosen based on previous research, showing that this dosage has effects on the gait characteristics of lame broilers (Caplen et al., 2013a). Furthermore, Caplen et al. (2013b) reported evidence for the effect of carprofen (15 and 25 mg/kg) on nociceptive thermal threshold in broilers with experimentally induced articular pain. The injections were performed by an animal technician (holding a FELASA category B certificate) that had been trained on beforehand and supervised during the experiment by the inspecting veterinarian. After all injections, all personnel exited the room, leaving the birds undisturbed in their home pens. Infrared cameras (CCTV Camera, D1325, Dahua Technology, Hangzhou, China) were fitted above the home pens, allowing a full view of 2 adjacent pens. Recordings of the behavior of the broilers were performed for 24 h.

Three observers, all trained in assessing broiler behavior but blinded to the treatment of the experimental birds (i.e., GS, sex, and injection solution), performed focal sampling using continuous recording (Altmann, 1974) of each experimental bird in 3 periods of 2 h: 1) before administration of injection solution (time of day: 4.30 to 6.30); 2) during expected peak effect of administration of the injection solution (3.5–5.5 h post injections); and 3) approximately 12 h post administration of injection solution (time of day: 20.30–22.30). The injection solutions were administered between 8.00 and 9.15; that is, observations for period 2 would start between 11.30 and 12.45 and end 2 h later. The decision on this timing was based on the knowledge that plasma concentrations of carprofen peak at 3 hours (and stay at this level until 6 h) following subcutaneous injections (Hothersall et al., 2012). During their observations, the observers noted behavior of the birds following the ethogram presented in Table 1. In addition, the observers made notes on the position of the birds, that is, on the litter or perch.

Statistical analysis

Statistical analyses were performed using the software R (version 3.6.3). The study was designed to allocate broilers to treatment groups according to their gait on day 27. It was expected that their gait would worsen with age. Indeed this happened, for example, with several GS0 broilers scoring as GS1 in the days following 27 d of age. However, several broilers also showed improved gait in relation to previous scores. As a result, while the mean GS from days 27–38 was significantly different between the groups ($F_{1,186}$ = 288.6; P < 0.0001; GS0 estimated marginal means (CI) = 0.80 (0.66–0.94); GS2 estimated marginal means

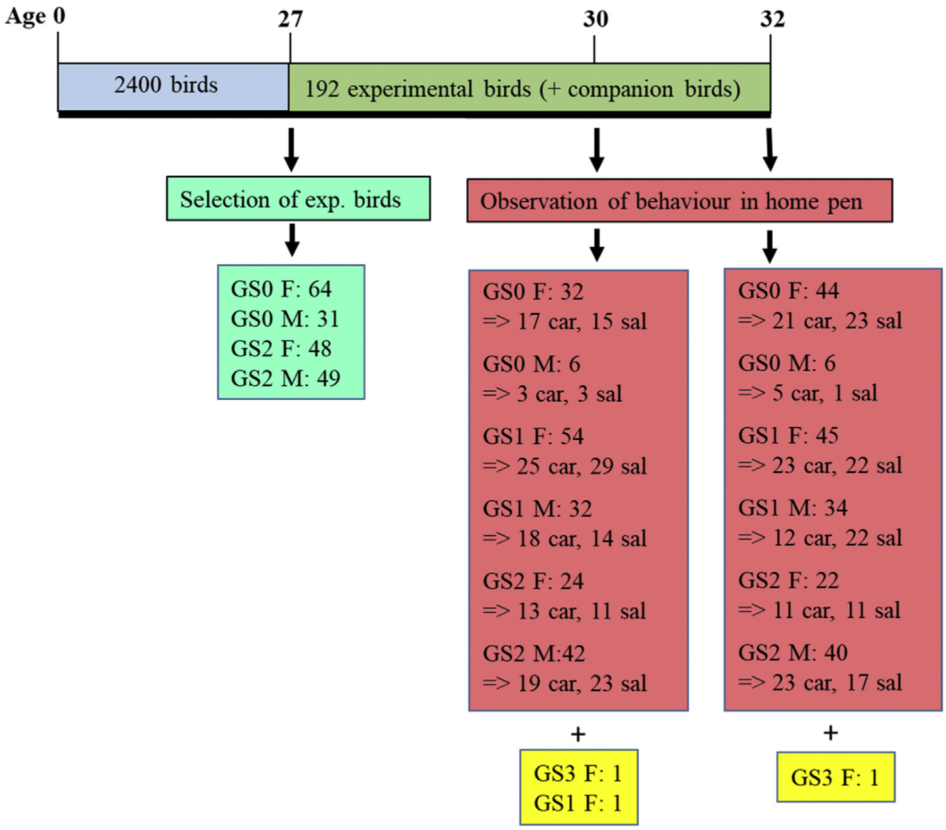

Figure 1. Flow chart of 1) the distribution of GS and sex of the 192 experimental birds (48 per block) selected from the 2400 birds (600 per block) on day 27 and 2) the distribution of GS, sex, and injection solution (carprofen/saline) of the experimental birds on days 30 and 32, respectively, when the behavior was observed in the home pens. One female was excluded from analysis due to a development into GS3 and data were lost for one female on day 30, resulting in 190 experimental birds on day 30 and 191 experimental birds on day 32.

(CI) = 1.62 (1.48–1.76)), the groups did not truly represent GS0 and GS2, mainly due to the large number of GS1 birds in both groups. Therefore, the statistical analysis of the behavioral data used the actual GS for each bird scored on days 30 and 32, and GS1 birds were included in their own category.

The following behaviors were analyzed: inactive behavior (i.e., time spent sitting or lying), sitting while feeding, perching, foraging, comfort behavior, locomotion, and dustbathing. Due to the large number of zeros for some of these behaviors (i.e., observation periods without any observation of a particular behavior), the statistical analysis in that case aimed to answer 2 questions: Firstly, is one experimental group of broilers more likely to perform a specific behavior? Secondly, when a specific behavior is performed, do the experimental groups differ in the time spent performing it? To answer the first question, the repeated measures were analyzed by a separate mixed effects logistic regression for the derived dichotomous 0/1 variable (0 if the duration is zero, 1 if it is > 0). The second question was answered using a generalized linear (mixed) model with Gamma distribution (and log link function) for the strictly positive durations (i.e., only those observation periods where the specific behaviors were performed for more than 0 s). The model, therefore, presents the estimated probability of a behavior being performed and the estimated mean duration of the performance of the behavior when the behavior was performed. Results are presented as estimates from the Gamma part after back-transformation with the natural exponential function and for the binomial part after application of the inverse-logit function; that is, $f(x) = \exp(x)/(1+\exp(x))$, which will return the estimated probability of a strictly positive (>0) duration.

For inactive behavior, a normal distribution could be assumed and a linear mixed model was used. Finally, locomotion and comfort behavior only had 2 zero records within the 2-h windows of observation, and the Gamma model described above was applied after addition of 1 to the 2 zero observations. Therefore, the results for inactive, locomotion and comfort behavior are presented as performance duration only, whereas the analysis of probabilities of performing the different behaviors was performed in addition to the analysis of duration for sitting while feeding, perching, foraging, and dustbathing.

The models included the fixed factors GS, sex, injection solution, age, period of the day, block, and if statistically significant, their interactions. Moreover, random effect of bird ID was included to account for correlation between repeated measures of the behavioral observations. Due to the large number of fixed effects and relative low sample size for each experimental group, a forward inclusion procedure for model building was followed to avoid false positives in higher-order

Table 1. Ethogram used for scoring of behavior of broilers while in the home pen.

Behavior	Description
Lying	The bird's body is resting on the floor with at least one leg stretched to the side.
Sitting	The bird's body is resting on the floor with both legs under the body while not engaged in other activities.
Standing	The bird is upright, both legs stretched, maintaining the body elevated from the floor while not engaged in other activities.
Locomotion	Horizontal or vertical movement of body, such as running, walking, jumping, and hopping without performing any other type of behavior.
Comfort behavior	Preening (manipulating own plumage with the beak), wing flapping, stretching legs or wings, feather ruffling/shaking (outside the context of dustbathing). Includes the pauses between each of the described elements of comfort behavior (= bouts).
Dustbathing	Rubbing the head and body against the ground, raking the bill on the ground, vertical wing shaking, pecking and scratching the ground with beak or body while lying on the side, shaking off dirt from the plumage. Includes pauses between the described dustbathing elements (= bouts).
Feather pecking	Striking or pulling, with the beak, the feathers of another individual. Includes the pauses between each peck (=bouts), may involve following the recipient bird.
Aggressive behavior	Hopping toward another bird, frontal threatening (the 2 birds have an upright position toward each other). Leaping toward another bird (= hopping on the place), may involve kicking, wing-flapping, and aggressive pecking (generally directed toward the head of another bird). Includes the pauses between each of the described elements (= bouts).
Submissive behavior	Avoidance response to aggressive behavior. Submissive bird moves away from aggressor and/or squats (stands with head low and wings partially open). Includes the pauses between each of the described elements (= bouts).
Escape behavior	Running from frightening stimuli, standing alert, squatting, and freezing.
Explorative behavior	Striking, with the beak, at the walls or perch. Includes the pauses between each peck (= bouts).
Foraging	Striking (with the beak) or scratching (i.e., using feet or toes to move the litter) on the floor. Includes the pauses between each peck (= bouts).
Feeding	Having the head in/striking with the beak at feed in the feeder. Includes the pauses between swallows (= bouts).
Drinking	Having the beak in touch with the drinker. Includes the pauses between sips (= bouts).

interactions. Nevertheless, the final model was forced to include all main effects for the fixed factors, statistically significant or not. *Post hoc* pairwise comparisons were performed between categories for any significant factors with Tukey adjustment for multiple testing for factors with three categories or more. The significance level (alpha) used was 0.05. Results for the fixed effects are presented as χ^2 likelihood ratio test (LRT), *P*-values, estimated marginal means, and 95% confidence intervals. However, results on interactions between fixed factors are only presented when significant.

Body weight was analyzed using a linear mixed effects model including as fixed effects the factors GS, sex, age, and the statistically significant two-way interactions GS by sex and sex by age. The analysis was adjusted for block as a fixed effect and included random effect of bird ID to account for birds being in experiment at the same time (block) and the correlation between the 2 weights of the same bird on days 30 and 32, respectively. In addition, the model allowed for variance heterogeneity in GS, sex, and age. Results are presented as estimated marginal means with 95% confidence intervals.

RESULTS

Inactive behavior was performed by 100% of the birds in each observation period. Correspondingly, 86% performed sitting while feeding, 79% performed perching, 96% performed foraging, 100% performed comfort behavior (a variable combining different preening, stretching, flapping, and rustling behaviors (Table 1)), 100% performed locomotion, and 14% performed dustbathing behavior.

Effects of GS on probability of performing different behaviors

An effect of GS was found on the probability of perching (LRT χ^2 = 13.1, df = 2; *P* = 0.001). GS2 birds were less likely to perch (probability (95% CI): 0.78 (0.69–0.85) compared to both GS1 (0.90 (0.86–0.93); *P* = 0.001) and GS0 (0.91 (0.85–0.95); *P* = 0.012). No effects of GS were found on the probability of the other behaviors (sitting while feeding (GS0: 0.94 (0.89–0.97), GS1: 0.93 (0.90–0.96), GS2: 0.95 (0.91–0.97)); foraging (GS0: 0.98 (0.95–0.99), GS1: 0.98 (0.96–0.99), GS2: 0.98 (0.95–0.99)), dustbathing (GS0: 0.10 (0.06–0.15), GS1: 0.14 (0.11–0.18), GS2: 0.11 (0.08–0.15)).

Effects of injection solution on probability of performing different behaviors

An effect of injection solution was found on the probability of sitting while feeding (LRT χ^2 = 8.40, df = 1; *P* = 0.004). Birds injected with carprofen had a lower estimated probability of sitting while feeding compared to those injected with saline (0.92 (0.88–0.95) v. 0.96 (0.93–0.97); *P* = 0.004). No effect of carprofen was found on the probability of perching (carprofen (0.88 (0.84–0.92), saline: 0.86 (0.81–0.90)) and foraging (carprofen: 0.98 (0.97–0.99), saline: 0.98 (0.96–0.99)). An effect of the interaction between age and injection solution was found on the probability of dustbathing (LRT χ^2 = 5.65; df = 1; *P* = 0.017) where birds injected with carprofen (0.15 (0.11–0.21)) tended to be more likely to dustbathe than those injected with saline (0.09 (0.06–0.13)) on day 30 (*P* = 0.066). The corresponding probabilities on day 32 were 0.10 (0.07–0.14) and 0.13 (0.09–0.18) for birds administered carprofen and saline, respectively. Any other effects of this interaction were lost during post hoc analysis due to the large number of comparisons.

Effects of sex on probability of performing different behaviors

No effect of sex was found on the probability of perching (F: 0.89 (0.84–0.93), M: 0.86 (0.78–0.91)), foraging (F: 0.99 (0.97–0.99), M: 0.98 (0.94–0.99)), and dustbathing (F: 0.12 (0.09–0.16), M: 0.11 (0.08–0.15). An interaction between sex and age was found for the probability of sitting while feeding (LRT $\chi^2 = 5.26$, df = 1; $P = 0.022$). Males were more likely to sit while feeding than females both on days 30 and 32 (F-age30: 0.88 (0.82–0.92), F-age32: 0.86 (0.80–0.91); M-age30: 0.96 (0.92–0.98), M-age32: 0.98 (0.96–0.99); $P < 0.014$ and $P < 0.0001$, respectively). Within sex, no difference was found between days.

Effects of period of day on probability of performing different behaviors

No effect of period of day was found on perching (P1: 0.86 (0.80–0.90), P2: 0.88 (0.83–0.92), P3: 0.88 (0.83–0.92)), foraging (P1: 0.98 (0.96–0.99), P2: 0.98 (0.96–0.99), P3: 0.98 (0.96–0.99)), and dustbathing (P1: 0.10 (0.07–0.13), P2: 0.13 (0.10–0.18), P3: 0.11 (0.08–0.15)). There was an interaction between period of day and age for the probability of sitting while feeding (LRT $\chi^2 = 7.27$, df = 2; $P = 0.026$). However, none of the post hoc pairwise comparisons were statistically significant after Tukey correction for multiple testing (P1: 30: 0.92 (0.86–0.95), 32: 0.97 (0.93–0.98); P2: 30: 0.92 (0.86–0.95), 32: 0.95 (0.91–0.98); P3: 30: 0.95 (0.90–0.97), 32: 0.93 (0.87–0.96)).

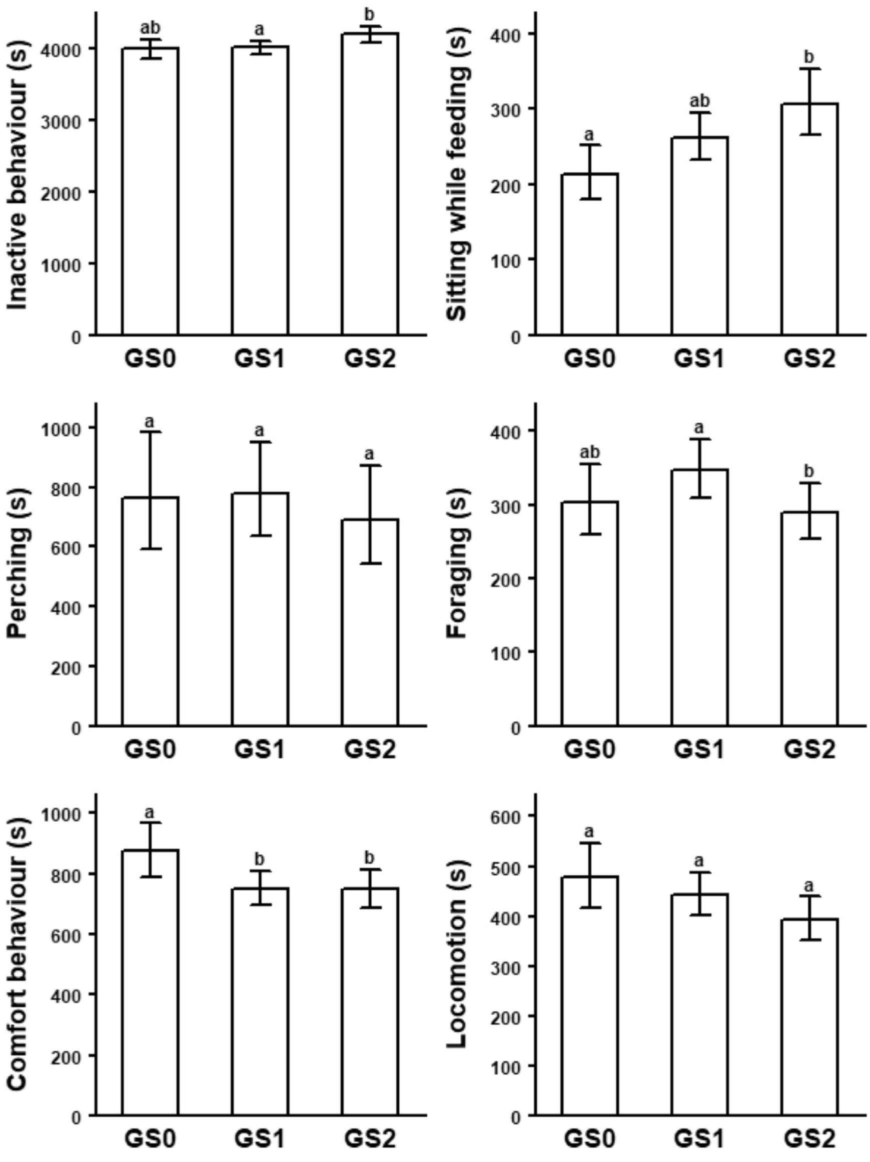

Figure 2. Duration of behaviors (back-transformed estimated marginal means (s) and 95% CI) performed by GS0, GS1, and GS2 birds, respectively. Letters indicate statistical significance.

Effects of age on probability of performing different behaviors

Age had no effect on any of the behaviors with the exception of the interactions including age mentioned earlier.

Effects of GS on time spent on different behaviors

The time spent on the different behaviors according to the GS is shown in Figure 2. An effect of GS was found on time spent inactive (LRT χ^2 = 8.06, df = 2; P = 0.018), sitting while feeding (LRT χ^2 = 10.7, df = 2; P = 0.005), foraging (LRT χ^2 = 7.19, df = 2; P = 0.027), and comfort behavior (LRT χ^2 = 9.01, df = 2; P = 0.011). GS2 birds spent more time inactive than GS1 (P = 0.022) and tended to be more inactive than GS0 (P = 0.055), whereas GS0 and GS1 birds did not differ. Furthermore, GS0 birds tended to spend less time sitting while feeding compared to GS1 birds (P = 0.051) and significantly less than GS2 birds (P = 0.026). Birds with GS2 spent less time foraging compared to GS1 (P = 0.047) but did not differ from GS0. GS0 birds performed more comfort behavior than GS1 (P = 0.009) and GS2 (P = 0.043) birds. There was a tendency for an effect of GS on time spent on locomotion (LRT χ^2 = 5.07, df = 2; P = 0.079) with GS2 birds tending to spent less time on locomotion than GS0 birds (P = 0.070). No effect of GS was found on time spent perching. A significant effect was found of the interaction between GS and period of day in the analysis of time spent dustbathing (Table 2; LRT χ^2 = 11.8, df = 4; P = 0.019) where GS0 birds spent more time dustbathing in the morning compared to GS1 birds (P = 0.036). The durations for the other combinations of GS and period of day did not differ from these or each other, but the time spent dustbathing by GS0 birds was generally higher compared to the other GSs (except for GS1 in P3), though not significantly.

Effects of injection solution on time spent on different behaviors

The time spent on the different behaviors according to the injection solution administered is shown in Figure 3. An effect of injection solution was found on time spent inactive (LRT χ^2 = 5.66, df = 1; P = 0.017) and locomotion (LRT χ^2 = 4.12, df = 1; P = 0.042). Birds were less inactive and spent more time on locomotion after injection of carprofen compared to those injected with saline. No effect of injection solution was found on time spent sitting while feeding, perching, and foraging. Injection solution was included in 2 significant interactions; an effect of the interaction between injection solution and age was found for time spent performing comfort behavior (Table 2; LRT χ^2 = 4.69, df = 1; P = 0.030), but none of the post hoc comparisons were significant after adjustment for multiple comparisons. Furthermore, an interaction between injection solution and sex was found to be significant for time spent dustbathing (Table 2; LRT χ^2 = 7.99, df = 1; P = 0.005) where males injected with carprofen spent less time dustbathing compared to males injected with saline (P = 0.027), while the effect of injection solution was in the opposite direction for females though not significantly.

Table 2. Duration of behaviors (back-transformed estimated marginal means (s) and 95% CI) for which significant interaction between explanatory variables was found. Data were analyzed with a Gamma model, including only data from birds that performed the behavior during the observation periods.

Behavior & explanatory variable	Level	Duration (s)	95-% CI (s)
Comfort behavior	30–Carprofen	844[a]	771–924
Age * Drug[1]	32–Carprofen	737[a]	674–806
	30–Saline	746[a]	681–817
	32–Saline	839[a]	767–919
Dustbathing	Female–carprofen	106[a,b]	66–171
Sex*Drug[2]	Male–carprofen	51[a]	29– 89
	Female–saline	90[a,b]	56–144
	Male–saline	114[b]	63–206
Dustbathing	GS0–P1	188[b]	86–409
GS*Period of day[3,4]	GS1–P1	40[a]	22– 71
	GS2–P1	71[a,b]	34–149
	GS0–P2	137[a,b]	58–323
	GS1–P2	83[a,b]	53–132
	GS2–P2	101[a,b]	54–188
	GS0–P3	72[a,b]	34–151
	GS1–P3	97[a,b]	57–165
	GS2–P3	62[a,b]	32–119

[a,b]Different letters within explanatory variable indicate significant pairwise difference (Tukey adjusted $P < 0.05$).
[1]LRT χ^2 = 4.69, df = 1, P = 0.030.
[2]LRT χ^2 = 7.99, df = 1; P = 0.005.
[3]LRT χ^2 = 11.8, df = 4; P = 0.019.
[4]P1: before injections, P2: during expected peak effect of carprofen injections, P3: approximately 12 h post injection.

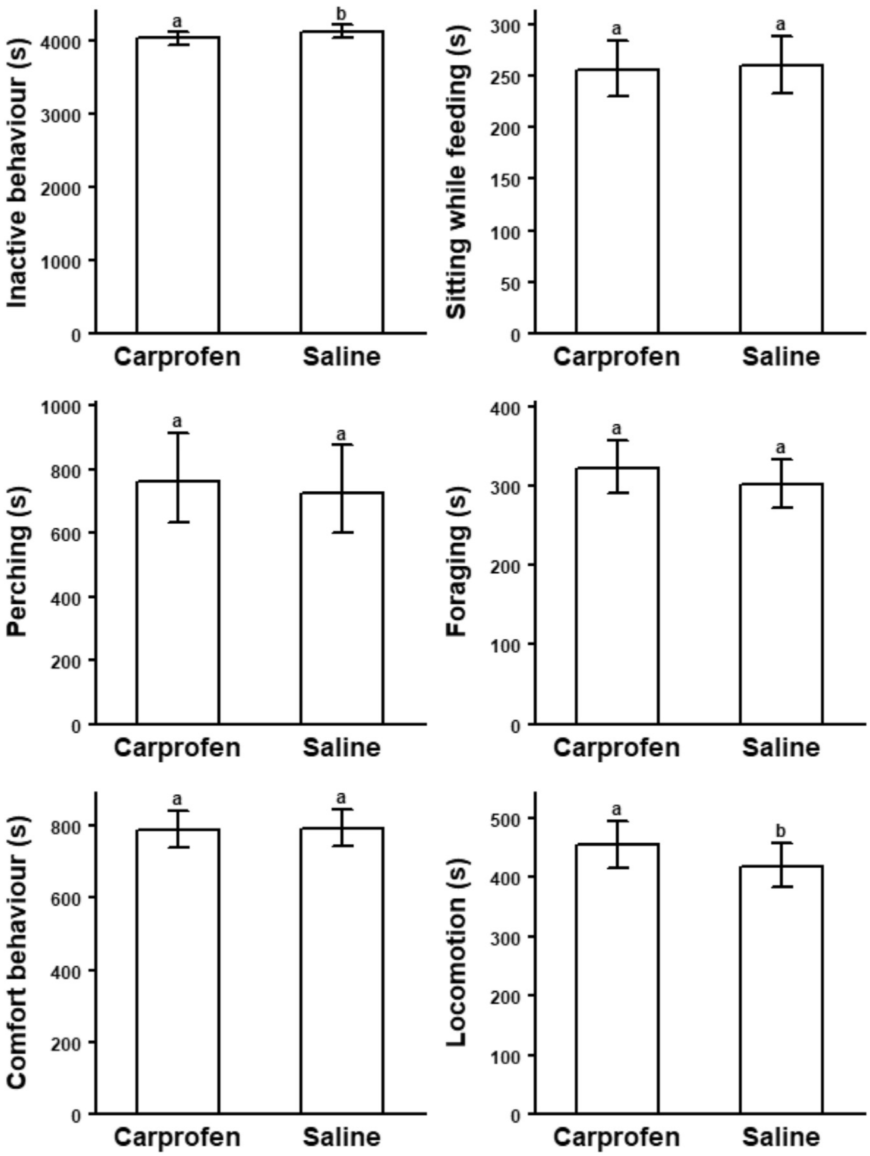

Figure 3. Duration of behaviors (back-transformed estimated marginal means (s) and 95% CI) performed by birds administered carprofen and saline, respectively. Letters indicate statistical significance.

Effects of sex on time spent on different behaviors

The time spent on the different behaviors according to the sex is shown in Figure 4. An effect of sex was found on time spent sitting while feeding (LRT $\chi^2 = 16.8$, df = 1; $P < 0.0001$) and comfort behavior (LRT $\chi^2 = 6.14$, df = 1; $P = 0.013$). Males spent more time sitting while feeding than females, and females performed more comfort behavior compared to males. No effect of sex was found on time spent inactive, perching, and foraging. As mentioned earlier, an effect of the interaction between sex and injection solution was found for time spent dustbathing (Table 2). Furthermore, an interaction between sex and period of day tended to be significant in the analysis of time spent on locomotion (LRT $\chi^2 = 5.98$, df = 2; $P = 0.050$) with females spending less time on locomotion during the morning (P1) and noon (P2) compared to the evening (P3; $P = 0.002$ and $P < 0.001$, respectively), whereas time spent on locomotion did not differ between the periods of day for males.

Effects of period of day on time spent on different behaviors

The time spent on the different behaviors according to the period of day is shown in Figure 5. An effect of the period of day was found on time spent inactive (LRT $\chi^2 = 15.4$, df = 2; $P < 0.001$), foraging (LRT $\chi^2 = 7.17$, df = 2; $P = 0.028$), and comfort behavior (LRT $\chi^2 = 8.60$, df = 2; $P = 0.014$). In the last part of the day (P3), the birds were less inactive compared to in the morning (P1; $P = 0.0499$) and in the afternoon (P2; $P < 0.001$). Furthermore, the birds spent more time foraging in the evening compared to in the morning

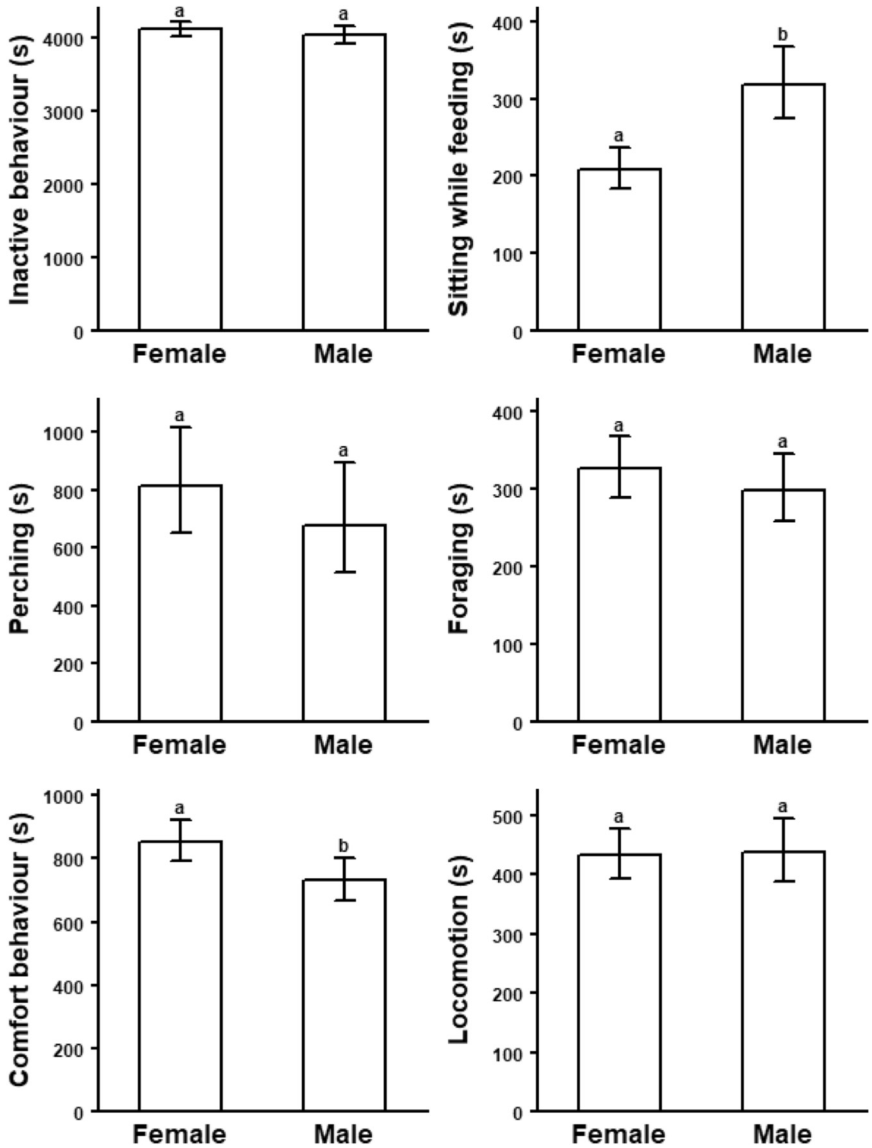

Figure 4. Duration of behaviors (back-transformed estimated marginal means (s) and 95% CI) performed by females and males, respectively. Letters indicate statistical significance.

($P = 0.022$). The birds performed more comfort behavior in the morning compared to in the evening ($P = 0.011$). No effect of the period of day was found on time spent sitting while feeding and perching. As mentioned earlier, an interaction between the period of day and GS was found in the analysis of time spent dustbathing (Table 2), and the interaction between sex and period of day tended to be significant in the analysis of time spent on locomotion (LRT $\chi^2 = 5.98$, df = 2; $P = 0.050$).

Effects of age on time spent on different behaviors

Age was not found to have a significant effect on the time spent on any of the behaviors with the exception of being included in the significant interaction between age and injection solution in the analysis of comfort behavior where none of the post hoc comparisons were found to be significant after adjustment for multiple comparisons (Table 2).

Body weight and associations with GS, sex, and age

In the analysis of body weight, significant interactions were found between sex and GS (LRT $\chi^2 = 11.7$, df = 2; $P = 0.003$; Figure 6A) and between age and sex (LRT $\chi^2 = 22.6$, df = 1; $P < 0.001$; Figure 6B). The body weight increased with GS for males (GS0–GS1: $P = 0.040$; GS1–GS2: $P < 0.001$; GS0–GS2: $P < 0.001$), whereas the increase for females was found nonsignificant for GSs one score apart (GS0–GS1: $P = 0.51$; GS1–GS2: $P = 0.21$), but significant when 2 scores apart (GS0–GS2: $P = 0.037$). Furthermore, an increase in body weight with age was found for both sexes (M: $P < 0.001$; F: $P < 0.001$).

DISCUSSION

Overall, the investigation of the behavior of broilers in their home environment suggests that birds assessed as

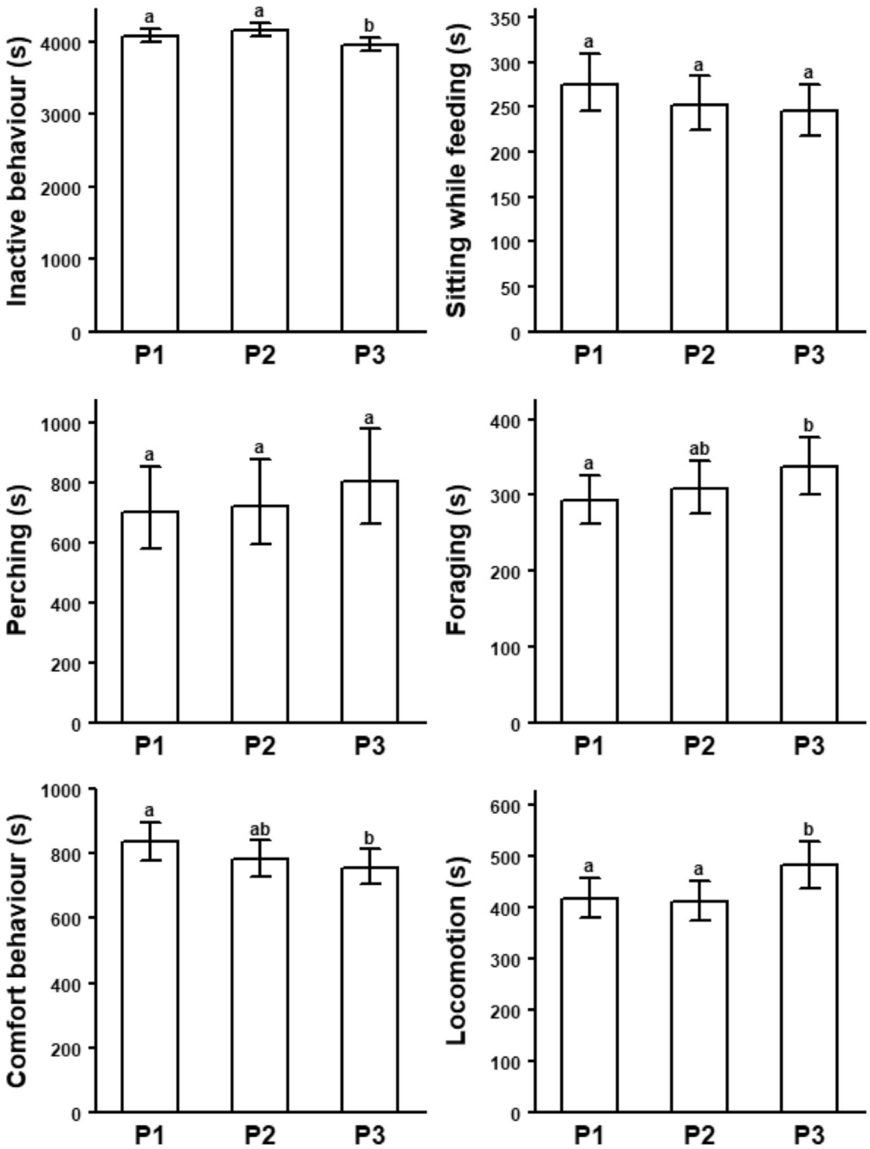

Figure 5. Duration of behaviors (back-transformed estimated marginal means (s) and 95% CI) performed during P1, P2, and P3, respectively. P1: before injections, P2: during expected peak effect of carprofen injections, P3: approximately 12 h post injection. Letters indicate statistical significance.

GS2 differed in time budget from birds with lower GSs. The behavior of GS2 birds in their home environment was characterized by inactivity, more performance of abnormal behavior in terms of sitting while feeding (as opposed to the normal avian standing while feeding), and less expression of natural behaviors such as perching, foraging, and comfort behavior. Even though not all behaviors were significantly affected by GS, these results suggest that behavioral elements involving greater physical exertion were performed less by birds of GS2 than the lower GSs. These results are further supported by results from a runway test performed as part of the present project (reported in Tahamtani et al., 2021) where birds showed reduced locomotor ability in the runway with increasing GS.

The types of behavior included in the present study ranged from core behaviors (such as feeding) to behaviors of lower resilience (behaviors that typically decrease if the cost involved in the activity increases (McFarland, 1999). Mandel et al. (2017) suggested that comfort behavior should be considered a behavior of lower resilience. The inclusion of such behaviors in ethograms has been suggested to increase the sensitivity in studies of, for example, sickness behavior (Littin et al., 2008; Weary et al., 2009). For example, Mandel et al. (2017) suggested that monitoring of low-resilience behaviors of dairy cows, such as brush use, may be a useful indicator of progress of recovery from disease. In the present study, similar reasoning was applied to the choice and inclusion of the different types of such behaviors, that is, sitting while feeding, perching, and comfort behaviors.

As mentioned in the introduction, to the best of our knowledge, no previous published studies have focused on GS2 broilers compared to birds with lower GS. However, for studies examining overall effects of GS, results comparable to the ones found in the present study have been demonstrated. For example, Weeks et al. (2000) studied broilers with GS0-3, and although no pairwise comparisons between the different GSs were made, they noted that the time budget of the different behaviors

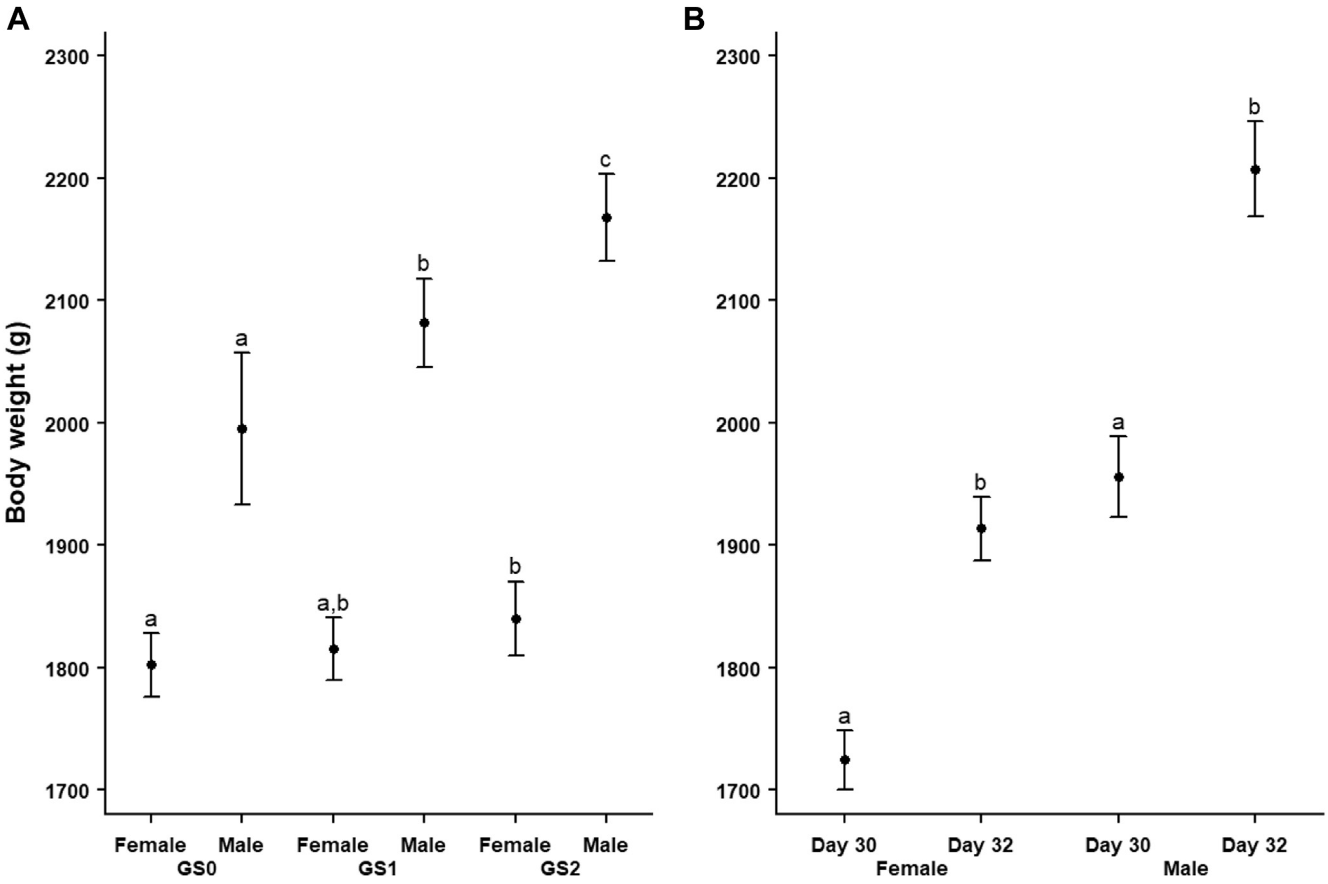

Figure 6. Body weight (estimated marginal means (g) and 95% CI) for the interaction between sex and GS (panel a) and the interaction between age and sex (panel b). [a–c]Different letters within sex indicate significant pairwise difference.

revealed little distinction between GS2 and GS3 birds, inferring that the overall significant differences found between GSs likely reflected a distinction between GS2-3 and lower GSs. They showed that increasing GS led to less activity and that with increasing GS, preening and feeding were performed progressively more while sitting, as compared to standing. Interestingly, the total time spent preening and feeding did not differ between GSs, only the posture (i.e., standing or sitting) adopted to perform the behaviors changed. In addition to sitting while feeding, Weeks et al. (2000) reported another behavior that increased in the time budget with increasing GS, that is, lying with one leg stretched to the side, which is considered abnormal if long-lasting in occurrence. Due to low representation in the current data set, this behavior was merged with sitting to the behavioral category "inactive" and therefore not analyzed separately. Furthermore, in a study of broilers reared commercially to 6 weeks of age and then moved to furnished cages in an experimental setting, Skinner-Noble and Teeter (2009) compared behavior of GS2 and GS3 birds and found the latter to rest more and stand less. In a recent study involving birds of more comparable genetics to the present-day population, Norring et al. (2018) reported less activity in birds with higher GSs. The authors investigated lying bouts, bouts of moving while lying, and walking bouts in broilers of known GS. The reported results revealed that birds with higher GS had longer total lying time and fewer walking bouts. However, the authors did not perform pairwise comparisons between GSs either. Of potential relevance is that the mean GS of the birds in the study by Norring et al. (2018) on day 32 of age was 2.3, indicating a lower walking ability in this study compared to our study. Hence, although no previous studies have carried out direct comparisons of birds assigned GS2 with birds of lower GSs, the present finding of differences in the time budgets depending on GS ≤ 2 seems to be supported by earlier studies using different statistical approaches.

As discussed by Weary et al. (2017) and Roughan et al. (2014), the interpretation of changes in the behavior of animals in their home environment in relation to underlying affective states such as pain is challenged by the fact that there are often possible alternative explanations for behavioral changes detected. Therefore, in the present study, addressing the behavior of the broilers in their home environment, we included a pharmacological intervention to facilitate inference about potential underlying affective states. Carprofen had significant effects on the duration of some of the behaviors performed by the broilers in their home environment. However, this was only shown as main injection solution effects and not as interactions between injection solution and GS. Thus, unlike our expectations, administration of carprofen affected the behavior of the birds across all GSs investigated in the study. Carprofen is an NSAID of the aryl propionic acid class with analgesic, anti-inflammatory, and antipyretic properties (Papich and Messenger, 2015). Hence, the observed effects of carprofen on the behavior of the broilers may in principle have been related to any of these effects. In the present study, 75% of the birds showed signs of at least one leg pathology (i.e., foot pad dermatitis, hock burns, femoral joint cartilage abnormality, femoral head necrosis, tenosynovitis, tibial dyschondroplasia, abnormal tibia angularity) at 38 days of age of which some may have been painful, and several are inflammatory (Riber et al., unpublished data). The present findings show that broilers administered carprofen were less inactive and spent less time on abnormal behavior in terms of sitting while feeding and more time conducting natural behaviors, such as dustbathing and locomotion as compared to the control treatment. This suggests that they benefitted from the actions of carprofen, possibly by relieving inflammation, pain, or both. Further studies, including the distribution of pathologies within and across GSs, as well as taking the time in relation to the onset and development of tissue pathologies into account, are needed to clarify whether pain is involved.

Differences in behavioral time budget between sexes have previously been reported for broilers. For example, Skinner-Noble and Teeter (2009) reported that locomotion occurred more often in GS2 females than in GS2 males, although only significantly on one of the 2 observation days. While we found no difference in respect to locomotion, males and females differed in another behavior linked to capability of physical exertion; females spent less time sitting while feeding than males. The lower time spent by males on behaviors requiring more physical exertion may be associated to the fact that males are heavier than females (Aviagen, 2019), which was also the case in the present study.

In broilers, the behavioral pattern varies throughout the light period of the day even if the light intensity is kept constant (Alvino et al., 2009). In the present study, we found foraging to occur less often in the morning than in the evening. Feeding consists of an appetitive phase, that is, searching for food or, in other words, foraging, and a consummatory phase, that is, ingestion of feed. It is well known that in the period immediately after lights-on, ingestion of feed is the main activity performed by broilers, likely resulting in foraging behavior being less prioritized as feed was available for *ad libitum* intake. Alvino et al. (2009) showed that when a distinct difference in light intensity between the light and the dark periods of the day exists, a peak in time spent preening occurs in the morning. This is in alignment with our findings, where more comfort behavior, including preening, occurred in the morning compared to the evening. Similarly, dustbathing has been found to occur more often during the morning compared to the remaining part of the light period of the day (Kristensen et al., 2007), which was also the time period where dustbathing was performed more in the present study, although only in GS0 birds. Unlike Kristensen et al. (2007), we found the broilers to be less inactive in the evening than in the morning.

Several studies have shown a change in the behavioral time budget of fast-growing broilers with age, but the time points compared have been separated by at least a week (e.g., Alvino et al., 2009; Wallenbeck et al., 2017; Bach et al., 2019). Generally, the older the broilers, the more inactive they are, typically reducing the time spent on locomotion, foraging, and standing. In the present study, we aimed for avoiding an age effect by having the least possible time interval between the 2 observation days, though ensuring a 24-h washout period (Hothersall et al., 2012) for carprofen in the broilers treated on day 30. The selected time interval was proven to be sufficiently short, as none of the behaviors were affected by age, except for comfort behavior where the interaction between age and injection solution was found to be significant, but none of the post hoc comparisons differed significantly after adjustment for multiple comparisons. Similarly, Weeks et al. (2000) found no significant behavioral differences between observations that were 2 days apart in fast-growing broilers aged 39 to 49 d, although the time spent on different behaviors changed significantly during the 11-d period.

Lastly, the causal background of walking impairment has been reported to be multifactorial, including factors such as suboptimal body composition, different leg health issues, and fast growth resulting in rapid achievement of high body weight (e.g., Corr et al., 2003a,b; Skinner-Noble and Teeter, 2009; Caplen et al., 2012; Granquist et al., 2019). Generally, the higher the body weight, the higher the GS (Sørensen et al., 1999, 2000; Kestin et al., 2001; Nääs et al., 2009). For example, Kestin et al. (2001) found a strong positive correlation between gait score and body weight, and when including body weight as a covariate in the analysis, the difference found between genotypes (differing in growth rate) disappeared. In the present study, the positive relationship between body weight and GS was not only demonstrated at the ages presented here, but also at slaughter when the birds were 38 d of age (Riber et al., unpublished data). A discussion of the effects of GS therefore also encompasses body weight/growth rate, as these factors are associated.

CONCLUSIONS

Overall, the results of the present study showed clear behavioral differences between birds of GS2 and those of a lower GS, when observed in their home environment. These results suggest that the behavior of GS2 birds is characterized by inactivity, more performance of abnormal behavior in terms of sitting while feeding, and less performance of natural behaviors such as perching, foraging, and comfort behavior when compared to birds of lower GS. In addition, administration of the analgesic drug carprofen affected the behavior of the birds across GSs, suggesting that their behavioral expression may have been limited by pain. These findings are of relevance to animal welfare, but the underlying causes are still not fully understood.

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