Preferences of Laying Hens for Three Perches of Varying Physical Characteristics

By

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Abstract

As production systems for laying hens in the United States move towards more complex environments with greater behavioral freedom for the bird, questions about how the environment influences the welfare of the bird have arisen. Due to its nature as a highly-motivated behavior, perch design is of particular interest, with both spatial arrangement and physical characteristics impacting the hen's health and affective state. This study sought to determine which perch type hens prefer by conducting a simple choice test using 77 week old Dekab hens. Observations were taken during both the morning and evening, with percent of time spent perching by hens significantly higher in evening observations ($P > 0.001$) Hens were given a choice between two perches available in commercial settings, a round metal perch, a mushroom-shaped plastic perch as well as shorter rectangular wooden beams that held these perches. The position of the perches within the pen was rotated between observation days. Overall ($P = 0.25$) and during morning observations ($P = 0.57$), no difference in percentage of perch use was found between the three perch types. During evening observations there was a preference for round metal perches over the rectangular wooden beams, with mushroom plastic perches used an intermediate amount ($P = 0.03$). During the evening observations, the laying hens had a location preference within the pen and perched more frequently on one side of the pen independent of perch type offered ($P > 0.001$). These results indicate that hens did not have a strong preference between the round metal or mushroom plastic perches, and that in choosing a place to perch the physical characteristics of the perch are possibly less important than other factors such as location.

Introduction

In a scientific context, animal welfare seeks to objectively determine an animal's quality of life, by considering factors such as an animal's health, behavior, and affective states (Fraser, 2009). These factors can overlap and impact one another. For example, an animal in poor health may be unable to perform species-specific, highly-motivated behaviors; such as dustbathing in chickens (Fraser et al., 1997, Fraser, 2009). Additionally, the affective states of animals cannot be directly observed and are only able to be assessed using indirect measures such as physiological or behavioral measures (Dawkins, 1990; Fraser et al., 1997). When evidence from multiple measures all point to a particular management decision as supporting good welfare, it is easy to make a recommendation. However, these measures can conflict with one another, causing animal welfare scientists to carefully weigh the relative value of each (Dawkins, 2003; Fraser, 2009). In the face of conflicting measures, Dawkins (2003) suggests two key questions for animal welfare: ”Are the animals healthy?” and “Do the animals have what they want?”.

Commercial housing for laying hens (*Gallus gallus domesticus*) in the United States is rapidly moving away from conventional cage systems (also known as battery cages) to alternative systems, with a focus on cage-free production (Mench et al., 2011; Scrinis et al., 2017; Shields et al., 2017). Much of this change has been driven by consumer and animal advocacy groups' increased interest in the welfare of animals in commercial farming, with pushes for change in the form of both market-led initiatives and legislation at the state level (Mench et al., 2011; Scrinis et al., 2017; Shields et al., 2017). One of the largest welfare concerns with egg-laying hens housed in conventional cage systems is the barren environment and the lack of opportunity to perform species-typical behavior, which includes perching. In an effort to allay these behavioral and affective state concerns, a ballot measure passed in 2018 banned the use of caged systems for laying hen in California, a major egg producing state with an estimated 13,664,000 layers in 2020 (Cal. Health and Safety Code §§ 25990 - 25994; USDA National Agricultural Statistics Service). Califonia's regulatory code specifies that production systems should be designed to include "scratch areas, perches, nest boxes, and dust bathing areas" (Cal. Health and Safety Code §§ 25990 - 25994). Part of the increased behavioral freedom in alternative housing systems is the ability to perch on an elevated surface, which can have mixed animal welfare outcomes for the laying hen (Lay et al., 2011). With an increasing number of hens housed in complex environments, the impact of these environments on the welfare of laying hens is an important area of ongoing study.

Access to perches for laying hens improves the welfare of the bird because perching is a highly motivated behavior (Olsenn and Keeling, 2002). Laying hens are domesticated from the red jungle fowl, which are also shown to spend time perching (Dawkins, 1989). For laying hens, daytime perching behavior declines with increased group size. Perching possibly acts as an antipredator defense for both jungle fowl and laying hens, which would make it a behavior important to the bird's survival in natural settings (Dawkins, 1983; Newberry et al., 2001). Behaviors that animals are highly motivated to perform are considered behavioral needs, with the animals often attempting to perform them even when the environment is suboptimal for performing that behavior (Weeks and Nicol, 2006). If the causal mechanisms for the animal's behavior remain in the environment, the mechanism of behavior is unlikely to extinguish, even if uncoupled from the ultimate cause of that behavior (Dawkins, 1983, 1990). Studies that look at the behavior of domestic laying hens in commercial systems given access to perches have found that many of the birds do perch at night, indicating that the causal mechanism is still in effect (Brendler and Schrader, 2016; Campbell et al, 2016). While not all behaviors performed in natural conditions lead to suffering when animals are unable to perform them, when hens were deprived of access to perches during a dark period, they showed increased exploratory behaviors that could be interpreted as frustration, an affective state associated with poorer welfare (Olsson and Keeling, 2000). One experimental method that attempts to quantify motivation, involves titrating an animal's willingness to work to obtain a resource with their willingness to work to obtain a resource with a known motivation (Dawkins, 1983). Laying hens have been shown to be willing to work by pushing through heavier doors for access to perches during dark periods, showing that hens are highly motivated to perch (Olsson and Keeling, 2002). These experiments help establish perching as a behavioral need for laying hens, with hens likely experiencing a more negative affective state when denied access.

While alternative production systems increase behavioral freedom, studies that compare different management systems show that birds housed in alternative systems may have reduced health (Rodenburg et al., 2008; Wilkins et al., 2011; Petrik et al., 2015). Two major welfare concerns have been identified as being influenced by the addition of perches into a system—keel bone damage and footpad health. Keel bone damage includes keel bone deviations and keel bone fractures and is a widespread problem. Some studies report up to 89% of hens with damage at the end of lay (Wilkins et al., 2011). This complex problem has multiple influencing factors and is associated with high impact events such as crashes into system furnishings such as ramps and perches and possibly lower impact strain from perch use (Pickel et al. 2010, 2011; Wilkins et al. 2011). The keel bone of a laying hen includes both bone and cartilaginous material and is connected to muscles used for both flight and perching. A damaged keel bone affects the mobility of the hen and her ability to navigate her environment (Nasr et al., 2012). Laying hens with a keel bone fracture showed reduced latency to jump from a perch when given butorphanol, an opioid that reduces pain. This finding suggests that laying hens find keel bone damage painful (Nasr et al., 2012). Differences in the design of the housing system are correlated to differences in rates of keel bone damage. Keel bone damage happens at higher rates in cage-free systems, likely due to higher rates of falls and crashes against system furniture, but is also present in conventional and furnished cage systems (Rodenburg et al., 2008; Wilkins et al., 2011; Petrik et al., 2015). One of the factors that has been correlated with the rate of keel bone damage is the physical design of the perch (Käppeli et al., 2011; Stratmann et al., 2015).

A second welfare concern in laying hens is the health of the footpad. Footpad dermatitis is the inflammation of the footpad that can lead to ulceration, and ultimately the swelling of the foot, a condition known as bumblefoot. These abnormalities are thought to be painful, and also impede the mobility of the hen (Tauson and Abrahamsson, 1996; Lay et al., 2011). Footpad health was found to be worse in cage-free systems than in conventional cage systems, with litter quality being one of the greatest factors in footpad health, but footpad health has also been linked to poor perch design (Tauson and Abrahamsson, 1994, 1996). Not all alternative production systems are created equal, and to some extent, these problems can be mitigated by improving the system's design (Lay et al., 2011).

Due to the nature of perching as a behavioral need and its correlation to keel and footpad health, research has been conducted to investigate the design of perching in alternative systems. Multiple factors have been studied, and in addition to the arrangement of perches within a system, physical characteristics of perches have been looked at, with much of the literature focused on the health of the bird. In many studies, multiple factors of perch design, such as diameter, shape, and the material the perch is composed of have been altered between the perches, making it difficult to isolate the reasons for differences between treatments.

Correlations have been found between the material of the perch and the rate of keel bone damage (Käppeli et al., 2011; Stratmann et al., 2015). Laying hens kept in floor pens and given access to metal round perches with a thin rubber layer were found to have a significantly greater number of severe and moderate keel bone deformities than hens with access to plastic T-shaped perches (Käppeli et al., 2011). This finding is possibly due to the hardness of the metal material. Another study found a smaller proportion of laying hens with keel bone fractures in laying hens given access to round perches covered with a soft polyurethane layer when compared to ones that had access to round metal perches, although there were also differences in the width of the perches in the two treatments (Stratmann et al., 2015). No significant difference was found with keel bone lesions when comparing hens given access to plastic perches and wood perches of several shapes at 55 weeks of age (Tauson and Abrahamsson, 1994).

Plastic perches were shown to cause a greater number of foot problems in laying hens compared to wooden perches (Tauson and Abrahamsson, 1994, 1996). This could be due to the slickness of the material which makes it more difficult for the hen to grip. The shape of the perch is another factor that likely contributes to the ease of gripping the perch (Pickel et al., 2010, 2011). In a study that compared the frequency of balance movements of birds on rubber-coated, metal, and wooden round perches, hens on the rubber perches were found to have fewer balance movements, but no difference was found between the metal and wooden perches (Pickel et al., 2010). Another consideration in an industry setting is the hygiene of the perch, which is influenced by material. Latency to jump from the perches, which is important for balance and avoidance of collisions and falls from the perch, was studied with metal, wood, and PVC plastic materials by placing laying hens on a starting perch and recording their behavior as they jumped to an identical perch 0.75 m away. The study found that the hens took significantly less time to jump between the metal and wood perches than the plastic perches when they were clean, but there were no significant differences when the perch was dirty. For both the metal and the wood perches, the birds took a longer time to jump when the perches were dirty (Scott and MacAngus, 2004). This distinction is important because the amount of manure might affect the overall preferences for the perches and the cleanliness of the perch has not previously been taken into account (Scott and MacAngus, 2004). Plastic mushroom shaped perches were found to be more hygienic than wooden perches at 35 weeks, possibly due to hens leaving scratches in the surfaces of the softer wooden perches (Tauson and Abrahamsson, 1994). Both plastic and metal perching are non-porous and easier to disinfect between flocks. This is a health benefit that should be taken into consideration (Tauson and Abrahamsson, 1994; Scott and MacAngus, 2004).

How the shape and width of the perch affects the welfare of the bird is also an area of interest, with studies comparing multiple varieties of perches. The shape of the perch is described in reference to the cross-section of the perch. One of the most commonly studied shapes is a round perch with a circular cross-section. Laying hens given access to round perches were found to have more keel bone damage than a T-shaped perch at 62 weeks, although difference in material was a possible confounding factor (Käppeli et al., 2011). This is thought to possibly be because round and oval perches put more localized pressure on the keel bone, which possibly causes the increased damage, in comparison to a square perch with a flatter design (Pickel et al., 2011). Research has been conducted to further reduce the peak pressure on the keel, using an air-cushioned round prototype perch that significantly reduced the pressure on the keel compared two commercially available mushroom shaped plastic perches, a round metal perch, and a flatten-round shaped plastic perch (Pickel et al., 2011).

The relationship between the shape of the perch on footpad health is less clear, but the peak force on the footpad was lowest on the oval-shaped perches compared to round or square perches (Pickel et al., 2011). Hens given mushroom shaped plastic perching were found to have worse bumblefoot scores when compared to a rectangular and a flattened-round wooden perch, but this was thought to be primarily due to the material (Tauson and Abrahamsson, 1994). A good portion of the research varied on multiple physical characteristics, such as width, material, and shape, making interpretation of these factors on health more difficult. Information about the effect of width on the health of the birds has not been studied in isolation from other perching characteristics.

When there is conflict or no clear consensus between different measures concerning what is best for the animal's welfare, asking what the animal wants is an important factor to consider when judging the relative importance of the measures (Dawkins, 2003). Preference testing seeks to understand an animal's preference among alternative resources by allowing animals the chance to decide what resource to utilize (Kirkden and Pajor, 2006). This can be a powerful tool, as what is important to the animal may not be adequately addressed solely through other welfare measures. In this way, behavioral measurements can be used to infer the subjective feelings of the animals, allowing for direct comparisons between the attractiveness of different choices from the animal's point of view (Kirkden and Pajor 2006; Fraser and Nicol, 2018). In a simple choice test, this is done by allowing the animals to choose between substitutes, meaning that all choices satisfy the same underlying motivation (Kirkden and Pajor, 2006).

Several assumptions underlie the use of preference testing, and it is subject to limitations. Preference testing assumes that the animal will make the choice that best promotes its own welfare (Fraser and Nicol, 2018). This can break down with animals in human care, whose current environment and conditions can differ significantly from what is found in the wild (Kirkden and Pajor, 2006; Fraser and Nicol, 2018). Another limitation of this testing is that it is dependent on the experience of the animal, and familiarity with one of the choices could influence the results. The ability of each animal to learn must be taken into account when interpreting results, as well as early life experiences (Kirkden and Pajor, 2006). This can influence the applicability of preference results to different populations within the same species. The perching behavior by laying hens raised in different conditions as pullets is variable (Pullin et al. 2020). To date, no research has considered whether or not the laying hens undergo a critical time period for the development of perch preferences. Motivation to use said resource can vary throughout the day, or the lifespan of the animal, so the behavioral time budget of the animal species should be studied, and observations must be comprehensive enough to take the variability into account (Fraser and Nicol, 2018). Perch use by laying hens was found to be greater during 'dark' periods with no or little lumination but also happens throughout the day (Brendler and Schrader, 2016; Campbell et al., 2016). Another major limitation of simple choice tests is that it is only able to give us a ranking of the substitutes, but not the overall valence of the choices. It is possible for the substitutes given to all be poor choices, or all adequate for the animals needs. In light of these limitations, preference research should not be the sole measure of welfare. Instead, the animal's desire should be fit into the additional context of how their desires influence their health (Kirkden and Pajor, 2006; Fraser and Nicol, 2018).

In order for the provided perches to be beneficial behaviorally to the birds, they need to be frequently used, but relatively few studies have explored the physical characteristics of perches that laying hens prefer. Behaviorally, a difference in material has not had an effect on time spent on the perch when the birds are not given a choice. In a study that looked at behavioral differences in birds when given access to perches of different materials, the perching durations of the birds were not found to be significantly different between wooden, metal, and plastic materials (Lambe and Scott, 1998). This result is similar to findings about the amount of time spent on perches by laying hens that were given access to either metal, wood, or rubber perches (Pickel et al., 2010). Hens given access to either soft-layered or metal perching did not differ significantly in time spent perching (Stratmann et al., 2015). Because perching is so highly motivated, a direct comparison in a choice test may be necessary to gauge the suitability of perching material. Laying hens are willing to use different perching materials available, but when given a choice between wood, plastic, and metal perching, wooden perches were found to be preferred over metal or plastic perches, while the birds showed no preference between metal and plastic perches (Chen et al., 2014).

In regards to the shape of the perch, laying hens had no preference when given a choice between round and hexagonal perches (Liu et al., 2018), nor between round and rectangular perches (Lambe and Scott, 1998; Chen et al., 2014). In another experiment that looked at the preferences of breeder birds with round, square, flat, and peaked perches, birds of 20 months who had been previously raised with square perches continued to prefer square perches followed by round and flat perches when given access to all four kinds. When the same experimental setup was conducted with perch naive birds, the square and round perches were equally the most preferred (Muiruri et al., 1990). Several studies have looked at preferences for the width of perches. Muiruri et al. (1990) found that 5 cm diameter round perches were preferred over 2.5 cm and 3.8 cm diameter perches . On rounded top perches, 4.5 cm wide perches were preferred over 1.5, 3.0, and 7.5 cm perches during the day, but no preference was found during the night (Struelens et al., 2009). With rectangular perches, 3.0 cm wide perches were preferred over 5.0

cm wide perches when hens were in groups of one and four, but not when they were in groups of eight (Chen et al., 2014).

It is possible that the suitability of the physical perch is reliant on several different factors that influence each other, including but not limited to material, shape, and width. Studies are needed to identify these factors in isolation, and the relationship, if any, between these factors remains unclear. While studies that systematically look at several different factors are needed, it may be more beneficial in the short-term to look at perch designs that are already in use commercially and for which some information about their effects on the bird's health exists.

The objective of the current study is to identify if laying hens have a preference between two different commercially available perches: a mushroom-shaped plastic perch and a round metal perch. The round metal perches cause more keel bone damage than t-shaped plastic perches, meanwhile plastic perches were found to have greater bumblefoot than in similar shaped wooden perches (Tauson and Abrahamsson, 1996; Käppeli et al., 2011). While some studies compare the health benefits of these materials and shapes, no study has looked to see if hens have a preference between mushroom and round perches. Both of these perches have potential commercial benefits; they are both made of non-porous material that can be sanitized easily between flocks. Additionally, both of these perch materials are more durable than wood, which can have a build up of manure as it gets scratched by the birds, and therefore, become less hygienic at 35 weeks compared to plastic perches (Tauson and Abrahamsson, 1994). This study used older hens, who possibly had some degree of keel bone damage. Because it is less damaging to the keel, it is possible that the mushroom plastic perches will be considered more comfortable to the bird, and a greater overall percentage of perching will be observed on them compared to the round metal perches.

Methods

The experiment was performed at Hopkins research facility at University of California, Davis, in Davis, CA. The protocol was approved by the University of California, Davis institutional animal care and use committee prior to the start of the experiment under protocol number 21936.

Fifty-three DeKalb laying hens, 77 weeks of age, were used during the experiment. The hens were housed in twelve 3.05 m x 3.05 m floor pens. Each pen contained two round feeders, a nipple water line and two perching structures. Each perching structure consisted of a metal frame 122 cm long by 61 cm wide, with a platform made of plastic slats at the height of 61 cm. Two lengths of perches 25 cm apart were attached to the structures 30 cm above the platform via wooden rectangular beams. One structure had two 122 cm lengths of round cylindrical metal perching (3.8 cm in diameter) and the other perch structure had two 122 cm lengths of plastic perches with a mushroom shaped cross-section (Lubing Systems LP). Perching structures were on opposite sides of the pen, with one of the 122 cm sides of each structure arranged alongside the eastern or western wall, and one 61 cm side placed along the pen doors. Natural light was used during the experiment. Sunrise occurred between 05:45-06:05 and sunset occurred between 20:00-20:30 during the experimental period.

Before this study began, the hens were housed in pens with a multitiered aviary system in groups ranging from 7-10 birds. On day zero, the hens were divided and transferred into new pens, with all social groupings consisting of hens that had been previously housed with one another. Six pens contained 5 hens, five pens contained 4 hens, and one pen contained 3 hens. The plastic mushroom and round metal perches were swapped at 16:00-17:00 to the opposite structure every 3 days (on days 3, 6, 9, 12, 15, 18, and 21 of the study), to control for possible location effects. Birds were given time to acclimate to the perches in both locations, with observations taking place on the days before perches were switched.

Behavioral observations began on day 8 and continued every 3 days (on days 11, 14, 17, 20, and 23). Pens were equipped with two video cameras (Lorex 1080p Security Camera Systems,Lorex Technology Inc, 250 Royal Crest Court Markham IN L3R 3S1 Canada, www.lorextechnology.com) positioned to give a view of both perching structures, and up to 6 pens could be viewed simultaneously on a video monitor using a network video recorder (Lorex 4K Ultra HD Network Video Recorders, Lorex Technology Inc). Observations were scored in real time twice a day, with a morning window from 09:00-11:00 and an evening window from 19:00-21:00. The experimental unit for the observations was the pen level, with no distinctions between individuals. Two observers collected behavioral data, with observer CL performing morning observations and observer CM performing evening observations. Interobserver reliability between CL and CM was determined by both scoring the first AM observation on day 8. Each two-hour observation window consisted of 30 minutes of instantaneous scan sampling at 5 minute intervals looking at all 12 pens, followed by a period of 10 minutes of continuous sampling, during which 6 pens were monitored simultaneously. These periods were alternated for the 2 hour window, giving a total of 90 minutes of scan sampling data for each of the 12 pens, and 30 minutes of continuous sampling per each of the 6 pens. During the scan sampling period, the number of birds currently perching was recorded for birds on the mushroom and round perches. Perching was defined as any bird sitting, standing, or moving along the perches. Due to the platform and perch design, laying hens were able to access 61 cm lengths of rectangular wooden beams (3 cm by 9 cm) on one side of the perches that held the two 122 cm lengths of perching for each of the two platforms. During the experiment, perching activity on these beams was observed. Observations of birds perching on the wooden beams was recorded with note of which perching material the beams were holding up. Perching activity on the wooden beams on both sides of the pen were added together to create a wooden treatment category to test if wood was preferred for perching.. During the continuous sampling period, birds in 6 pens (4 pens N=5, 2 pens N=4 hens) were continuously monitored for falls and pushes while on the perches for a 10-minute period. Pushes were defined as contact with another hen that caused one of them to move away. Falls were defined as displacement caused by another hen that resulted in the hen leaving the perch.

Statistical analysis

Statistical analysis was conducted in R (R core team, 2020) using the agricolae package (de Mendiburu and Yaseen, 2020) and the dunn.test package (Dinno, 2017). Figures were created in R using the ggplot2 package, and labels were adjusted using Inkscape (Wickham, 2016; Inkscape Project, 2020). For each 2 hour observation window the number of hens counted perching for each perch type was tabulated for each pen, and the total count for each treatment in each pen was converted into a percentage of time spent perching on that treatment by the hens. Data did not meet normality assumptions when a Shapiro-Wilks test was conducted for frequency of perching, therefore, non-parametric tests were performed. Statistics are displayed in results as median percentage, and interquartile range (IQR). The total percentage of birds perching for each pen during each observation period was tabulated and a Mann-Whitney test was used to check for differences in perching frequencies during the morning and evening observations. Due to the observed perching on the rectangular wooden cross beams, the percentage of hens perching on the wooden beams on both perching structures was combined and tabulated for each pen. A Kruskal-Wallis test was performed to see if there were differences in perching frequencies between the three perches, with post hoc testing done using a Dunn test with a Bonferroni correction. Due to differences in perch use during the morning and evening observation periods, each test was performed using overall, morning, and evening data.

Each pen contained two perching structures on opposite sides. The percentage of observation time the birds were observed to be perching on each side of the pen was tabulated by summing the number of birds on the experimental perches with the number of birds on the rectangular wooden beams for that perching treatment to get the total for structure. Because treatments were rotated, the observation totals for the two treatments were assigned to sides based on observation date. Average perch use for both sides of each pen was calculated in order to assign the side that birds spent more time on as the preferred side for each pen. To check for a location preference, a Mann-Whitney test was used to test if the percentage of time spent between the preferred side and the side less frequently used in the pen was significantly different. Observations of falls and pushes were infrequent, and therefore, a large enough sample size was not observed for meaningful statistical analysis based on the data collected during continuous observations..

Results

Perches were used more frequently during the evening (24.722%, IQR=16.667-31.319%) than during the day (0.00%, IQR=0.00-0.00%) ($W = 28.5$, $P<0.001$) (Figure 1). For all observations, hens did not show a preference for perch type (Figure 2) among the mushroom plastic (0.00%, IQR=0.00-8.33%) round metal (0.00%, IQR=0.00-11.11%), or rectangular wood perches (0.00% IQR=0.00-3.33%) ($\chi^2 = 2.81$, df=2, $P = 0.245$). For morning observations, hens did not show a preference for perch type among the mushroom plastic (0.00%, IQR=0.00-0.00%), metal round (0.00%, IQR=0.00-0.00%), or rectangular wood perches (0.00%, IQR=0.00-0.00%) (χ^2=1.11, df=2, *P*= 0.574). For evening observations, there was a significant difference between treatments (χ^2=7.04, df=2, *P*=.0296). A Dunn test with a Bonferroni correction was conducted to test for differences between treatments. There was no difference between metal round (7.50% IQR= 0.00-27.2%) and mushroom plastic (8.33 IQR= 0.00-22.5%) (Z = -1.04, P = 0.448) and mushroom plastic and rectangular wood(2.50% IQR=0.00-10.00%) perches (Z = 1.59, P = 0.166), but there was a difference between the metal round and rectangular wood perches, with the wooden perches used significantly less (Z = 2.63, P = 0.013).

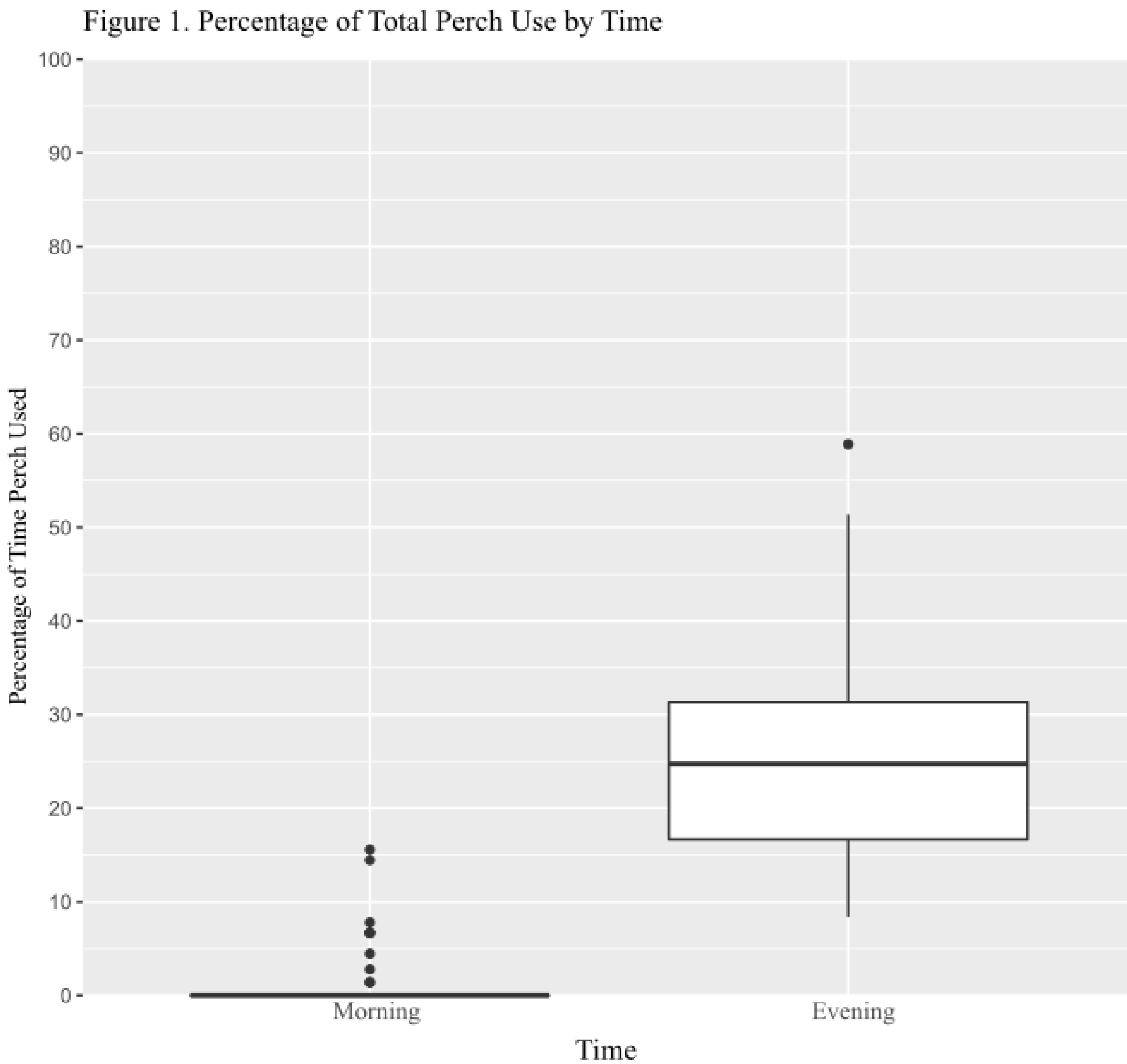

Figure 1. The percentage of time hens were recorded to be perching on any perch type (wood, metal, plastic) for observations during the morning and the evening period. The birds spent a larger percentage of time perching in the evening (W = 28.5 , P<0.001).

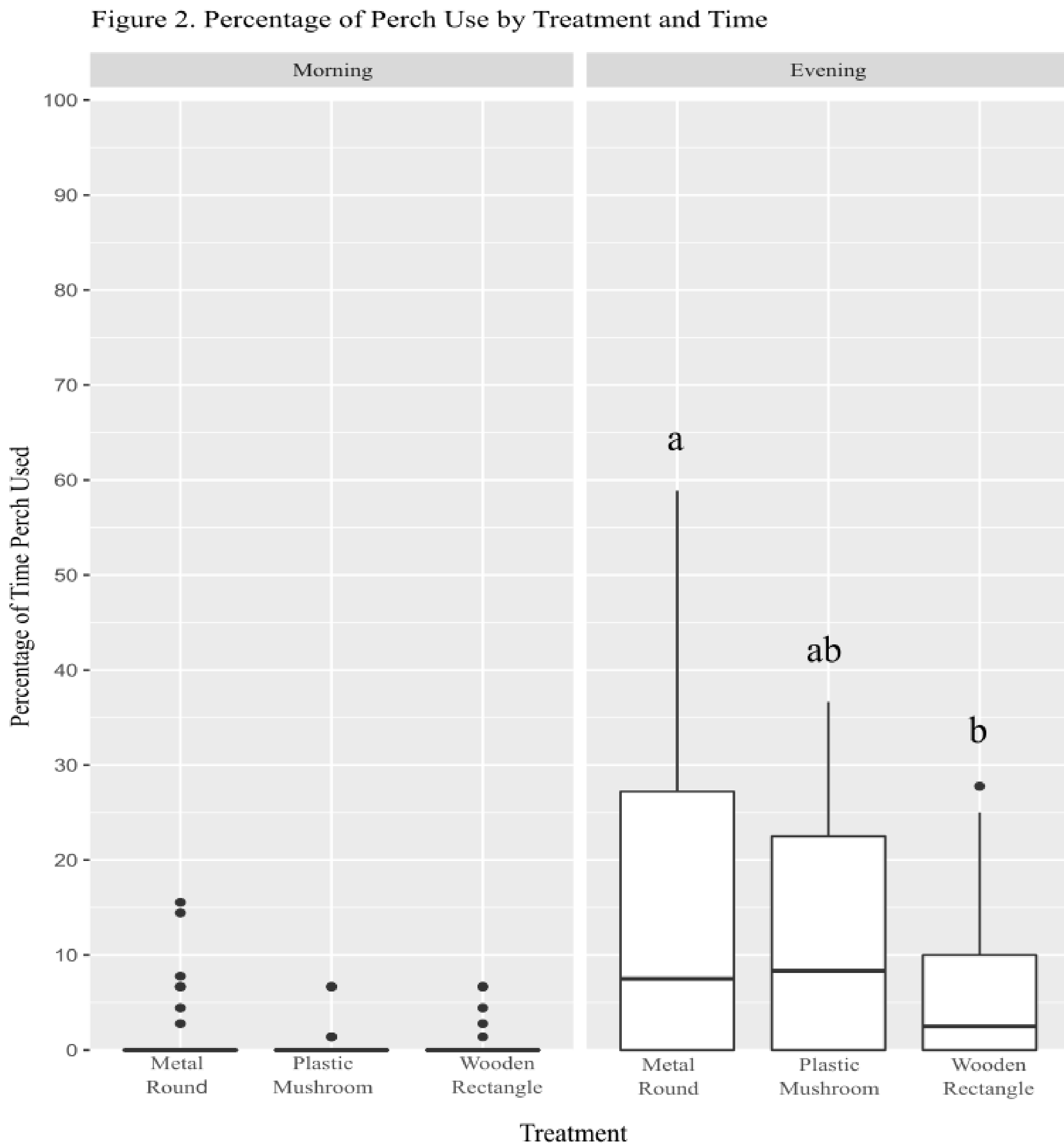

Figure 2. The percentage of time hens were recorded to be perching on each perch type for both the morning and afternoon time frames. There was no difference between treatments for the morning (*P* = 0.574). For the evening, use of perch type was significantly different (a, b; P=0.01).

Overall, laying hens showed a strong preference for one side of the pen, with the laying hens spending more time perching on one side (8.33% IQR=0.00-23.89%) than the other (0.00%, IQR=0.00-0.00%) (W = 14,768 , P<0.001). During morning observations, (Figure 3) hens did not show a preference (W = 2,487, P=0.365) for the side more frequently used (0.00%, IQR=0.00-0.00%) or the other side (0.00%, IQR=0.00-0.00%). During evening observations, hens showed a strong preference for the side they more frequently used (24.44%. IQR=15.28-31.11%) than the side opposite it (0.00%, IQR=0.00-1.11%) (W = 5,140, P<0.001).

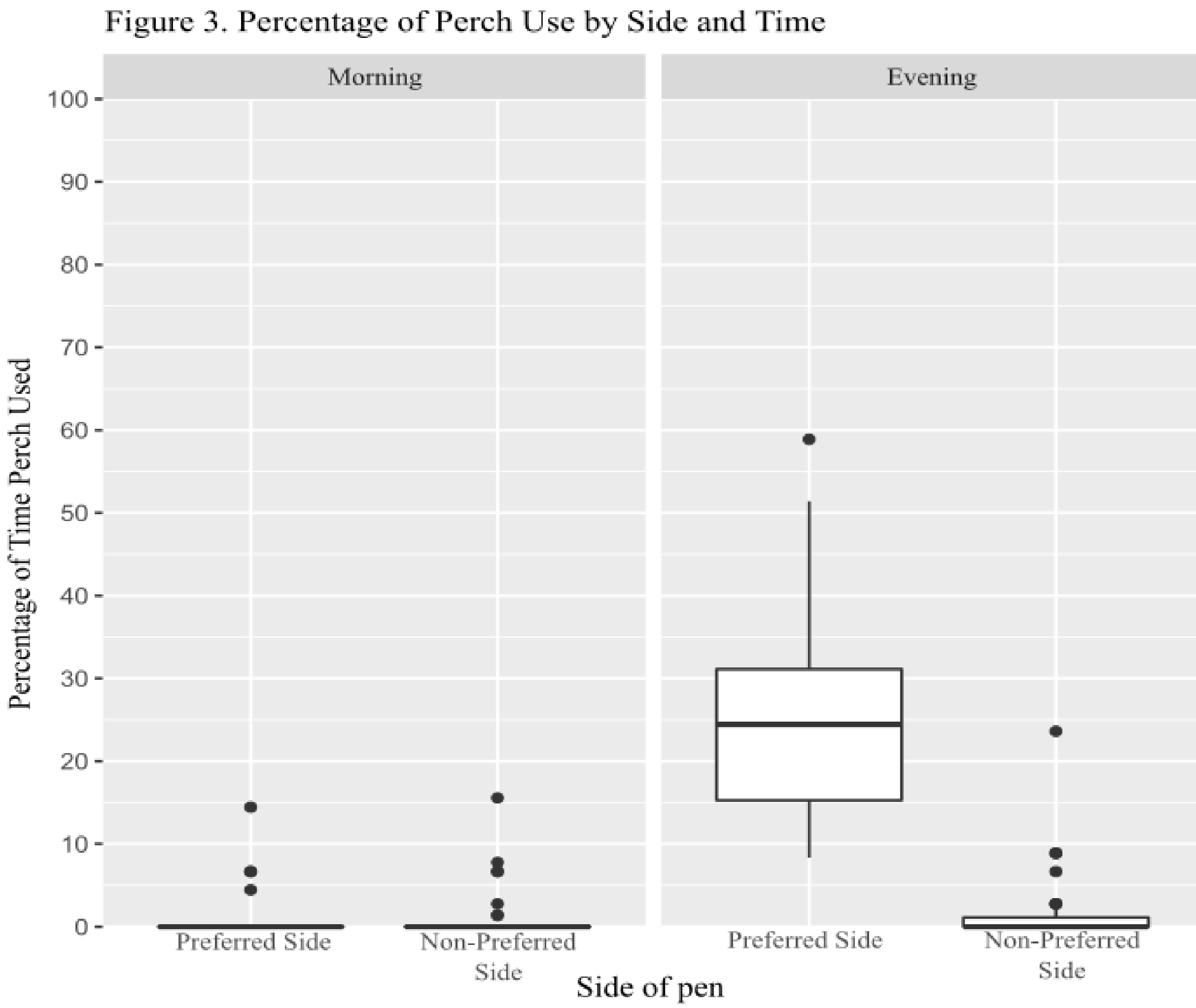

Figure 3. The percentage of time the hens were recorded to be perching on each side of the pen (commonly used, or less commonly used), as well as by time of observation (morning and evening), regardless of perch type. During the morning there was no significant difference between sides used to perch (W= 2478, P=0.365) During evening observations there was a significant difference (W = 5140, P<0.001) between the side of the pen used most frequently and the other side of the pen.

Discussion

One limitation of choice testing is that the motivation to use said resource can be variable throughout the day, or the lifespan of the animal (Fraser and Nicol, 2018). The behavioral time budget of the animal species should be studied, and observations must be comprehensive enough to take the variability into account (Fraser and Nicol, 2018). Perch use by laying hens has been found to be greater during dark time periods with no or little illumination, but also happens throughout the day (Brendler and Schrader, 2016; Campbell et al., 2016). Percentage of time spent perching during the day was less than percentage of time spent perching during the evening observations for the current study. When broken down into morning and evening, no significant differences were observed for the morning observations between treatment groups or location. While it is possible that the hens have different preferences for perches used during the day, this could also be because of how infrequent perching behavior was observed during the morning. Sixty-one out of 72 morning observations had no perching activity observed. It is possible that the observation window was too short to accurately account for the bird's daytime preference. There was no preference for perch type overall or in the morning, however, the infrequency of perching during the morning could have created a floor effect and masked the preferences seen when only the evening data was tested. While more observations and data could reveal that laying hens' preferences for perches are different in the morning compared to night, further testing should focus on the birds preferences during the evening and dark periods, when perches are most utilized.

Effects of perch type on perch use in the evening

For the evening observations, the frequency of perching by laying hens in this study on mushroom plastic or round-metal perches was similar. This result could indicate that the hens do not have a strong preference between the mushroom plastic and round metal perches.

Previous studies have shown that wood is a preferred material in comparison to plastic and metal for perching (Chen et al., 2014). When rectangular wood was included as a treatment category, perching on wood was not different from the mushroom plastic perches, but was significantly less than the round metal perches. Due to platform design, the rectangular wooden beams were not offered equally as an alternative to the other perches, which limits the conclusions that can be drawn. In preference testing resources should be offered equally, because in simple choice testing there is no way to account for competition among resources or the animal's motivation to access resources (Kirkden and Pajor, 2006). The limited nature of the wood as a perching material (e.g., the shorter perch lengths, the perches affixed on top of the wood causing obstructions, and the alternate arrangement of the perches) could all be factors that influenced their use during the experiment. It is possible that given equal access to wooden perches, the birds would have preferred the wooden rectangular beams in alignment with previous findings.

Perch preference has been shown to be influenced by the size of the group studied. Chen et al. (2014) found that laying hens preferred 3 cm widths of perchs while in groups of one and four hens but not in a larger group size of eight hens. This result could be due to social factors; it has been found that birds in larger groups perch less when controlled for stocking density (Newberry et al., 2001). Another possibility is that it could be due to increased competition for the perch as the perch length per bird decreases. Due to mortality before the study, the number of hens in each pen was variable, with a stocking density ranging from 3.10-1.86 m^2 per hen. While this stocking density is variable, it is still extremely low compared to commercial settings. Due to wooden perches being an accidental treatment, the length of perch per bird was variable, with the two mushroom and two round perches providing a range of 48-81 cm of total perch length per bird per treatment. This length of perching is generous enough that competition for perches should be extremely low. The wooden square treatment consisted of two perches located on opposite perching structures, for a total length of 24-41 cm per bird for the wooden treatment, though that doesn't account for interruptions in the perch length caused by the other perches being affixed on top of the wood. Along with the obstruction caused by the other perching structures, birds spent the majority of their time on only one of the perching structures instead of utilizing both, which could have increased the competition for the wooden perch and limited its use. While displacements were too rare for statistical analysis, it is possible that the three 10 minute focal observations using only 6 pens during each observation window was not enough to capture the behaviors, and that competition was a limiting factor for rectangular wood perch use.

A major limitation of simple choice tests is that it is only able to give us a ranking of the substitutes (Kirkden and Pajor, 2006; Fraser and Nicol, 2018). It does not give us the ability to tell if the overall valence of the substitutes is positive, or the degree to which the animal is motivated to obtain the resource (Kirkden and Pajor, 2006). It is possible that laying hens find all of these perches as adequate resources to fulfill their motivation to perch. This is aligned with previous findings in the perching behavior of laying hens, with the time hens spent perching not differing between metal, plastic, and wood perches when given no choice in material (Lambe and Scott, 1998). Due to the limitations of perch shape options and varying confounding factors related to group size and differences in material and width, preference research has not yielded a clear consensus into which perch shape is most preferred by hens. One study of 20 month old breeder hens, that had previously had access to rectangular perches for several months, showed a preference for rectangular perches, while perch naïve hens of a similar age had an equal preference between round and rectangular perches (Muiruri et al., 1990). In another study, rectangular perches were found to be preferred over round perches by Hyline Brown hens 18 weeks of age (Chen et al., 2014) . This could possibly be due to the shape creating less strain on the keel bone, or a more comfortable position for the foot of the bird (Pickel et al., 2011). The rectangular wood perch in the current study was less preferred than the round metal perches, though this could be due to flaws in experimental design, such as differences in group size and differences in offered perch lengths for the three treatments. Another study found no difference in preference between hexagonal and round perchs by Lohmenn White hens 17 weeks of age (Liu et al., 2018). It is possible that the physical characteristics of the perch are not as important to the bird as other characteristics such as the height, arrangement, or location of the perch.

Another limitation of choice preference tests is that it is dependent on experience. The ability of each animal to learn must be considered when interpreting results, as well as early life experiences (Kirkden and Pajor, 2006). This can influence the applicability of preference results on different populations within the same species. The environment in which laying hens are raised can affect their perching behavior (Pullin et al., 2020), and the extent to which the hen's prior environment and experience influenced their behavior during the experiment is unknown. In the current study, the birds had prior exposure to the metal round perches, as well as some familiarity with wooden "perches" that were used in the multitiered aviary the birds were previously housed in. This could have influenced the results of the preference tests, with the possibility that the birds could be attracted to the familiarity of the round metal perches and rectangular wood, or the novelty of the mushroom plastic perches. In order to account for this, a six day acclimation period was used, and observations took place on the day before the perches were to be rotated. While it is possible that this was too brief a time to acclimate, it was likely sufficient based on the result of perch use not being significantly different between the round metal and mushroom plastic perches. As previously mentioned, another study found that 20 month old breeder hens that had previously had access to rectangular perches had a preference for the rectangular perches, while perch naïve hens of a similar age had an equal preference between round and rectangular perches (Muiruri et al., 1990). The hens in the current study did not show a strong preference for the perches that they had previously been exposed to compared to the mushroom plastic perches, possibly because they are not highly valued as a substitute by the birds, even with the previous exposure.

Effects of location on perch use

Hens in the current study showed a preference for perch location in the pen, with the majority of perching happening on one side of the pen, regardless of which perch type it offered. Due to the design, while the mushroom plastic and the round metal treatments were rotated, rectangular wooden perches were always offered on both sides. This is another confounding factor in the design, because there is no way to tell if the birds on the wood were there because they preferred that perching structure, or if they preferred wood to the other offered treatments. The preference for location could be the result of the group behavior of the birds. As previously mentioned, social grouping can have an effect on preference testing; in one experiment laying hens preferred 3 cm widths of perchs while in groups of one and four hens but not in a larger group size of eight hens (Chen et al., 2014). The birds in this experiment had been housed with one another before the experiment began so that they were familiar with one another and to minimize aggression. Perching is often a synchronous activity and antipredator behavior (Newberry et al., 2001), and it is possible that the ability to perch in locations close to other hens at night might be more important to the hen than the ability to perch on a preferred substance.

It is possible that this preference developed before the start of the study, and is possibly related to the multi-tiered aviary structure that was on one side of the pen the birds were previously housed in. Habituation to perching on one side of the aviary could have happened, but limited conclusions can be drawn about the reason for the location preference. Because birds were not identified on the individual level in this experiment, the extent of their past experience is unknown. The birds had been regrouped between studies, and it is possible that birds in this study had previously been given access to multi-tiered aviaries on both sides of the pen, or on only one side of the pen. The design of the experiment did not attempt to balance for what side of the pen the multi-tiered aviary was on for the hens before the start of the experiment, and the effect, if any, of moving the hens into new pens across the aisle of the building. It is also possible that the location preferences were related to external factors unrelated to former experience. Factors that could have influenced the results include the amount of available light or the side of the pen the door was opened on during animal husbandry. While steps were taken to standardize the pens, it is possible that one location was more suited for perching based on a factor that was not taken into consideration.

General Discussion

Several assumptions underlie the use of preference testing, and it is subject to limitations. Preference testing assumes that the animal will make the choice that best promotes its own welfare (Fraser and Nicol, 2018). This can break down with animals in human care, whose current environment and conditions can differ significantly from what is found in the wild (Dawkins 1983, 1990; Kirkden and Pajor, 2006). Laying hens have been highly selected for egg production, and therefore might be subjected to different welfare concerns compared to their ancestor, the red jungle fowl. A small study looking at red jungle fowl at 112 weeks of age found only one keel bone fracture in a sample size of 29 birds, and eleven of the 29 birds with keel bone deviations (Kittelsen et al., 2020). Keel bone damage was found to be higher in white leghorns than in red jungle fowl raised in identical conditions, with 10% of red jungle fowl and 69% of the white leghorns having keel bone damage at 80 weeks of age (Kittelsen et al., 2021). The value of the hen's preferences for differing perch design in light of the low level of keel bone fractures in the ancestral population means that their choices may not line up with what is best for them (Kirkden and Pajor, 2006).

Conclusions

Time of day was found to have a significant effect on perching behavior, with birds spending more time on perches during evening observations, this aligns with previous research, and is expected given its use as an antipredator defense (Brendler and Schrader, 2016; Campbell et al., 2016). No preference was found between the metal round and mushroom plastic perches during the evening observations, while the metal round perches were found to be preferred over the wooden perches. This does not align with previous findings that found wood to be a preferred perching material. Due to the nature of simple choice preference tests, unequal competition for substitutes and the prior experiences of the birds, the applicability of the information gained could be limited. Location was found to have a significant effect, the reason for this is unknown, but possibly is also related to prior experience. More research is needed in order to untangle the effects of location on perch preference, and find what physical characteristics make a perch the most suitable for laying hens. A systematic approach to shape, material and width should be conducted in order to see how these attributes affect the hens preferences. The use of younger, perch naïve hens should be used, ideally with pullets the same age as commercial hens when they are first exposed to perches. The preferences of the hens should be viewed within a larger framework for the hens welfare. In addition to preference testing, an evaluation of perches for their effects on hen health should be undertaken, with both footpad health and keel bone damage measured.

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