ABSTRACT

Title of Dissertation:	EFFECTS OF ENVIRONMENTAL ENRICHMENTS ON WELL-BEING MEASURES IN COLONY-CAGED JAPANESE QUAIL (*COTURNIX JAPONICA*)
	Chirantana Vikas Mathkari, Doctor of Philosophy, 2022
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Breeding purpose Japanese quail (*Coturnix japonica*) are often maintained in barren cages with little environmental complexity. These conditions can lead to unwanted social interactions and stress-related behaviors that can cause serious injury, mortality, and reduce productivity. Use of environmental enrichments has shown to improve poultry well-being; however, the optimal enrichments for quail have not been widely studied. The aim of this project was to evaluate the effectiveness of various enrichments on the well-being and productivity of breeding quail colonies (1 male, 2 females/cage). The enrichments studied were designed to reduce unwanted interactions and stress-related behaviors by either providing a shelter (protective enrichments) or by providing mental stimulation (stimulatory enrichments). In Experiment 1, using an incomplete Latin square design, each cage received one protective enrichment (Hut, Plastic leaves, or Grass), or one stimulatory enrichment (Mirror, Feeder toy, or Mat), or no enrichment (control) (average n=17/treatment). In Experiment 2, using an incomplete Latin square design, each cage received one of the following enrichment combinations: Hut + Mirror, Hut + Feeder toy, Hut + Mat, or only Hut (average n=14/treatment). Parameters measured included behaviors, body weight, Hen Day Egg Production (HDEP), egg weight, physical scores, and fecal corticosterone metabolites (FCM) levels. Measures were analyzed using a one-way ANOVA or Chi-square test on SAS 9.4. The results indicate that exposure to a single enrichment reduced stress-related damaging behaviors and increased resting; while exposure to a combination of enrichments reduced a larger variety of stress-related damaging behaviors more efficiently. Two of the three protective enrichments (Hut, Grass) exerted a notable impact on production, while combinations of protective and stimulatory enrichments were more efficient in decreasing physical scores as compared to a single enrichment. The Hut + Mat combination notably reduced the FCM levels as compared to only Hut. Our study identified enrichments which are optimal for improving breeding quail well-being, and exhibit the potential to improve the economics of the quail industry.

EFFECTS OF ENVIRONMENTAL ENRICHMENTS ON WELL-BEING MEASURES IN COLONY-CAGED JAPANESE QUAIL (*COTURNIX JAPONICA*)

by

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Dedication

To every non-human animal whose life is impacted by the actions of mankind. It is through knowing the lives of animals that I have become a humbler being. May they be freed from the cycle of death and re-birth.

Acknowledgements

Remember the last time you visited an amusement park? Upbeat pop music, aroma of the fried, sweet foods, rattling of the fast rides, and the adrenaline rushing through your blood as you fasten your seatbelt for the roller-coaster! You begin eagerly, run from one ride to another with your friends, and get so engrossed in the experience, that even before you know, the day has come to an end! This precisely describes my Ph.D.: a journey which provided me the wonderful platform to explore new ideas, to re-think norms, to live in the moment; and most importantly, to interact with new people. Most of these individuals have indeed made my journey an enthralling and fulfilling experience. Now as my day at the amusement park comes to an end, I would like to take this opportunity to thank each of them who were instrumental in making this journey worthwhile.

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Chapter 1: Introduction

Introduction

Production animals, especially poultry, form a major part of the food production system worldwide. The birds are reared under various housing and management conditions, each of which can impact their well-being differently. Safeguarding the well-being of domestic poultry, although the birds are ultimately utilized for consumption, is important for both, ethical and economic reasons. It can be ethical to ensure the well-being of farm animals, and certain laws might require a society to do so. Consumer preferences towards products from humanely treated animals is another reason to enhance animal well-being. Equally importantly, negative well-being can influence the birds' physical health, and can therefore be detrimental to production-based industrial profits.

Breeding quail form an important part of the quail industry and typical housing conditions form a major determinant of their well-being and productivity (Cheng et al., 2010; Narinc et al., 2013). A typical quail colony consists of males and females grouped in the ratio of 1:3 or 1:4, that share a barren cage continuously (Narinc et al., 2013). The lack of a stimulatory environment, coupled with continuous close exposure to the opposite sex, results in unwanted social and reproductive interactions such as feather pecking and excessive sexual mounting (Laurence et al., 2015) and self-damaging behaviors such as pacing, and over-vigilance (Blokhuis et al., 1991). Such behaviors can lead to feather loss, injuries, lameness, chronic stress, compromised immunity, and death (Jones, 2004; Meseret, 2016). Barren cages impact the birds' productivity (Blas, 2015) and the health of their offspring (Bertin et al., 2008; Houdelier et al., 2018). Therefore, environmental modifications that can decrease unwanted interactions and self-damaging behaviors by providing protection and/or mental stimulation, can improve breeding quail well-being.

One such environmental modification is the use of environmental enrichments. Environmental enrichments are additions to the birds' environment, that help them perform more natural behaviors, improving their biological functioning and productivity (Newberry, 1995). They provide the birds more control over their environment (Weinberg and Levine, 1980), and improve their capacity to cope with challenges, by reducing stress (Cheng et al., 2002). Several enrichments have been shown to improve birds well-being previously, yet are not implemented commercially mainly due to practicality issues (Riber et al., 2018). Thus, for an effective commercial implementation of enrichments, understanding the exact effects each enrichment category has on the birds' well-being is essential.

The aim of this project is to analyze the impact of two categories of environmental enrichments on breeding quail well-being. Three protective enrichments, that provide shelter from unwanted interactions, namely an acrylic hut (Hut), a patch of live grass (Grass), and plastic leaves (Plastic leaves); and three stimulatory enrichments, that keep the birds engaged and distract them from performing self-damaging behaviors, namely octagonal mirror (Mirror), rubber mat (Mat), and feeder toy (Feeder toy), were used to study the effect of enrichment exposure on quail behavior, physiology and production.

Chapter 2: Literature Review

Japanese quail

The domestic Japanese quail originated from the wild Japanese quail (Chang et al., 2007), and are presently used for varied purposes such as agricultural animals, laboratory models, and pets. Generally weighing between 100 to 150 grams (Mizutani, 2003), these small birds are sexually dimorphic, with the females exhibiting dark spots among the pale breast feathers (Balthazart et al., 1983). The spots are absent on the males' breast feathers, which are uniformly reddish-brown (Balthazart et al., 1983). The birds have short feathers that limit their flying ability; and upon being frightened, quail tend to scurry away rather than fly (Chang et al., 2005). However, unlike most of the other domestic poultry species, quail are migratory birds, and tend to migrate from breeding sites such as East and Central Asia, to the southern parts such as Vietnam, Southern China, and even Africa (Barilani et al., 2005). A prey species, they prefer low-laying vegetation habitats including grass and shrubs, and tend to seek hiding areas even under domesticated settings (Guesdon et al., 2011). This hiding behavior distinguishes them from most of the other domestic poultry that are naturally motivated to perch (Duncan, 1998). Japanese quail exhibit vocalizations that differ between the sexes; and a typical crow is characterized by two short parts followed by a final major trill (Barilani et al., 2005). Similar to most of the other domestic poultry, dust-bathing is a highly motivated behavior in quail; and occurs approximately corresponding to the third quartile of the photoperiod when given access to dust-bathing material (Statkiewicz and Schein, 1980). Quail exhibit a distinct and specific mating ritual, where the male grabs the female’s neck and mounts her, followed by a distinctive strut upon insemination (Correa et al., 2011). Excess of the mounting attempts, which are common in commercial quail breeding farms, have shown to results in injuries and mortalities in the female birds (Schmid and Wechsler, 1997). The egg color is seen to range from white to blue to pale brown, and weighs 10 grams on average (Kirkwood and Hubrecht, 2010). Feeding and drinking occurs in alignment with the photoperiod, and preferred feed include grass seeds and small insects (Mills et al., 1997). Compared to the commercial chicken, quail have a smaller body size, faster maturation, shorter incubation period, and are easier to handle and maintain (Shanawany, 1994).

The domestication of the Japanese quail goes back to at least 1500 years. As early as 770 BC, they were reared as song birds and used as sacrificial animals in China (Chang et al., 2005). Around 960 AD, Chinese rulers started rearing quail in captivity and breeding them for fighting and entertainment (Chang et al., 2005); with the Greek and Roman cultures exhibiting a similar trend contemporarily (Morgan, 1975). The first evidence of quail farming for food dates back to the mid-16th century, and the use of quail meat and eggs for medicine began a century later (Genchev, 2014). The extensive selection of Japanese quail in Japan during the early 20th century led to a population exhibiting substantially better egg-laying and meat traits than the other domesticated quail (Mills et al., 1997, Donchev & Angelov, 1971). This selection was a milestone in quail domestication (Genchev, 2014), making them considerably different from their wild counterparts- morphologically, behaviorally, and productively.

Today, the domesticated Japanese quail are used for a variety of purposes including meat and egg production, as laboratory models, as a pet bird, and occasionally as a fighting bird. The domestic quail population involved in production of meat and eggs represents around 11.8% of all production birds, and contributes to around 10% of all table eggs in the world (Conway, 2017) and 0.2% of the global poultry meat produced (Lukanov, 2019). This makes quail production second only to chickens. Quail farming has flourished in various parts of the world, with East Asia, Brazil, and Canada leading in egg production, and China, Europe and US leading in meat production (Minvielle, 2004; Bertechini, 2012). Quail farming is considered an ideal solution to the problem of animal protein shortage in developing countries, and an alternative to chicken in developed nations (Shanawany, 1994). Their small size, fast maturation, robustness, and laying ability make them an ideal laboratory model for animal science, developmental biology, veterinary and human medicine (Huss et al., 2008; Tserveni-Goussi and Fortomaris, 2011). The quail have been used as an experimental animal for producing vaccines against several diseases which they themselves are resistant to (e.g., Newcastle disease) (Shanawany, 1994). The quail genome has been entirely sequenced (Kawahara- Miki et al., 2013), which makes them suitable for genetic and epigenetic research, replacing the domestic chicken (Andraszek et al., 2014). Japanese quail are easy to handle and maintain, easy to tame and are small in size; all of which makes them a popular pet bird in many regions worldwide (Lukanov et al., 2018). Bird breeder associations across nations include the Japanese quail as a pet bird, and some like the European association of poultry, pigeon, cage bird, rabbit and cavy breeders are even preparing a European pet bird standard for them (Lukanov, 2019). In some Asian nations, including China, Afghanistan, and Pakistan, quail males are still used for fighting today (Mills et al., 1997).

The US is one of the biggest producers of quail products, with about 19 million birds raised annually (da Cunha, 2009). Quail farming is common in several US states, with Georgia, North Carolina, South Carolina, Alabama, and Mississippi leading the charts (USDA, 2011). A majority of the Japanese quail are raised for their meat and eggs in backyard settings (100 to 15,000 birds each year), with only a small number of commercial quail farms (producing 20,000 to 300,000 birds annually) present in the nation (USDA, 2011). The average life span of the quail ranges from 3 to 4 years, and the birds reach maturity around 6 weeks of age (Vali, 2008). They are processed for meat at around 8 weeks, upon attaining a weight of 100 to 150 grams (Mizutani, 2003). Egg laying begins around 8 weeks of age, and the females lay 250 to 300 eggs over a two-year production cycle, after which their production begins to decline (Mizutani, 2003). A typical quail egg is weighs roughly 10 grams (Mizutani, 2003). The management of quail is similar to that of other poultry species (Shanawany, 1994), and involves breeding stock, hatchery and brooding, and caged housing system.

Breeding quail form the backbone of the quail industry, as they are the source of the meat and egg laying birds. In an industrial setting, breeding quail are typically reared in small barren cages, where females and males are grouped in the ratio of 1:3

or 1:4, and share the cage continuously (Narinc et al., 2013). The lack of a stimulatory environment, coupled with continuous close exposure to females results in unwanted sexual behavior in males, such as excessive and sometimes injurious mountings causing injuries to the females who cannot escape (Galef et al., 2006). Such an environment causes unwanted social and reproductive interactions, like feather pecking, cannibalism, chasing and excess mounting with grasping, which decrease the time the bird can spend on productive routine activities like eating, drinking, egg laying, and resting (Laurence et al., 2015); ultimately taking a toll on the birds' productivity. Exposure to an enriched environment that provides protection from unwanted interactions and stimulates mental engagement with the environment can improve the birds' well-being.

Role of Welfare in Production Animals

Animals have been reared for food since centuries, but the methods of animal farming have gradually advanced in order to maximize the animals' output (Vigne, 2011). The rise of urbanization in the 1940s witnessed a shift from backyard farming to mechanized commercial farms (Dimitri et al., 2005). Commercialization helped increase the animals' output but their well-being was not necessarily cared for until the second half of the 20th century (Broom, 2011). Awareness of animal well-being likely grew as science advanced alongside the study of ethics. By the 1960s, scientific developments increased the acceptance of the fields of neurology and ethology (Broom, 2011). Parallelly, social movements like the women's suffrage movement and the civil rights movement gained momentum (Morris, 1999). Together, these developments conceptualized animal sentience, animal welfare and animal rights, and proved pivotal in the development of animal welfare standards (Fischer, 2004). A milestone that catalyzed the formalization of the standards was Ruth Harrison's 1964 book, Animal Machines, which exposed the inhumane treatment of commercial farm animals (Broom, 2011). A year after the book's release, the British government commissioned an investigation into the welfare of commercially farmed animals. The commission, led by Professor Roger Brambell, came to be known as the Brambell Committee, that developed the Five Freedoms of animal welfare as guidelines to help monitor farm animal welfare (Broom, 2011).

The Five Freedoms acted as a base to measure animal welfare at a given stage in the animal's life (Brambell, 1965). The freedoms enumerated were the 'Freedom from hunger and thirst', concerning the provision of fresh water and a balanced diet to ensure optimal health; the 'Freedom from discomfort' requiring the provision of an appropriate environment including a shelter and a comfortable resting area; the 'Freedom from pain, injury or disease', for the prevention and/or a timely diagnosis and treatment of factors which can cause pain, injury or disease; the 'Freedom to express normal behavior' dealing with sufficient space, enrichment, and company of conspecifics; and the 'Freedom from fear and distress', concerning the provision of conditions and treatment that would prevent psychological suffering (DEFRA, UK Government archive). The freedoms are regarded worldwide as the pillars of animal welfare and are used commonly to measure welfare even today (McCulloch, 2013).

The advancements in measuring animal welfare seem to have helped materialize several animal welfare laws in various parts of the world. Among these, some laws are solely dedicated to farm animal welfare. For example, the Welfare of Farmed Animals Regulation, 2007 in the U.K. sets the minimum welfare standards for farm animals while reared on the farm (Welfare of Farmed Animals Regulation, 2007). Other laws are dedicated to non-human animals in general, and are therefore appliable to farm animal well-being. For instance, the Prevention of Cruelty to Animals Act, 1960 in India, which prohibits the infliction of unnecessary pain or suffering on all non-human animals, including farmed ones (Prevention of Cruelty to Animals Act, 1960). Some laws apply nationwide, such as the Twenty-Eight Hour Law, 1906 in the U.S. that requires unloading and the provision of rest, feed and water if the animals are being transported for 28 hours or more (Twenty-Eight Hour Law, 1906). Some others are not mandated federally but are followed at state levels. An example is the Prevention of Farm Animal Cruelty Act, 2008, followed in California, U.S. which requires pregnant sows, veal calves, and layers to be provided adequate space to stand up, lie down, move around freely, and fully extend all limbs (Prevention of Farm Animal Cruelty Act, 2008).

Ensuring optimal welfare might not be achievable through these laws given the diverse farming techniques implemented and the current gap in measuring welfare accurately. Several laws tend to use a more generalized approach while accounting for welfare standards, which although applicable across numerous farming methods, is likely to result in loopholes. For instance, India's Prevention of Cruelty to Animals Act, 1960 mentions the provision of reasonable movement opportunity in cages but does not discuss species-specific requirements that may vary within species including birds and mammals, preys and predators (Prevention of Cruelty to Animals Act, 1960). Therefore, making the current welfare measures more accurate and developing new welfare parameters to measure welfare can help make such laws more specific and tailored for improving farm animal welfare.

Evaluating Welfare Assessment Methods

Measuring behavior through ethograms

Measuring behavior is a non-intrusive technique to assess welfare, as it reflects the animal's current state of physical and mental health (Weary et al., 2009). One of the methods of measuring behaviors is through an ethogram. An ethogram is a catalogue of defined behaviors performed or expected to be performed by an animal or group of animals (Mench, 1992), and therefore aids observing and categorizing behaviors. It helps assess behavior based on the duration and frequency of a particular activity listed in the ethogram. Activities can be of two types, namely, state and event. A state is an activity which is exhibited for a longer duration and can include standing, sitting, and lying down. Unlike states, events are the activities performed in bouts and include sexual mounting, feather pecking, and preening.

Ethograms are generally built based on both behavioral states and events, and are built in alignment with the study objectives (Banks, 1982). For example, a study examining the effects of barrier perches in broilers had an ethogram of consisting of three behaviors- perching, activity, resting (Bench et al., 2016). While the ethogram of an environmental enrichment study in quail included behaviors like physical contact with or nearness to the enrichment in addition to routine activities like resting, preening, moving (Miller and Mench, 2005). It can be useful to design species-specific ethograms which clearly define and distinguish each of the species' behaviors. For example, water-fowl may exhibit different behaviors than land birds given their environment (Johnsgard, 1965), and the ethogram for them can include behaviors like paddling, resting near water source, and bathing. The definitions of some poultry behaviors lack standardization, making them open to interpretation. For instance, a study on chickens defined preening as 'when the bird arranged its feathers with its beak' (Villagra et al., 2014), while another defined preening as 'the bird preens its feathers with the beak and stimulates the uropygial gland' (Tavares et al., 2015). Some less common behaviors including severe feather pecking, are studied commonly but tend to lack a complete description of each state or event seen during these behaviors (McGlone, 1986). Therefore, species-specific ethograms should include the less common behaviors in addition to the more regular ones, in order to improve standardization.

In certain non-poultry livestock species, attempts seem to have been made to categorize ethograms to make their implementation easier. For example, Hall in 1989 divided cattle behaviors as follows- maintenance, self-expression, and social. This has made a significant contribution in understanding cattle behavior better (Kilgour, 2012). Specific ethograms are also being used to develop precision tools for behaviors whose etiology is not entirely known. Some examples include a software to measure ear biting in pigs (Diana et al., 2019), and real-time behavioral tracking in cattle to aid in the design of better ethograms (Dao et al.,2015). The use of technology exhibits the potential to improve the reliability and validity of ethograms.

Physical Scores

Physical scores are commonly used as a welfare assessment technique on farms (Blokhuis et al., 2007). They are indicative of physical health, and are measured as a visual assessment of body parts or regions including the head, neck, back, toes (Blokhuis et al., 2007). Some common assessment methods are discussed below.

1. *Injury score*

Injury score measures the physical damage or harm to the animal caused by various factors including accidents, wounds, fractures, and bleeding (Bilcik and Keeling, 1999). The scale helps quickly diagnose the animal's physical health and/or carcass quality by measuring the extent and severity of external and internal injuries respectively (Grandin, 2015). The technique can be implemented remotely, especially in live large animals.

In poultry, injuries may occur during various managemental stages including handling, transport and slaughter. Injuries can be self-induced or a result of conspecific-related activities including crowding, piling and aggression (Jones, 1996) or a result of improper management and housing (Grandin, 2015). Welfare Quality's assessment protocol for poultry provides a guideline for measuring injuries in chicken broilers and layers on farm and at the slaughterhouse (Arnould and Niekerk, 2009). The scoring system concentrates on specific body-regions including the breast, hock, and wings, with each part scored on a scale of 0 to 2, where 0 indicates absence of injuries, and 2 indicates severe injuries covering more than 50% of the body part (Arnould and Niekerk, 2009). The scale has been implemented by broiler researchers such as Federici et al.,2016, de Almeida et al., 2017. However, the system excludes the scoring of other body parts like the head, which, if injured, can have a severe impact on the birds' health. Additionally, the system is not applicable to other poultry species such as quail, ducks, and turkeys, which seems to have resulted in the use of varying injury scoring systems by researchers who study these species (Robison et al., 2015; Hanafy et al., 2018; Grün et al., 2021). Designing a general examination system of transport injuries has been suggested (Grandin, 2015), but no standard guidelines currently exist. A more nuanced and detailed injury scoring system that accounts for different body areas, and various managemental stages can prove useful in better evaluating poultry welfare.

A lack of a standard injury scoring system has been reported in non-poultry livestock species (Baxter et al., 1983), making it difficult to compare and analyze one injury score study with the others. Similar to poultry, insufficient standardization has resulted in the use of non-standard and/or modified injury scoring systems in livestock species (Eldridge and Winfield, 1998; Livesey et al., 2002; Cook et al., 2016). Species-specific injury scoring systems which address specifications including the animal's life stage, rearing purpose, anatomy, and physiology, should be developed.

2. *Feather score*

Feather score is a reflection of the feather cover of the bird and is closely linked to feather pecking (Webster and Hurnik, 1990). Feather pecking is the act of pecking a conspecific, and can result in severe feather pecking, which harms the receiver's welfare (Rodenburg et al., 2004). Feather scores are commonly used as a flock measure of welfare as they are a good indicator of the level of aggression and/or cannibalism present in the flock (Webster and Hurnik, 1990). A scale of 1 to 5, with 1 being fully feathered and 5 being poorly feathered with naked spots is seen to be used commonly by researchers (Taylor and Hurnik, 1994; McAdie et al., 2005; Mahmoud et al., 2015). However, different scales have also been suggested. For example, a study on layer hybrids used a reverse scoring scale of 1 to 4, where a score of 1 symbolized bare skin, while that of 4 meant complete feather coverage (Yamak and Sarica, 2012). Both the systems are prone to subjectivity and can also cause poor inter-study comparisons. Welfare Quality's poultry assessment protocol (Arnould and Niekerk, 2009) seems to overcome the barrier of subjectivity to a considerable extent. It analyzes the feather cover in three pecking-prone body regions including head and neck, back and rump, and belly, by measuring the bare skin area in centimeters (Arnould and Niekerk, 2009). The use of this protocol in poultry species apart from chicken needs to be done with caution, as the bare skin area symbolizing a particular score may vary from species to species depending on the total body area. Developing a software that can photograph and measure the bare skin areas from various body regions and interpret them species-wise, can likely help obtain more reliable feather scores.

3. *Footpad/Feet Score*

Footpad score measures the physical damage to the plantar surface of the bird's feet and reflects several foot health issues (Ekstrand and Algers, 1997). For example, the score can provide a quick assessment of the existence and severity of footpad dermatitis, a welfare problem in production flocks (Mayne, 2005).

Additionally, the score can help identify other lesions, which can potentially impact the birds' ability to access food and water. A footpad scoring system has been proposed by a Swedish study on broilers (Ekstrand et al., 1998), where the lesions are scored from 0 to 2, with 0 indicating no lesions, 1, mild lesions and 2, severe lesions. This system is seen to be followed in a few broiler studies in Europe such as Meluzzi et al., 2008 and Nielsen, 2009. However, several other studies on chicken welfare tend to use different systems such as a 1 (no lesion) to 9 (very severe lesions) score (Kjaer et al., 2006). Another system was designed and used by Martrenchar et al., 2002, where the lesions were scored from 0 (no lesion) to 3 (lesions covering more than 50% of the footpad); and has been followed by some other researchers including Pagazaurtundua and Warriss, 2006, and Jacob et al., 2016. A lack of standardization in measuring footpad scores is visible across poultry species. For instance, the scoring system for turkeys used by Hocking et al.,2008, scored the feet from 0 (no lesion) to 4 (necrosis of more than 50% of the footpad); while a study performed in Japanese quail (Mohammed et al., 2017), scored the feet solely on the presence or absence of lesions. The commonly used but non-standardized footpad scoring led to a review for validating its use. The study by Heitmann et al., 2018, was conducted to evaluate a scoring system of 1 (no lesion) to 4 (large and deep lesion/s) against the backdrop of previous literature. The analysis was done based on visual scoring, interobserver reliability, macroscopic and microscopic measurements, and the interobserver reliability held valid for only half of the observations; indicating the need for a simplified version of the scale (Heitmann et al., 2018). Development of a standard scoring system for every poultry species, with a clear line of segregation between each level, and accounting for the width and depth of the lesions can improve interobserver reliability and therefore, the system's validity.

Feet scoring scales in non-poultry livestock seems fairly standardized for the individual species. For instance, a scale of 0 to 9 is seen in cattle and pigs, and 0 to 4 in sheep. The scales are nuanced, which can actually improve the accuracy of the scoring system (Norton et al., 2019). For instance, Van der Waaij et al., 2005 analyzed the feet scores in cattle using the 0 to 9 scale, but measured the rear and the side views of the feet separately before obtaining an average score. Another study divided each hoof into five parts and scored each part individually before averaging the hoof score (Logue et al., 1994). However, the increased scoring time due to the details can impact the inter-observer reliability, and the validity of the system needs to be weighed against its reliability.

Production parameters

Production is closely linked to the animal's physical and mental health, and can therefore reflect its well-being state (Thornton, 2010). Production measures are largely non-intrusive, easy to perform (Botreau et al., 2007), making them a convenient technique to gauge the animal's welfare. Some common production measures have been discussed below.

1. *Feed efficiency*

Feed efficiency is an important production parameter as feed accounts for 60-70% of the costs of the poultry system (Williams et al., 2013). Feed efficiency is largely dependent on the birds' body weight and feed intake. Body weight and feed intake are impacted by the presence of stress (Gross and Siegel, 1980; Lin et al., 2006) and are therefore a reflection of the bird's well-being state.

Measuring body weight periodically provides an estimate of the birds' growth (Turner et al., 1984) and is performed using diverse techniques. Weights are measured in groups which is often seen in chicks, and are measured individually in adult birds and experimental birds (Noy and Sklan, 1999). Weighing methods can be manual, where the birds are placed on a digital scale and the weight is recorded; or less intrusive, with the use of hanging scales and platform scales, where the birds get weighed upon stepping on them (Doyle and Leeson, 1989). Research on developing newer, non-invasive weighing techniques is seen as manual handling is labor intensive and can be subject to error (Lott et al., 1982). Examples include wireless environmental sensors which measure the air experienced by the bird and use the data to estimate the average body weight (Jackman et al., 2015), and the use of an electromagnetic waves to estimate body composition and weight (Scott et al., 2001).

Body weight in livestock is performed either by a weighing scale or as an estimation of morphometric parameters like chest girth and body length (McCulloch and Talbot, 1965). A drawback of the manual methods is that they can be cumbersome and can injure the farmer and/or the animals. Automatic methods such as interactive analysis software (Guo et al., 2017) have been developed, but their practical implementation has been questionable due to the high cost.

Feed intake measures in poultry reflect the birds' nutrition and health but may not be performed as regularly given the practical restraints such as manually collecting and measuring feed wastage (Aydin et al., 2014). The measure is observed to be performed at varying frequencies, as per the requirement of the study. For example, a study which observed the effects of an additive fed until 13 days of age, measured the feed intake only at the 13th day (Leeson et al., 2000); while another feed additive study measured the feed intake on a daily basis (Chen and Yu, 2020). The method of measuring the feed intake might impact the accuracy of the measure. Some studies measure individual feed intakes (Gleaves et al., 1977; Akhtar et al., 2003), whereas many other measure pen/group feed intake (Marks, 1985; Pettersson and Aman, 1989; Cave, 1982). Therefore, improving the design of this measure by considering nuances including the feed texture, the housing system, the bird's age, the time gap maintained between offering the feed and measuring the unconsumed feed, can help standardize the measure.

Feed intake measures for livestock are more feasible on an individual basis, however, the practice can prove labor intensive and uneconomical in some cases (Arthur and Herd, 2005). Automation in measuring feed intake can help over these barriers and are being researched in the recent decades (Edan et al., 2009). For instance, a real-time monitoring and control system was developed by Banhazi et al., 2012, to collect the feed intake information in a timely and cost-effective way. However, the systems exhibit some downsides which can prevent the on-farm implementation of the systems. Brown-Brandl and Eigenberg's work on livestock feeding behavior monitoring systems (2011) revealed that the systems showed high accuracies (98.3% in cattle and 98.7% in pigs) but affected the animal's natural feeding behavior.

2. *Egg characteristics*

The study of the effects of hen welfare on egg production and quality began in the early 21st century (Sossidou and Elson, 2009). A majority of the early welfare studies focus on the egg quantity, which is measured as the hen day egg production (HDEP) (Sossidou and Elson, 2009). This measure is a valid parameter of the total eggs laid by a hen or a group of hens over a certain time period, and is less prone to error (Sossidou and Elson, 2009). However, HDEP may not be an indicative of the individual hen's welfare due to the common method of group housing the hens. The reliability of the measure may dwindle especially in a free-range setting, where the hens have more freedom of space and eggs can be laid even on the floor. The quality of the eggs is an equally important measure given the impact stress can exert on it (Roberts, 2004). Egg quality is determined by certain external and internal indices, which seem fairly standardized. An example is egg weight, the measuring ease of which make it a highly standardized egg quality measure (Roberts, 2004). Similarly, shell thickness, albumin height and yolk height are measured using standardized methods (Roberts, 2004). However, different instruments have been utilized to measure the same parameter. For example, a study used a tripod micrometer was measuring the albumin and yolk heights (Tadesse, 2012), but another study used vernier caliper to measure the same (Dorji and Phurba, 2014). The rationale of using the different instruments (minimum length each can measure is different) cannot be justified by the subject characteristics as both the studies used exotic chickens. Such differences can make the comparison of the respective indices difficult. Measures which can help assess welfare better, like presence of red spots, smell of the egg, chalaza characteristics, have not been well-defined on scales in relation to hen welfare, likely due to subjectivity issues. New methods of measuring egg quality (Dunn, 2011) and the use of technology to measure them (De Ketelaere et al., 2004) can prove useful in assessing hen welfare and trans-generational welfare better.

Role of Environmental Enrichments in Poultry Well-being

Until the first quarter of the 20th century, most domestic poultry were reared in backyards (Bessei, 2018), where the expression of natural behaviors was feasible. However, with the rise in urbanization in the 1940s, mechanization of the farms started, where birds were reared indoors in closed and barren spaces (Bessei, 2018), reducing the birds' exploratory opportunities. Gradually, as the link between the lack of exploratory opportunities and stress was established (Nicol and Guilford, 1991; Newberry, 1999), the concept of enriching the birds' environment surfaced (Jones, 1996).

Environmental enrichments play an important role in enhancing poultry well-being as they offer the birds an opportunity to express natural behaviors in the current unnatural commercial housing conditions. The term 'enrichment' is an improvement or enhancement in quality (Cambridge English dictionary); and therefore, an environmental enrichment is a modification of the bird's environment done with the aim of improving their life quality (Newberry, 1995). Environmental enrichments include additions to the environment which help increase the occurrence and range of the bird's normal or species-specific behavior, prevent the development of abnormal behavior or reduce its extent and complexity, increase the positive exploitation of the environment, and increase the bird’s ability to handle behavioral and physical challenges (Newberry, 1995). Thus, environmental enrichments improve the bird’s biological functioning by reducing stress (Newberry, 1995). Reduction in stress is known to improve the birds’ immunity, liveability, and production potential (Hill, 1983) and therefore the use of enrichments can help improve the farm economics.

Environmental enrichments are of various types including foraging, sensory, structural and social. Foraging enrichments promote foraging and comprise of feed-related modifications including treats filled in tubes (Lumeij and Hommers, 2008), as well as the provision of whole food items including vegetables and fruits (Lima et al., 2019). A foraging enrichment for domestic poultry can also involve access to pasture (Dal Basco et al., 2014) or exposure to a foraging mat (Sandilands et al., 2021). Sensory enrichments help stimulate the bird’s senses including olfaction, sound, touch and sight. Examples of sensory enrichments include exposure to radio or music (Gvaryahu, 1989), access to a sand box (Bubier, 1996), exposure to ultra-violet light (Jones et al., 2001), use of compact discs (Veldkamp, 2012) and mirrors (Zahoor et al., 2022), and exposure to computer screens (Jones, 2001). Structural enrichments are physical additions to the bird’s environment and may attempt to mimic the bird’s natural habitat. In domestic poultry, a wide range of structural enrichments exist such as perches, swings, nest boxes, hay, and artificial leaves (Riber et al., 2018). The use of structures that help the bird hide including a hut or a wall separation are also a part of structural enrichments. Social enrichments involve modifications to the bird’s social environment, and entail exposure to conspecifics in varying group sizes and varying stocking densities (Meseret, 2016). Several environmental enrichments have been studied previously, and their impact on well-being as a measure of behavioral, physical, and production measures are discussed below.

Effect of Enrichments on Behavior

Behavior has been commonly used as a measure of understanding the impacts of environmental enrichments on poultry well-being (Riber et al., 2018, Campbell et al., 2019). Behaviors may be categorized as maintenance behaviors including eating, drinking and sitting; exploratory behaviors including cage pecking and litter pecking; and damaging behaviors comprising severe feather pecking, excessive sexual mounting, and stereotypies like pacing and vigilance.

Maintenance behaviors like feed and water consumption exert a direct impact on the bird's well-being, and therefore measuring them is crucial while evaluating the effects of environmental enrichments (Bloomsmith et al., 1991). Several studies show that environmental enrichments do not impact the birds' eating and drinking behaviors in species including turkeys (Crowe and Forbes, 1999; Sherwin et al., 1999; Huff et al., 2003), chicken (Bizeray et al., 2002; Aksit et al., 2017; Vasdal et al., 2019;), ducks (Chen et al., 2015) and quail (Miller and Mench, 2005; Laurence et al., 2015). However, changes in the feed presentation can impact the birds' eating and drinking behaviors. For example, exposure to grain distributed over litter material (instead of being placed in feeder) resulted in more feeding behavior in broiler chickens (Stadig et al., 2017) and layers (Hartcher et al., 2015). Similarly, aiding the birds' ability to view the feed also seems to have an effect on their feeding behavior. For instance, use of natural light as an environmental enrichment in broiler chickens was seen to increase the time the birds spend eating (Bailie et al., 2013; de Jong and Gunnink, 2019).

Exploratory behavior is a natural behavior in birds, and is generally performed by pecking a surface or an object (Duncan, 1998). It is a highly motivated behavior and is considered an indicator of a positive welfare state (Duncan, 1998). The impact of environmental enrichments on exploratory behaviors seems to be largely dependent on the characteristic of the enrichment. Food-related enrichments are seen to positively impact exploratory behaviors in various poultry species. For example, the exploratory behaviors increased in broilers upon exposure to peat and bales of lucerne hay (Vasdal et al., 2019) and through the use of scattered whole wheat as an environmental enrichment (Pichova et al., 2016). Similarly, environmental enrichments which can be pecked by the bird increase their exploratory behavior. For instance, an increase in the exploratory behavior was observed in laying hens who were exposed to pecking strings (Hartcher et al., 2015), quail that were exposed to plastic green grass (Laurence et al., 2015), and turkeys that were exposed to blunt metal objects (Martrencher et al., 2001). In contrast to these, environmental enrichments which do not provide a suitable pecking surface, do not tend to impact exploration. An example is the use of panels and elevated platforms in broilers, which led to the exhibition of a very low frequency of exploratory behaviors (Bach et al., 2019). However, how the long-term use of non-pecking friendly environmental enrichments can impact the birds’ desire to explore other surroundings remains largely unknown.

Damaging behaviors are the behaviors performed by the bird that can damage the bird and/or its conspecifics (Duncan, 1998). They can ultimately result in serious injury and mortality, impacting the flock's production. Some examples of damaging behaviors include severe feather pecking, pacing, and excessive sexual mounting (Riber et al., 2017). The use of environmental enrichments has shown to significantly reduce such behaviors, and improve the birds' welfare (Relic et al., 2019). One such damaging behavior, which is seen to be decreased across species with the use of environmental enrichment is severe feather pecking. In laying hens, the frequency of severe feather pecking was reduced two-fold when supplemented with an environmental enrichment (van Staaveren et al., 2021). In broiler breeders, materials that promote foraging and dustbathing have successfully reduced severe feather pecking (Riber et al., 2017). Environmental enrichments that are stimulatory in nature tend to decrease severe feather pecking in other poultry species too, as they likely either distract the birds' attention or re-direct their pecking. Such enrichments have shown to reduce severe feather pecking in ducks (Colton and Fraley, 2014), turkeys (Crowe and Forbes, 1999; Lindenwald et al., 2021) and quail (Miller and Mench, 2006; Laurence et al., 2015; Taskin and Karadavut, 2017). Another damaging behavior which can be impacted by environmental enrichments is pacing. Environmental enrichments have decreased pacing behavior especially in quail (Miller and Mench, 2005; Laurence et al., 2015). Environmental enrichment studies that do not measure pacing behavior may instead measure resting behavior as an indicator of decreased pacing. Environmental enrichments are seen to improve resting (standing/sitting) in domestic poultry including broilers (Ohara et al., 2015; Bach et al., 2019) and layers (Mishra et al., 2005; Moroki and Tanaka, 2015). At times, increased durations of walking may be an indicator of pacing, when no distinction is made in the ethogram. However, in some cases, like broiler chickens who often exhibit locomotory ailments, increased walking can be regarded as an indicator of positive welfare. The use of laser pointers as an environmental enrichment in broilers increased their activity levels, including the time spent walking (Meyer et al., 2019). Therefore, walking behavior may not always be a reliable indicator of pacing. A third damaging behavior, especially common in breeding birds, is excessive sexual mounting. Eighty percent of the mating acts are forced in broiler breeders (de Jong et al., 2009), and the percentage is likely to be similar in other poultry species, given the common housing methods. However, not many enrichment studies seem to measure mating behavior, as it is less frequent in meat birds which are generally processed before reaching sexual maturity and egg-laying birds which are housed in same sex colonies. Environmental enrichment studies that have been conducted in breeder birds indicate an impact of environmental enrichments on decreasing sexual mounting. For example, sexual mounting has been observed to reduce in broiler breeders exposed to perches (Adeniji, 2012), cover panels (Leone and Estévez, 2008) and a moving fence between the males and females (Van Emous, 2010). Apart from physical and visual separations, environmental enrichments related to light changes have also shown promising results. Exposing broiler breeders to UV-A light has shown to modulate their sexual behavior positively (Jones et al., 1999; Jones et al., 2001).

Therefore, it can be concluded that enriching the birds’ environment can alter their behaviors and social interactions positively.

Effects of Enrichments on Physiology

Environmental enrichments are known to affect the birds' biological functioning (Newberry, 1995); and this can be measured through the changes in their physiology. Techniques used to measure physiological changes include physical scores such as feather score, footpad score, and injury score; and hormonal changes such as levels of corticosterone, serotonin and adrenaline.

Feather scores can reflect of certain unwanted behaviors including severe feather pecking and excessive sexual mounting, and has been measured in environmental enrichment studies along with these behaviors (e.g., Mason et al., 2010; Hartcher et al., 2015; Tahamtani et al., 2016). The effect of environmental enrichments on feather score seems to be more widely observed in layers as compared to broilers, plausibly due to the low aggressive behavior in broilers and the later onset of severe feather pecking and cannibalistic behaviors in domestic poultry (Rodenburg et al., 2013). A recent meta-analysis of layer studies concluded that the addition of a stimulatory or a foraging enrichment resulted in a significant decrease in the feather scores (indicating better coverage) of the birds (Staaveren et al., 2021). The feather score remained unchanged in broilers in a study that measured the impact of exposure to bunches of strings and bales on welfare (Hocking and Jones, 2006), likely due to the enrichment's structural role. However, structural enrichments such as elevated plateaus improved the feather score in turkeys (Lindenwald et al., 2021). The positive effects of environmental enrichments on feather scores are visible in other poultry species including ducks, where improvements in feather scores have been observed upon exposure to free range (Reiter et al., 1997), and stimulatory objects such as balls (Colton and Fraley, 2014; Chen et al., 2015) and ribbons (Chen et al., 2015); and in quail exposed to enrichments such as saw-dust bedding and sand-bathing substrates (Nordi et al., 2012) which could re-direct pecking.

Injury scores help determine the extent of skin lesions present on the bird at a particular time (Bilcik and Keeling, 1999), and maintaining a record of the scores can serve as an index of the bird's long-term welfare condition. In environmental enrichment studies, injury scores can serve as a measure of safety in the use of the enrichment by the birds. Similar to feather scores, the effects of injury scores seem more prominent in layer birds as compared to broilers, possibly due to the later onset of damaging behaviors. Layer birds enriched with pecking objects such as pecking stones and pecking blocks exhibited lower injury scores than the unenriched ones (Liebers et al., 2019; Schreiter et al., 2020), but such effects were not seen in broilers exposed to pecking objects (De Jong and Gunnink, 2019). A variety of enrichment categories are seen to improve injury scores in turkeys. Structural enrichments such as perches were seen to improve injury scores in turkeys (Crowe and Forbes, 1999; Martrenchar et al., 2001; Lindenwald et al., 2021), and a similar improvement was observed on exposure to foraging enrichments including scattered grain (Crowe and Forbes, 1999) and stimulatory enrichments like metal objects (Martrenchar et al., 2001).

Footpad scores are a technique to assess the physical condition of the birds' footpad (Ekstrand and Algers, 1997), which in turn can impact its walking ability. The footpad conditions are impacted by various environmental factors including the litter quality, drinker design, season, and stocking density (Shepherd and Fairchild, 2010) and can therefore prove to be a good measure in analyzing the impacts of several enrichments such as structural, foraging, and stimulatory. In broilers, the use of structural enrichments has shown to improve footpad conditions. Examples include perches (Groves and Muir, 2013; Yildirim and Taskin, 2017), vertical panels (Cornetto and Estevez, 2001), natural light (Bailie et al., 2013) and straw bales (Baxter et al., 2018). However, other studies show that the use of such environmental enrichments in broilers have not necessarily improved their overall leg health (Nielsen, 2004; Karaarslan and Nazligul, 2018), and further studies are required to understand the mechanisms through which the enrichments impact feet. Relatively more consistency is seen in the impacts of structural enrichments on leg health and footpad in layers. For example, improved footpad and leg conditions were seen in layers with the use of ramps between perches (Heerkens et al., 2016) and a combination of dry litter and perches (Wang et al., 1998). Footpad scores seem to be used commonly in assessing the effects of environmental enrichments on ducks, and show changes based on the enrichment's moisture level. Footpad scores were higher (worse condition) in ducks exposed to deep litter (Abdel-Hamid et al., 2020) and water troughs (Schenk et al., 2016), while those in the ducks exposed to a plastic ball were lower than the control (Colton and Fraley, 2014).

Corticosterone is the major corticosteroid in birds and plays an important role in the bird's stress response (Etches, 1976; Wodzicka-Tomaszewska, 1982; Donker and Beuving, 1989). It exhibits temporary elevation in response to acute stressors, and helps the bird re-gain a state of homeostasis (De Schraaf and Kloet, 2000).

Higher and sustained levels of corticosterone are seen in response to chronic stressors, and prove detrimental to the bird's health (De Schraaf and Kloet, 2000). Corticosterone levels can be measured through various analyses including blood, feces, feathers, and egg. However, sample collection is intrusive in some of these methods (e.g., blood sample), and can potentially decrease the reliability of the results if not performed quickly after restraint (Blas, 2015). Environmental enrichment studies in poultry which measure corticosterone levels have commonly done so using blood plasma samples (Kannan and Mench, 1996; Al-Aqil et al., 2009; Adeniji, 2012; Hafizah et al., 2015). The use of feather samples to assess corticosterone has also been performed in birds (Fairhurst et al., 2011; Weimer et al., 2018). Environmental enrichment obtained by controlling the room temperature is seen to reduce the birds' plasma corticosterone as compared to hotter environments (Al-Aqil et al., 2009; Adeniji, 2012). However, exposure to music increased the plasma corticosterone levels in broilers initially, before making them no different than the control (Hafizah et al., 2015). These results were similar to the initial increase in the feather corticosterone levels in nutcrackers (Fairhurst et al., 2011), which indicate towards the need to evaluate the levels more often during the experimental period. Increasing sampling frequency is more feasible through the use of non-invasive sampling techniques such as fecal samples, which reflect the corticosterone levels within the last few hours (Blas, 2015). Additionally, fecal corticosterone measures surpass the need for handling and therefore do not cause a handling stress induced corticosterone rise which can interfere with the results (Palme et al., 2005). Corticosterone is extensively metabolized in the liver and is therefore measured as metabolites in the feces (Klasing, 2005). The effects of enrichments on fecal corticosterone remain largely unknown with varying results observed. For instance, the hormone levels remained unchanged after exposure to a nest box (Engel et al., 2019), however, another study exhibited an increase in levels when birds were deprived of a nest box (Alm et al., 2016). Exposure to foraging enrichments which acted as a fiber source increased fecal corticosterone levels (Dawkins et al., 2004; Alm et al., 2014), but exposure to pumice stone and alfalfa hay decreased the levels significantly (Son et al., 2022).

Effects of Enrichments on Production parameters

Production parameters including body weight and egg production play a key role in the economics of poultry farming and are therefore a regular measure of assessing the impacts of environmental enrichments on poultry (e.g., Leone and Estevez, 2008; Hartcher et al., 2015; Yildirim and Taskin, 2017). There is a perception that the use of environmental enrichments can reduce productive performance (Estevez, 2009), which could further encourage researchers to include production measures in enrichment studies. In chicken studies, several environmental enrichments have been found not to have a negative impact on the birds' productivity (Nicol, 1992; Appleby et al., 1993; Fiscus-le Van et al., 2000; Pettit-Riley and Estevez, 2001) although some exceptions exist. According to a recent review by Riber et al. (2018), barriers, panels and novel objects did not impact the broilers' body weight, but a few enrichments which increased activity (e.g., scattering food) did reduce body weight. Contrary to this, body weight gains have been noticed in sound-based environmental enrichments including classical music (Gvaryahu et al., 1989) and playback of hen vocalization (Woodcock et al., 2004). In turkeys, similar to chickens, several studies reported no effect of environmental enrichments on the birds' body weight (Sherwin et al., 1999; Lewis et al., 2000; Martrenchar et al., 2001; Lindenwald et al., 2021), with the exception of a study that demonstrated an increase in body weight after exposure to a combination of stimulatory enrichments such as plastic balls and mirrors (Huff et al., 2003). The effects of environmental enrichments on egg production are seen assessed through certain egg parameters including egg number, cleanliness and eggshell quality. For example, in chicken broiler breeders, the use of perches decreased the number of eggs laid on the floor (Appleby et al., 1998), and the use of wood-shaving bales improved their eggshell quality (Edmond et al., 2005), without impacting the total egg produced. However, similar to the effects of enrichments on body weight, the effects on egg production also exhibit certain exceptions. In layers, exposure to pecking stones and alfalfa bales during the laying stage increased the egg weights (Schreiter et al., 2020). Therefore, it can be concluded that a majority of the enrichments do not impact the birds' production negatively, but further research is required to analyze the impact of specific environmental enrichments on production parameters.

Gaps in Research on Environmental Enrichments

Based on this review, current literature indicates that much attention has been given to analyzing the effects of environmental enrichments on layers or broilers. In comparison, our knowledge of the environmental enrichments needed for the poultry breeding population is relatively limited. This situation is particularly true for breeding quail welfare, which seems to have received rather less focus. Currently, the well-being of breeding birds is quite compromised (Mench, 2002; Hristov et al., De Jong and Gunnink, 2019) and the use of optimal environmental enrichments in the colonies can help ameliorate the situation.

Optimal environmental enrichments should meet the need of the species and do so in accordance with the rearing purpose of the species. Given quail's preference for low-lying habitats (Balthazart et al., 1983), an enrichment that provides a perching space might not be as well fitted for quail as would one that provides them a hiding area. Appropriate enrichments would include those that help reduce damaging behaviors and unwanted interactions in the particular species. For example, a protective hut can be used in breeders to give the females and the less dominant males a place to escape the dominant males' pecking and/or mounting. A stimulatory enrichment, like a mirror, which would distract the birds from performing harmful interactions could prove useful in breeding colonies. Thus, tailoring enrichments to satisfy the birds' needs is required in order to improve the well-being.

Several environmental enrichments which have been studied previously are not free of lacunae, and this can deter their use on a commercial level. For example, enrichments like bales of substrates can accumulate moisture and give rise to biosecurity issues (Riber et al., 2018) and the use of whole fruits or vegetables would require regular labor engagement (Bhanja and Sahu, 2021). Such enrichments might impact production and biosecurity negatively, and further discourage their commercial use. Thus, optimal environmental enrichments also need to be economical and practical to use. Therefore, enrichments that satisfy all three criteria need to be tested on breeding quail to increase their commercial adoption.

Research Goal and Hypotheses

The research goal of the following body of work is to evaluate the impact of three protective environmental enrichments (Hut, Plastic leaves, Grass) and three stimulatory environmental enrichments (Mirror, Mat, Feeder toy) on the well-being parameters of colony-caged breeding Japanese quail. According to the author's knowledge, huts, mats, and feeder toys have not been used in quail before. The goal was achieved by testing the following hypotheses:

1. Hypothesis 1: Exposure to individual environmental enrichment in breeding quail will result in a) decreased unwanted social and sexual interactions, b) reduced stress-related damaging behaviors, c) increased positive behaviors, d) improved production, e) better physical scores, and f) lowered fecal corticosterone; ultimately improving their well-being and productivity.

Hypothesis 2 was dependent on Hypothesis 1, and excluded two protective enrichments, Plastic leaves and Grass, as the enrichments were observed to lack practicality when Hypothesis 1 was tested. Therefore, the only protective enrichment used to test Hypothesis 2, was the Hut.

2. Hypothesis 2: Exposure to a combination of a protective and a stimulatory enrichment will result in a) reduction of a larger variety of unwanted interactions and damaging behaviors, and b) an increase in more positive behaviors, c) better physical scores, and d) lowered fecal corticosterone, in comparison to exposure to a single enrichment; thereby proving more beneficial for improving well-being and productivity.

Chapter 3: Effects of Environmental Enrichments on the Behavior of Colony-Caged Japanese Quail (*Coturnix japonica*)

Abstract

Breeding purpose Japanese quail (*Coturnix japonica*) are often maintained in barren cages with little environmental complexity. These conditions can lead to unwanted social interactions and stress-related behaviors that can cause serious injury, mortality, and reduce productivity. Use of environmental enrichments has shown to improve poultry well-being; however, the optimal enrichments for quail have not been widely studied. The aim of this project was to evaluate the effectiveness of various enrichments on the well-being of breeding quail colonies (1 male, 2 females/cage). The enrichments studied were designed to reduce unwanted interactions and stress-related behaviors by either providing a shelter (protective enrichments) or by providing mental stimulation (stimulatory enrichments). In Experiment 1, using an incomplete Latin square design, each cage received one protective enrichment (Hut, Plastic leaves, or Grass), or one stimulatory enrichment (Mirror, Feeder toy, or Mat), or no enrichment (control) (average n=17/treatment). In Experiment 2, using an incomplete Latin square design, each cage received one of the following enrichment combinations: Hut + Mirror, Hut + Feeder toy, Hut + Mat, or only Hut (average n=14/treatment). Behaviors were recorded for 4 hours a day on the day of enrichment introduction, 14 days after introduction, and last day of exposure (day 42 in Experiment 1, and day 28 in Experiment 2). Behaviors were evaluated by scan sampling (1 scan/ 5 minutes), and were analyzed using a one-way ANOVA or

Chi-square test on SAS 9.4. Experiment 1 recorded a decrease in the frequencies of sexual mounting, vigilance, bird pecking, and cage pecking in all the enriched birds as compared to the control ($P<0.0001$, 0.0001, 0.0001, 0.0001). Compared to the control, the enriched birds exhibited lower walking frequencies ($P<0.01$) and higher standing frequencies ($P<0.001$). In Experiment 2, vigilance was lower in Hut + Mirror compared to only Hut ($P=0.013$). Compared to only Hut, cage pecking was lower in Hut + Feeder toy ($P=0.028$) and Hut + Mat ($P<0.001$). Significantly higher sitting was observed in the birds provided with a Hut + Mat than those exposed to the Hut ($P=0.014$). Sexual mounting did not vary between the treatments ($P > 0.05$). Bird pecking did not vary between the treatments ($P > 0.05$). To conclude, each enrichment exerted diverse behavioral impacts, however exposure to a single enrichment reduced stress-related damaging behaviors and increased resting; while exposure to a combination of enrichments reduced a larger variety of stress-related damaging behaviors. The use of optimal enrichments or combinations of such enrichments may increase quail well-being and improve economics of the quail industry.

Keywords: Japanese quail, Environmental enrichment, Behavior, Welfare, Housing

Introduction

Japanese quail (*Coturnix japonica*), compared to other poultry species, exhibit a faster growth rate and maturation, shorter incubation period, are easier to handle and maintain, and can be reared in an urban setting. These characteristics make them an ideal candidate for meat and egg production. The quail population involved in production of meat and eggs represents around 11.8% of all production birds, and contributes to around 10% of all table eggs number in the world (Conway, 2017) and 0.2% of the global poultry meat produced (Lukanov, 2019). The population of farmed quail is secondary only to chicken, making them an ideal solution to the problem of animal protein shortage in developing countries, and an alternative to chicken in developed nations (Shanawany, 1994). Quail are frequently used as laboratory models for animal science, developmental biology, veterinary and human medicine given their robustness, quick maturation, and good laying ability (Huss et al., 2008; Tserveni-Goussi and Fortomaris, 2011). Unlike that of some other poultry species, the quail genome has been entirely sequenced (Kawahara- Miki et al., 2013), which makes them suitable for genetic and epigenetic research, replacing the domestic chicken (Andraszek et al., 2014).

Breeding purpose Japanese quail are an integral part of the quail industry; however, they are often maintained in barren cages (Shanawany, 1994) which lack stimulation. These conditions commonly show stress-related behaviors that can cause serious injury, mortality, and ultimately reduce production. Excessive sexual mounting is common, where the female may not be receptive, and misdirected head grabs by the males result in head wounds and ocular injuries (Cheng et al., 2010). In extreme cases, frequent mounting attempts may result in the death of the female. The presence of two or more quail males in the same cage can increase the frequency of aggressive male-male interactions (Edens, 1987), resulting in injuries to and/or death of the submissive males. Exposing domestic poultry to a barren environment can cause repetitive behaviors (e.g. stereotypies) (Blokhuis et al., 1991) including pacing, over-vigilance, and severe feather pecking; and result in physical damage. Such stress-related damaging behaviors arising from a lack of stimulation can reduce the time spent on maintenance behaviors including resting and eating, and impact the birds' well-being. Chronic stress due to barren housing conditions reduces the reliability of the results obtained from laboratory quail (Edwards et al., 2018) and lowers well-being and productivity.

A potential solution to this welfare issue is the use of environmental enrichments. Environmental enrichments are additions to the environment which help increase the occurrence and range of the birds' normal or species-specific behavior, prevent the development of abnormal behavior or reduce its extent, increase the positive exploitation of the environment, and increase the birds' ability to handle behavioral and physiological challenges (Newberry, 1995). Thus, environmental enrichments improve the bird's biological functioning (Newberry, 1995), and their use has been suggested to reduce welfare issues in domestic poultry (Jones, 2001). There have been a number of studies of the effects of environmental enrichments on poultry behavior. Enrichments that provide structural complexity, including features like covered panels, perches, and fences, offer the birds opportunities to modulate their social interactions. Unwanted social interactions including excessive sexual mounting has been seen to be reduced through the use of such enrichments (Leone and Estévez, 2008; Van Emous, 2010; Adeniji, 2012). Enrichments that provide more foraging and pecking opportunities help re-direct damaging feather pecking behavior. The use of such enrichments has shown to reduce severe feather pecking in various poultry species (Riber et al., 2017; Taskin and Karadavut, 2017; van Staaveren et al., 2021). Enrichments that provide stimulation and novelty help create mental engagement, increase exploratory behaviors (Martrencher et al., 2001; Hartcher et al., 2015; Laurence et al., 2015) and reduce stereotypic pacing (Miller and Mench, 2005; Laurence et al., 2015). Thus, enrichments can prove pivotal in improving the birds' well-being through reducing unwanted interactions and stress-induced damaging behaviors.

Enrichments, despite their numerous benefits, are not employed commonly in commercial settings because a majority of the research evaluates the effects of a set of various enrichments, leaving the exact behavioral effects of each individual enrichment largely unknown. A need exists to create and evaluate individual enrichments that aim to improve the behavioral repertoire of poultry and therefore their well-being. Understanding the behavioral impacts of individual enrichments can pave the way to evaluate sets or combinations of those enrichments and identify the combinations that are the most effective. In breeding quail, commonly reared in mixed-sex colonies in barren cages, enrichments that could provide protection from unwanted social interactions (protective enrichments) and/or provide stimulation to elicit greater behavioral diversity (stimulatory enrichments), can aid improve well-being; and should therefore be studied.

The aim of this project was to evaluate the impacts of three protective enrichments (Hut, Plastic leaves, Grass), three stimulatory enrichments (Mirror, Feeder toy, Mat), and three enrichment combinations (Hut + Mirror, Hut + Feeder toy, Hut + Mat) on the behavior of breeding quail. Our Hypothesis 1 was that the use of a single enrichment in breeding quail will result in a) decreased unwanted social and sexual interactions, b) reduced stress-related damaging behaviors, and c) increased positive behaviors, ultimately improving their well-being and productivity. Hypothesis 2 was dependent on Hypothesis 1, and excluded two protective enrichments, Plastic leaves and Grass, as the enrichments were observed to lack practicality when Hypothesis 1 was tested. Therefore, the only protective enrichment used to test Hypothesis 2, was the Hut. Hypothesis 2 was that the use of a combination of a protective and a stimulatory enrichment will result in a) reduction of a larger variety of unwanted interactions and damaging behaviors, and b) an increase in more positive behaviors, in comparison to exposure to a single enrichment; thereby proving more beneficial for improving well-being and productivity.

Materials and Methods

The experiment was conducted in the Animal Sciences Wing of the Animal and Avian Sciences (ANSC) department of the University of Maryland, College Park (UMD). The protocol for the experiment was approved by the University of Maryland Institutional Animal Care and Use Committee (#981251-1).

Animals and Housing

A total of 120 in-house strain of Japanese quail (*Coturnix japonica*; Department of Animal and Avian Sciences, University of Maryland, College Park), hatched and raised in the University of Maryland Animal Wing facility were used for this experiment. The birds belonged to a closed population for over 25 years and have been shown to express low levels of aggressive behaviors (Ottinger, 2001) which reduced the mortality rate and enabled long behavioral observations. The birds were reared in brooding pens with litter and supplemental heat until 6 weeks of age, before being transferred into stainless steel colony cages (~71.12 x 25.4 x 22.86 cm) in the ratio of 1 male:2 females. Cage banks were quartered in a way that each cage shared two adjacent sides with other cages (Appendix 1). All birds received commercial feed and water *ad libitum*. A light schedule of 14L:10D was applied throughout the experimental period. The indoor climate was controlled using an automatic ventilation system that monitored and controlled temperature and humidity. The air humidity was 60 ± 2%, and the ambient temperature was kept at 32 ± 0.5°C from day of hatch and gradually decreased to reach and stabilize at 20 ± 0.5°C by day 35. All the subjects were housed in the same room.

Experimental Set-up

The experiment utilized three protective enrichments and three stimulatory enrichments. The protective enrichments formed a physical and/or visual barrier for the birds, and consisted of a) hand-made flat-topped acrylic hut measuring 25.4 x 16.51 x 15.24 cm (Hut) (Appendix 1, Figure 2), b) artificial plastic leaves hanging measuring 22.86 cm in height (Plastic leaves) (Appendix 1, Figure 3), and c) patch of live grass measuring 17.78 x 10.16 x 15.24 cm and placed in a green plastic tray (Grass) (Appendix 1, Figure 4). Grass also provided a foraging opportunity and was replaced every two weeks in order to maintain the protective effect of the enrichment. The stimulatory enrichments were designed to encourage mental stimulation and/or redirect pecking. These consisted of a) 12.7 cm wide octagonal mirror (Mirror)

(Appendix 1, Figure 5), b) 15.24 x 3.17 cm hand-made PVC pipe-style feeder toy (Feeder toy) (Appendix 1, Figure 6), and c) heavy-duty fingertipped rubber mat measuring 20.32 x 25.4 cm (Mat) (Appendix 1, Figure 7). Feeder toy contained the birds' regular commercial feed and was refilled every two weeks. The enrichments were secured with zip ties or metallic clips in the cage to avoid displacement.

Exposure to a Single Enrichment (Experiment 1)

The first experiment studied the behavioral effects of exposure to either a protective or a stimulatory enrichment. The treatments consisted of Hut, Plastic leaves, Grass, Mirror, Feeder toy, Mat, and a control (no enrichment). Treatments were randomly assigned to 40 cages (1 male and 2 females/cage), using an incomplete Latin square design, replicated thrice in time (average n=17/treatment). The experiment was conducted for 18 weeks, with each replicate lasting for 6 weeks. Replicate 1 was birds ages 7-12 weeks, replicate 2 was ages 13-18 weeks, and replicate 3 was ages 19-24 weeks. The birds maintained the same cages throughout the experiment, while treatments were re-assigned for each iteration of the Latin square design. The enrichment was placed on the side of the cage which was not shared with another cage.

Exposure to a Combination of Enrichments (Experiment 2)

The second experiment studied the behavioral effects of exposure to enrichment combinations consisting of one protective and one stimulatory enrichment. Grass and Plastic leaves were not used in Experiment 2 due to their lack of practicality and/or use by the birds observed in Experiment 1. Therefore, the treatments consisted of Hut + Mirror (Appendix 1, Figure 8), Hut + Feeder toy, Hut +

Mat (Appendix 1, Figure 9); and only Hut. Treatments were randomly assigned to 29 cages (1 male and 2 females/cage), using an incomplete Latin square design, replicated twice in time (average n=14/treatment). The experiment was conducted for 8 weeks, with each replicate lasting for 4 weeks. Replicate 1 was birds ages 26-29 weeks and replicate 2 was ages 30-33 weeks. The birds maintained the same cages throughout the experiment, while treatments were re-assigned for each iteration of the Latin square design. The Hut was placed on the shared side of the cage, while the other enrichments, if present, were placed on the side of the cage that was not shared with another cage.

Behavioral Observations

The behaviors were videotaped using cameras (Zmodo ZMD-DX-SIN8) attached in front of the cages and connected to digital recorders. In Experiment 1, the behaviors were recorded on the day of introduction of the enrichment (Day 0), two weeks after enrichment introduction (Day 14), and on the last day of enrichment exposure (Day 42). Observations were made every 5 minutes from 06:00 to 08:00 and 16:00 to 18:00 using the scan sampling technique and based on the ethogram presented in Table 1. All occurrences of every behavior were recorded for each bird. Behaviors were analyzed at the cage level where the average instances of the behavior per 5 minutes was calculated. In Experiment 2, the behaviors were recorded Day 0, Day 14, and on the last day of enrichment exposure (Day 28); followed by the procedures conducted in Experiment 1.

__Table 1:__ Ethogram used for behavioral observations

Behavior	Description
Standing	Standing on both legs with eyes open and no apparent movement of body
Sitting	Sitting with legs folded and body in contact with the ground and eyes open or shut
Walking	Moves two or more steps in a forward direction
Eating	Head extended towards the feeder and manipulating or ingesting feed
Drinking	Beak in contact with drinker and ingesting water
Vigilant	Neck stretched and moving from side to side while standing or walking
Sexual mounting a) Mounting b) Being mounted	Behaviors consists of a) and b) described below Bird approaches another bird from behind and climbs on the rear end of the latter Bird crouches in response to the climbing on its rear end
Preening	Self-manipulation of own feathers with beak while standing or sitting
Feather pecking	Use of the beak to peck the feathers of another bird
Cage pecking	Use of the beak to peck the floor or walls of the cage
Wing flapping	Both wings opened and moved up and down repeatedly
Floor-scratching	Scratching the cage floor with one or both feet

Interaction with enrichment	
Near enrichment	The bird is in the part of the cage which has the enrichment
On enrichment	The bird is sitting, standing or walking on the enrichment
Under enrichment	The bird is sitting, standing or walking under the enrichment
Pecking at enrichment	The bird is using the beak to peck a part of the enrichment
Viewing enrichment	The bird is near the enrichment and is viewing it

Statistical Analysis

Behaviors were analyzed using generalized linear mixed models in SAS 9.4 (SAS Institute Inc., 2016, Carey, NC), with cage as the experimental unit. PROC UNIVARIATE was used to assess normality. Square root transformation was used to normalize the interactions with enrichment(s), feather pecking, cage pecking, walking, sitting, and preening behaviors. Vigilance, floor scratching and standing behaviors were normalized using log transformation. Using PROC MIXED, behaviors were analyzed by accounting for the time past after enrichment exposure by including the day after exposure as a classification variable. Random effects accounted for included those of the time replicates, cage location, and the interactions between replicate, cage location, and the treatment. Behaviors that did not meet the assumptions of ANOVA, such as sexual mounting, wing flapping, and egg laying were analyzed using PROC FREQUENCY and CHI SQUARE. P-values were adjusted for multiple comparisons using the Bonferroni adjustment and significance was set at adjusted P-value < 0.05.

Results

Effects of Exposure to a Single Enrichment

Among the protective enrichments, interactions with the enrichment did not differ across treatments on Day 0 ($P > 0.05$; Figure 1). Interactions with Grass and those with Hut did not change from Day 0 to Day 42 ($P > 0.05$; Figure 1). Interactions with Plastic leaves decreased from Day 0 to Day 42 (1.33 ± 0.08 interactions per 5 minutes, P=0.001; Figure 1); and on Day 42, were lower than those with Hut and Grass (1.72 ± 0.06 and 0.60 ± 0.06 interactions per 5 minutes respectively, $P < 0.05$; Figure 1). Interactions with Grass were greater than those with Hut on Day 42 (0.49 ± 0.06 interactions per 5 minutes, P=0.049; Figure 1). In the stimulatory enrichments, on Day 0, interactions with the Mat were higher than those with the Mirror (0.52 ± 0.07 interactions per 5 minutes, P=0.004; Figure 1). Interactions with the enrichments remained unchanged from Day 0 to Day 42 in the Mirror, Feeder toy, and Mat ($P > 0.05$; Figure 1); such that on Day 42, interactions with Mat were higher than those with the Mirror and Feeder toy (0.50 ± 0.06 and 0.46 ± 0.06 interactions per 5 minutes, $P < 0.05$; Figure 1).

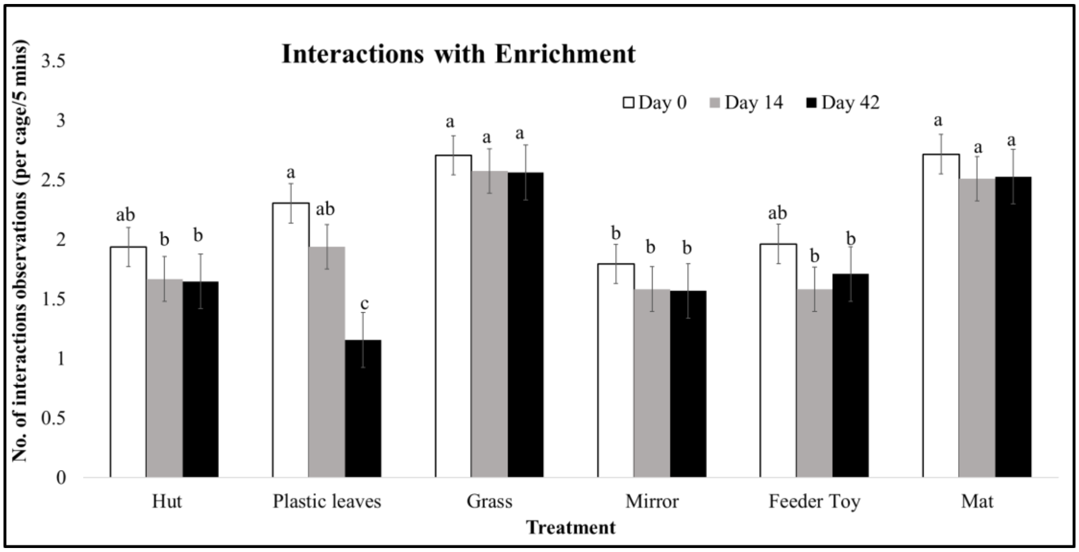

Figure 1*: Number of interactions observations (per cage/5 minutes) in hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of interactions with the enrichment over time (P < 0.05).

Sexual mounting on Day 0 was higher in the control as compared to Hut, Plastic leaves, Grass, Mirror, Feeder toy, and Mat (0.02 ± 0.007, 0.02 ± 0.007, 0.01 ± 0.007, 0.01 ± 0.007, 0.02 ± 0.007, and 0.01 ± 0.007 mounts per 5 minutes respectively, $P < 0.05$; Figure 2). No changes were seen in the behavior between Day 0 to Day 42 in any of the treatments ($P > 0.05$; Figure 2).

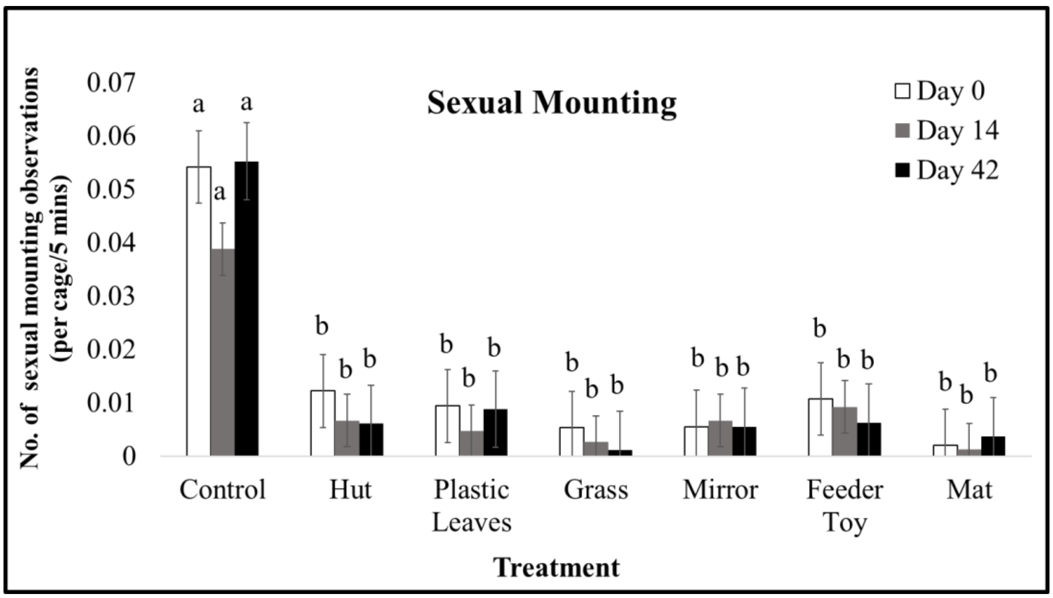

***Figure 2**: Number of sexual mounting observations (per cage/5 minutes) in the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of sexual mounting over time (P < 0.05).

Vigilance behavior was higher in control on Day 0 than Hut, Plastic leaves, Grass, Feeder toy, and Mat (0.19 ± 0.016, 0.14 ± 0.016, 0.14 ± 0.016, 0.10 ± 0.016, and 0.18 ± 0.016 vigilance per 5 minutes respectively, P < 0.001; Figure 3). Vigilance decreased from Day 0 to Day 42 in the Mirror (0.09 ± 0.020 vigilance per 5 minutes, P=0.041; Figure 3). Vigilance increased from Day 0 to Day 42 in the control (0.19 ± 0.018 vigilance per 5 minutes, P=0.045; Figure 3), making it higher than Hut, Plastic leaves, Grass, Feeder toy, Mirror and Mat (0.21 ± 0.044, 0.19 ± 0.044, 0.24 ± 0.044, 0.24 ± 0.044, 0.12 ± 0.044, and 0.20 ± 0.044 vigilance per 5 minutes respectively, P < 0.001; Figure 3) on Day 42.

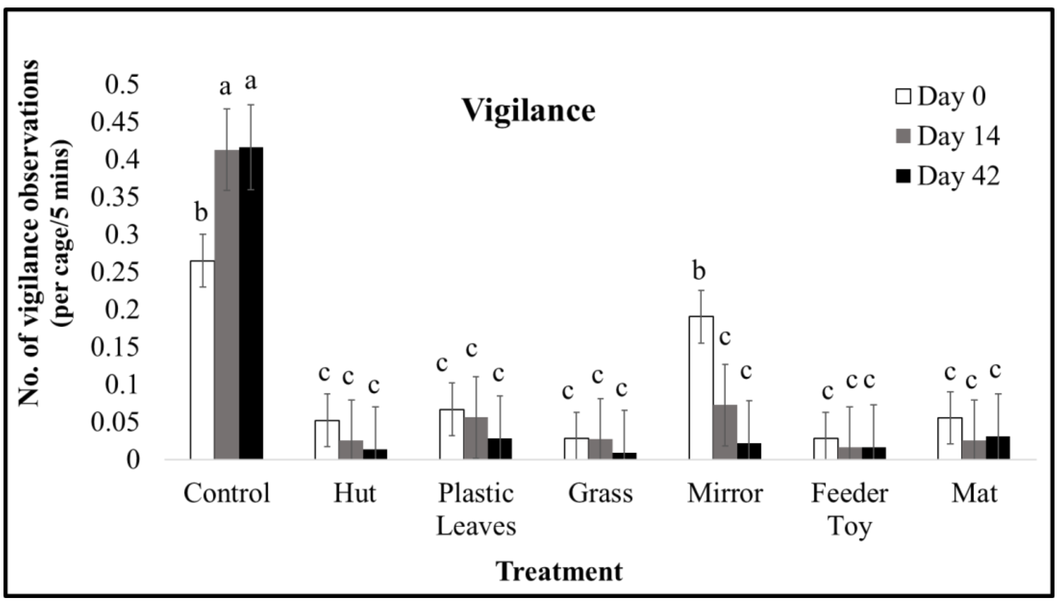

***Figure 3**: Number of vigilance observations (per cage/5 minutes) in the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of vigilance over time (P < 0.05).

Feather pecking on Day 0 was higher in the control as compared to Hut, Plastic leaves, Grass, Mirror, Feeder toy, and Mat (0.02 ± 0.003, 0.03 ± 0.003, 0.03 ± 0.003, 0.02 ± 0.003, 0.02 ± 0.003, and 0.02 ± 0.003 pecks per 5 minutes respectively, $P < 0.01$; Figure 4). No changes were seen in the behavior between Day 0 to Day 42 in any of the treatments ($P > 0.05$; Figure 4).

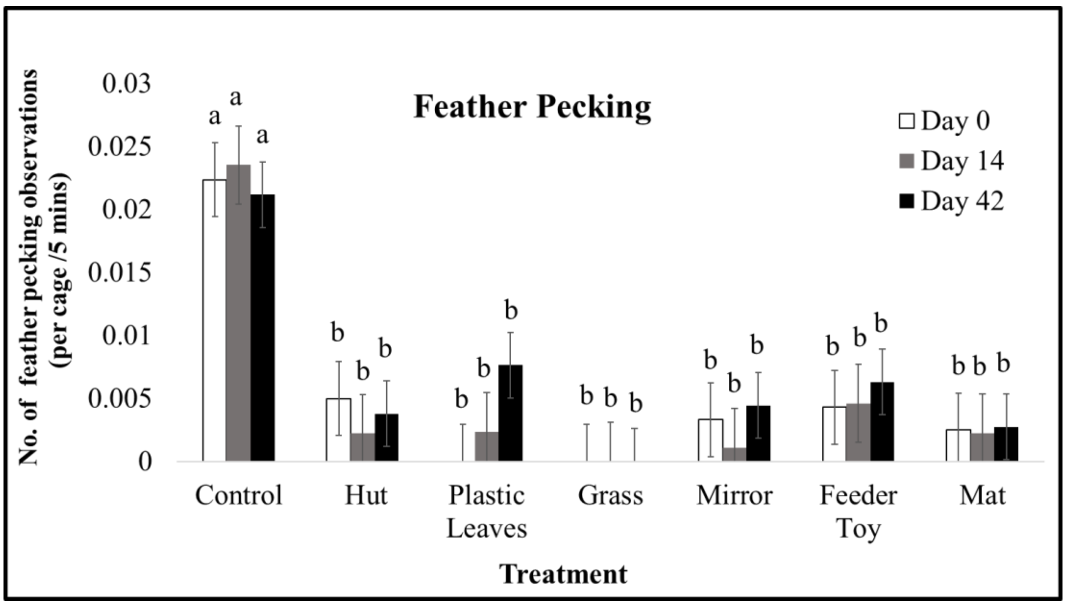

Figure 4: *Number of feather pecking observations (per cage/5 minutes) in the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of feather pecking over time (P < 0.05).

Cage pecking was higher in the control on Day 0 as compared to Hut, Plastic leaves, Grass, Mirror, Feeder toy, and Mat (0.04 ± 0.001, 0.03 ± 0.001, 0.07 ± 0.001, 0.07 ± 0.001, 0.06 ± 0.001, and 0.07 ± 0.001 pecks per 5 minutes respectively, $P < 0.05$; Figure 5), and did not change from Day 0 to Day 42 in the control ($P > 0.05$; Figure 5). The behavior was lower on Day 0 in the Hut compared to Grass, Mirror, Feeder toy, and Mat (0.01 ± 0.004, 0.01 ± 0.004, 0.01 ± 0.004, and 0.01 ± 0.004 pecks per 5 minutes respectively, $P < 0.05$; Figure 5). Cage pecking decreased from Day 0 to Day 42 in the Grass, Mirror, Feeder toy, and Mat (0.11 ± 0.02, 0.08 ± 0.02, 0.08 ± 0.02, and 0.09 ± 0.048 pecks per 5 minutes respectively, $P < 0.01$; Figure 5), making the difference between the enrichments insignificant on Day 42 ($P > 0.05$; Figure 5).

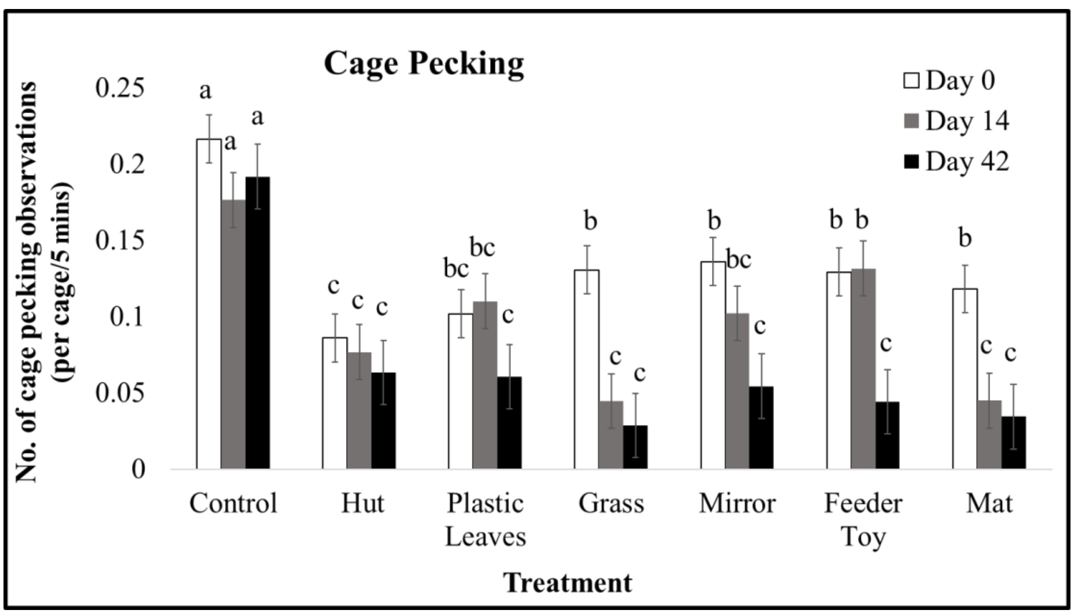

Figure 5: *Number of cage pecking observations (per cage/5 minutes) in the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of cage pecking over time (P < 0.05).

Floor-scratching was higher on Day 0 in the control as compared to Plastic leaves, Grass, Feeder toy, and Mat (0.07 ± 0.04, 0.06 ± 0.01, 0.03 ± 0.02, and 0.03 ± 0.02 scratching per 5 minutes respectively, P < 0.05; Figure 6), and did not change from Day 0 to Day 42 in the control (P > 0.05; Figure 6). The behavior remained unchanged from Day 0 to Day 42 in Plastic leaves (P > 0.05; Figure 6) and Grass (P > 0.05; Figure 6), and decreased over time in Hut (0.03 ± 0.04 scratching per 5 minutes, P=0.006; Figure 6). Floor-scratching was lower in the Mat on Day 42 as compared to Mirror and Feeder toy (0.02 ± 0.04 and 0.02 ± 0.04 scratching per 5 minutes respectively, P < 0.05; Figure 6). On Day 42, floor-scratching was higher in the control as compared to Hut, Plastic leaves, Grass, Feeder toy, and Mat (0.04 ± 0.02, 0.03 ± 0.02, 0.03 ± 0.02, 0.01 ± 0.01, and 0.03 ± 0.04 scratching per 5 minutes respectively, P < 0.05; Figure 6).

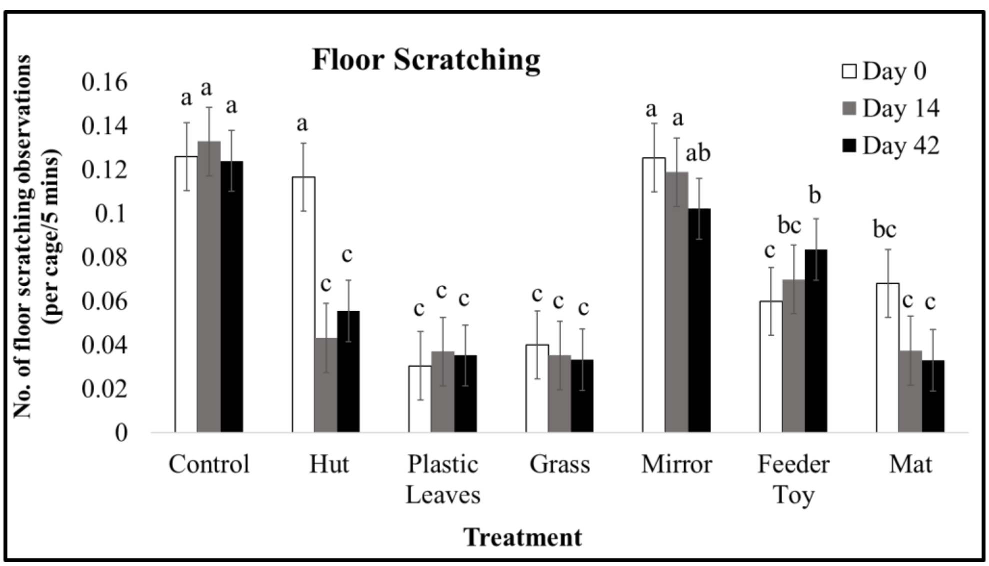

Figure 6: *Number of floor scratching observations (per cage/5 minutes) in the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of floor scratching over time (P < 0.05).

Walking frequencies on Day 0 were higher in the control as compared to Hut, Plastic leaves, Grass, Mirror, Feeder toy, and Mat (0.51 ± 0.04, 0.33 ± 0.04, 0.54 ± 0.04, 0.46 ± 0.04, 0.26 ± 0.04, and 0.25 ± 0.04 walking per 5 minutes respectively, $P < 0.05$; Figure 7). No changes were seen in the behavior between Day 0 to Day 42 in any of the treatments ($P > 0.05$; Figure 7). On Day 42, walking was higher in the Plastic leaves as compared to the Hut and the Grass (0.25 ± 0.01 and 0.28 ± 0.04 walking per 5 minutes respectively, $P < 0.05$; Figure 7).

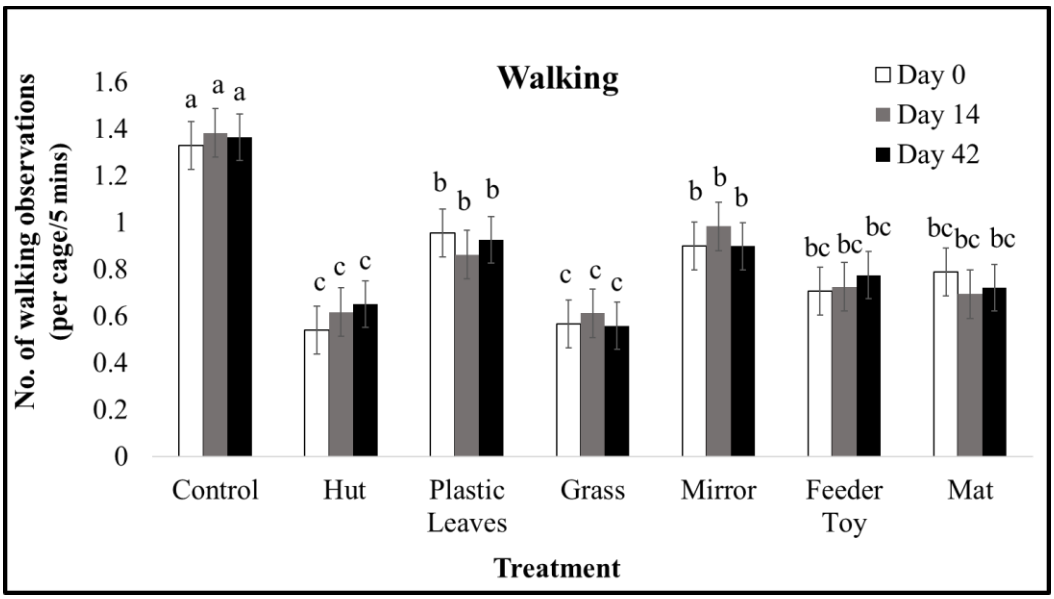

Figure 7: *Number of walking observations (per cage/5 minutes) in the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of walking over time ($P < 0.05$).

Standing was higher on Day 0 in the control as compared to Grass, Mirror, Feeder toy, and Mat (0.37 ± 0.04, 0.36 ± 0.07, 0.38 ± 0.04, and 0.46 ± 0.02 standing per 5 minutes respectively, P ≤ 0.001; Figure 8), and did not change from Day 0 to Day 42 in the control, Grass, Mirror, Feeder toy, and Mat (P > 0.05; Figure 8). The behavior was higher on Day 0 in Grass as compared to the Hut and Plastic leaves (0.42 ± 0.02 and 0.44 ± 0.02 standing per 5 minutes respectively, P < 0.001; Figure 8). Standing increased from Day 0 to Day 42 in the Hut and Plastic leaves (0.43 ± 0.02 and 0.48 ± 0.04 standing per 5 minutes respectively, P < 0.01; Figure 8), making it no different from Grass on Day 42 (P > 0.05; Figure 8).

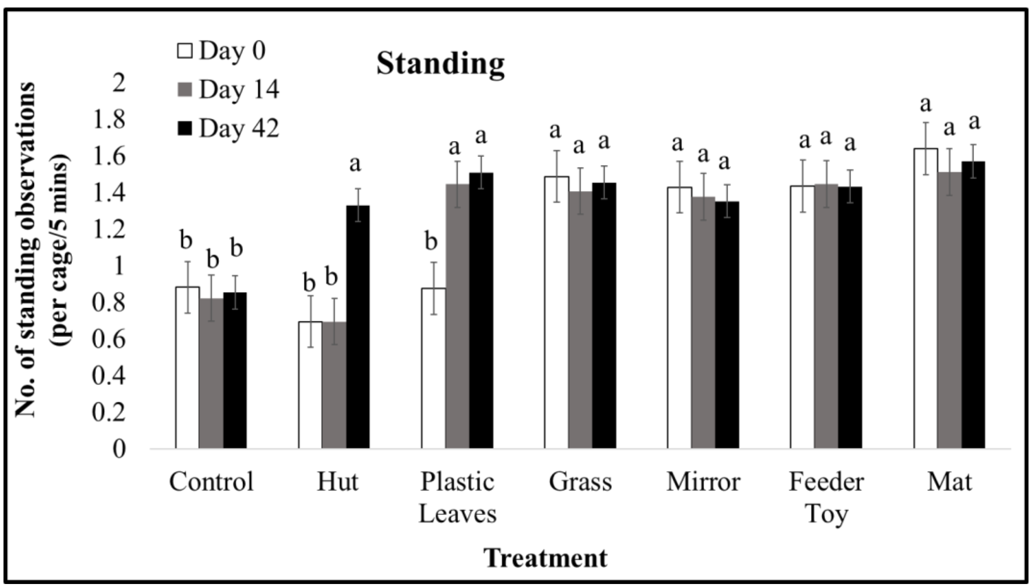

***Figure 8**: Number of standing observations (per cage/5 minutes) in the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of standing over time (P < 0.05).

Sitting was lower in the control on Day 0 as compared to Grass and Mat (0.32 ± 0.07 and 0.29 ± 0.07 sitting per 5 minutes respectively, P < 0.01; Figure 9), and the behavior did not change from Day 0 to Day 42 in the control, Grass, and Mat (P > 0.05; Figure 9). Sitting increased from Day 0 to Day 42 in Hut, Mirror, and Feeder toy (0.22 ± 0.07, 0.20 ± 0.07, and 0.27 ± 0.05 sitting per 5 minutes respectively, P < 0.01; Figure 9). It was higher than the control on Day 42 in Hut, Mirror, and Feeder toy (0.20 ± 0.07, 0.24 ± 0.07, and 0.21 ± 0.07 sitting per 5 minutes respectively, P < 0.05; Figure 9). On Day 42, the behavior was higher in Grass as compared to Hut, and Plastic leaves (0.20 ± 0.05 and 0.18 ± 0.05 sitting per 5 minutes respectively, P < 0.05; Figure 9). On Day 42, sitting was more in the Mat as compared to Mirror and Feeder toy (0.24 ± 0.07 and 0.22 ± 0.07 sitting per 5 minutes respectively, P < 0.05; Figure 9).

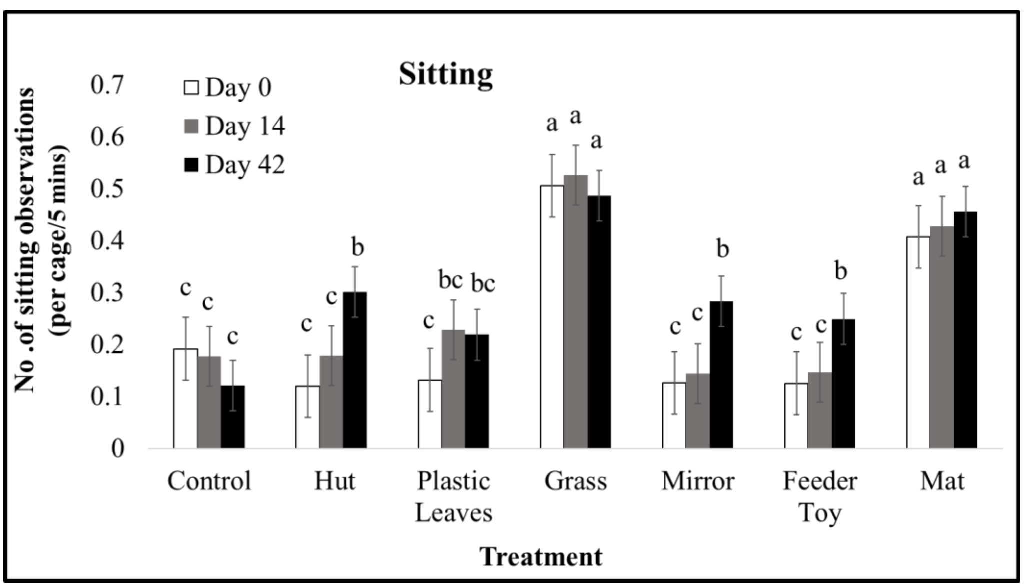

***Figure 9**: Number of sitting observations (per cage/5 minutes) in the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of sitting over time (P < 0.05).

The enrichments did not impact eating, drinking, wing flapping, or preening ($P > 0.05$, > 0.05, > 0.05 and > 0.05 respectively; Figure 10).

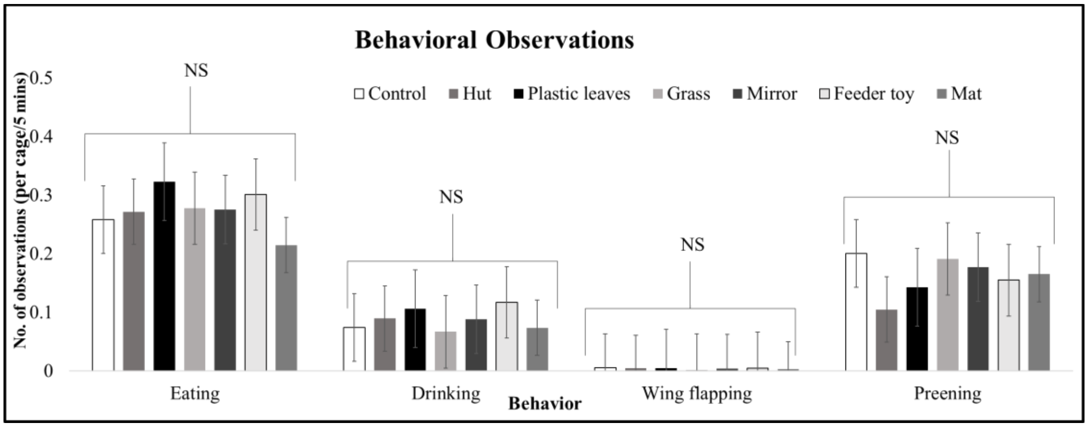

Figure 10*: Number of observations of eating, drinking, wing flapping, and preening (per cage/5 minutes) in the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. NS (non-significant) indicates a lack of significant difference between treatments.

Effects of Exposure to a Combination of Enrichments

Interaction with the hut was lower in the Hut + Mat as compared to Hut (0.28 ± 0.03 interactions per 5 minutes, P=0.015; Figure 11). Interactions with the hut did not vary between Hut, Hut + Mirror and Hut + Feeder toy (P > 0.05; Figure 11). Interactions with the mat were more in Hut + Mat as compared to the interactions with the mirror in Hut + Mirror (0.26 ± 0.07 interactions per 5 minutes, P=0.006; Figure 11).

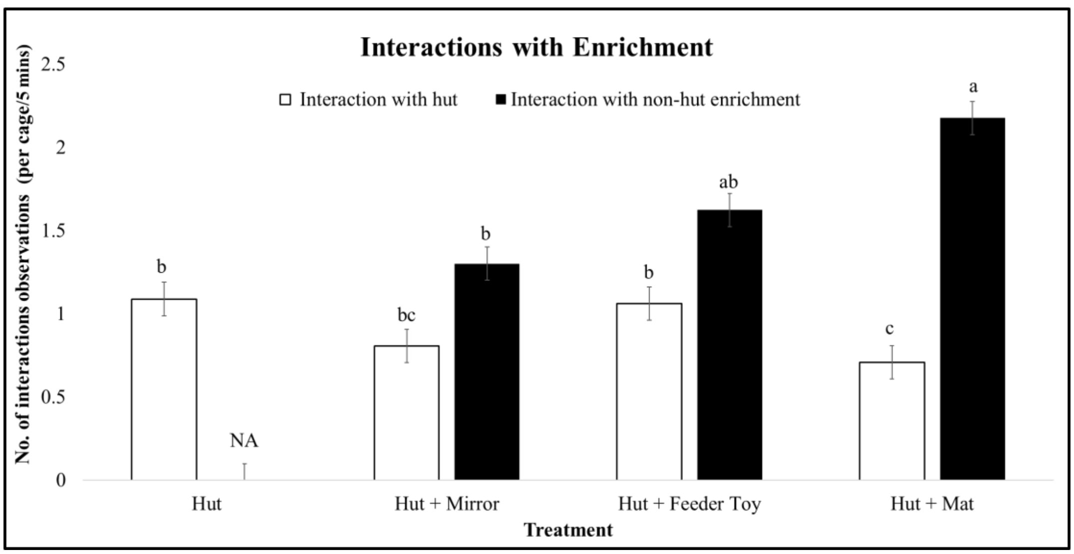

Figure 11*: Number of interactions observations (per cage/5 minutes) in the hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the interactions with the enrichments (P < 0.05). NA= not applicable.

Vigilance behavior decreased from Day 0 to Day 28 in Hut + Mirror, Hut + Feeder toy, and Hut + Mat (0.03 ± 0.04, 0.04 ± 0.01, and 0.06 ± 0.01 vigilance per 5 minutes respectively, P < 0.05; Figure 12) but remained unchanged across time in the Hut (P > 0.05; Figure 12). The behavior was higher on Day 28 in the Hut as compared to Hut + Mirror and Hut + Feeder toy (0.04 ± 0.01 and 0.04 ± 0.01 vigilance per 5 minutes respectively, P < 0.05; Figure 12).

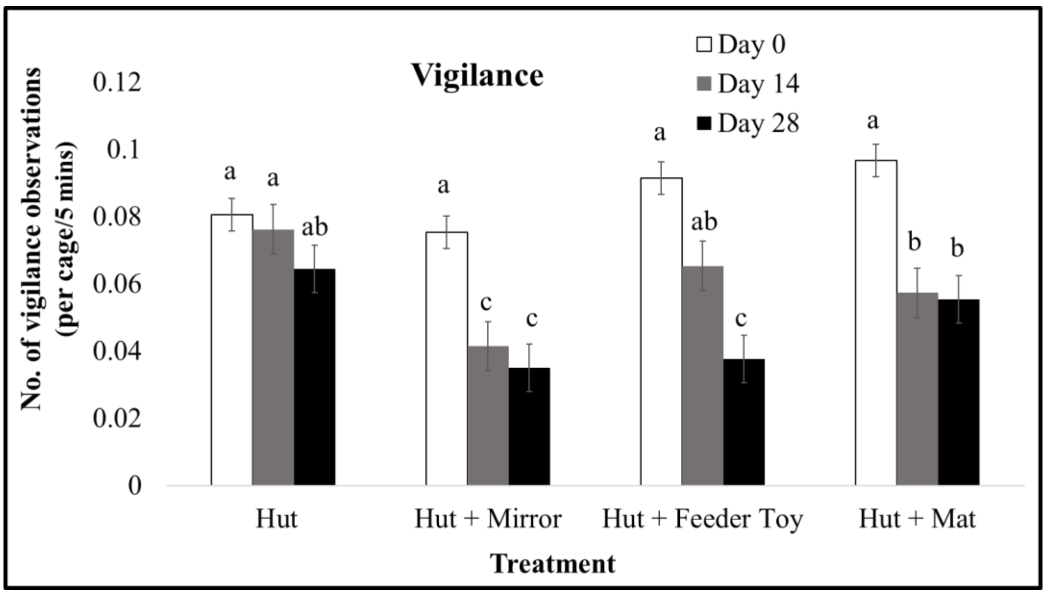

Figure 12*: Number of vigilance observations (per cage/5 minutes) in the hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of vigilance over time (P < 0.05).

Cage pecking was lower in Hut + Mat on Day 0 as compared to Hut and Hut + Mirror (0.36 ± 0.02 and 0.31 ± 0.02 pecks per 5 minutes respectively, $P < 0.05$; Figure 13). The behavior did not change from Day 0 to Day 28 in Hut ($P > 0.05$) but decreased over time in Hut + Mirror and in Hut + Mat (0.24 ± 0.02 and 0.16 ± 0.02 pecks per 5 minutes respectively, $P < 0.05$; Figure 13). Cage pecking was lower in the Hut +Mat on Day 28 as compared to the Hut, Hut + Mirror, and Hut + Feeder toy (0.33 ± 0.02, 0.11 ± 0.02, and 0.18± 0.02 pecks per 5 minutes respectively, $P < 0.05$; Figure 13).

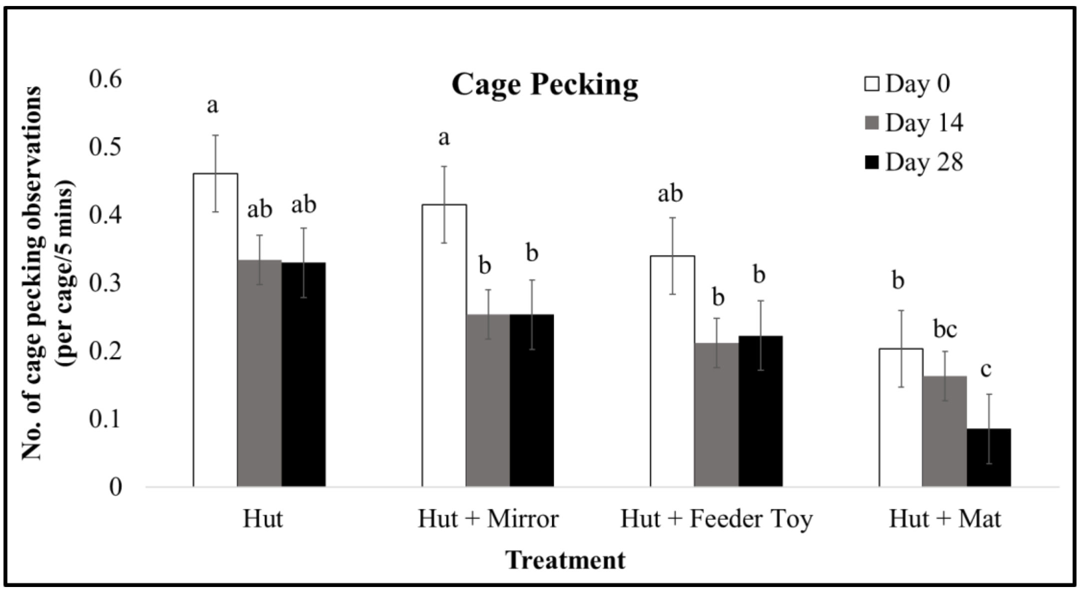

***Figure 13**: Number of cage pecking observations (per cage/5 minutes) in the hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of cage pecking over time (P < 0.05).

No differences were seen across the treatments in the frequencies of feather pecking, sexual mounting, and floor-scratching ($P > 0.05$, > 0.05, and > 0.05 respectively; Figure 14).

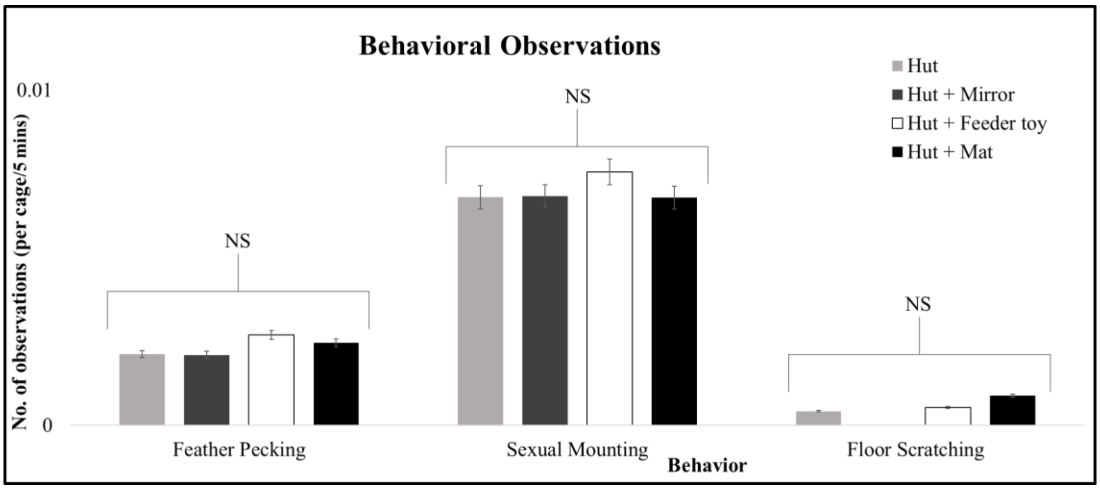

***Figure 14**: Number of observations of feather pecking, sexual mounting and floor scratching (per cage/5 minutes) in the hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. NS (non-significant) indicates a lack of significant difference between treatments.

Walking frequencies did not differ within and across treatments (P > 0.05 and > 0.05 respectively; Figure 15).

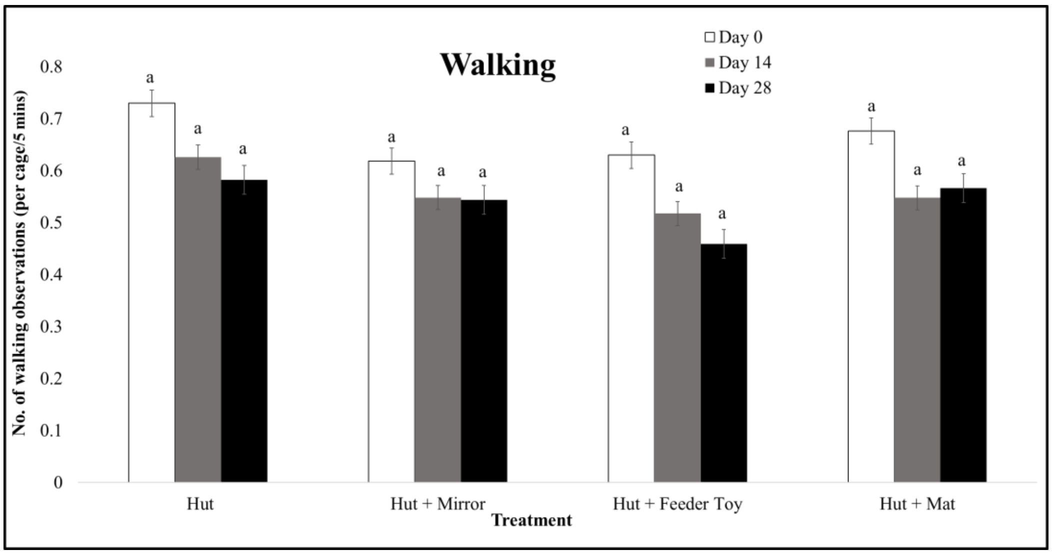

***Figure 15**: Number of walking observations (per cage/5 minutes) in the hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of walking over time (P < 0.05).

Standing frequencies did not differ across treatments on Day 0 (P > 0.05; Figure 16). The behavior increased from Day 0 to Day 28 in Hut, Hut + Mirror, Hut + Feeder toy, Hut + Mat (0.30 ± 0.05, 0.34 ± 0.05, 0.38 ± 0.08, and 0.36 ± 0.04 standing per 5 minutes respectively, P < 0.05; Figure 16), but did not vary across the treatments on Day 28 (P > 0.05; Figure 16).

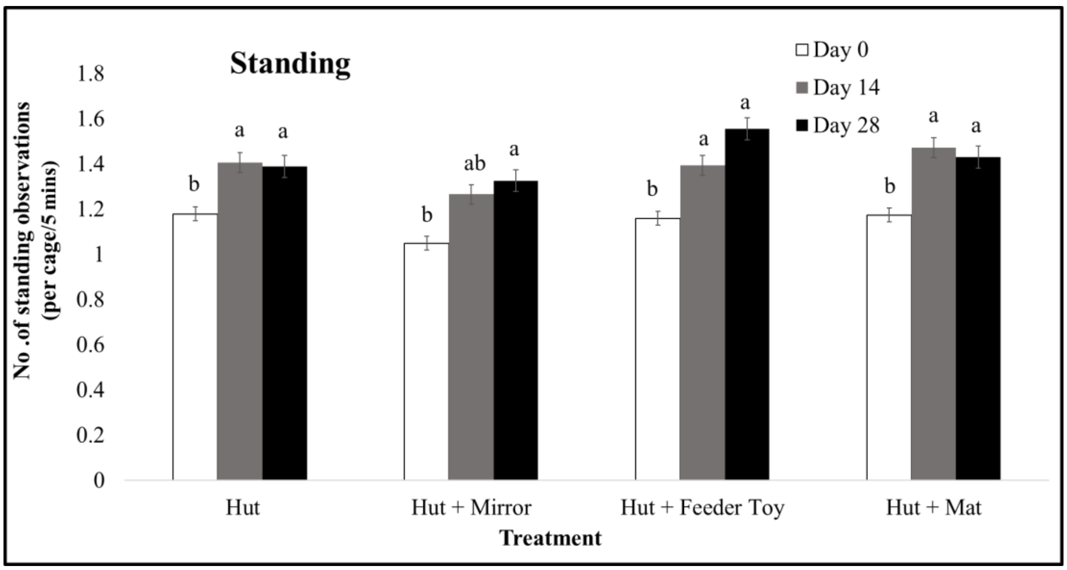

***Figure 16**: Number of standing observations (per cage/5 minutes) in the hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of standing over time (P < 0.05).

Sitting was higher on Day 0 in Hut + Mat as compared to Hut, Hut + Mirror, and Hut + Feeder toy (0.12 ± 0.05, 0.10 ± 0.05, and 0.20 ± 0.05 sitting per 5 minutes respectively, $P < 0.05$; Figure 17). The behavior increased from Day 0 to Day 28 in Hut + Mirror and Hut + Feeder toy (0.02 ± 0.05 and 0.05 ± 0.06 sitting per 5 minutes respectively, $P < 0.05$; Figure 17), but did not vary over time in Hut ($P > 0.05$; Figure 17), leading to higher sitting frequencies on Day 28 in Hut + Mirror and Hut + Feeder toy as compared to Hut (0.01 ± 0.05 and 0.01 ± 0.05 sitting per 5 minutes respectively, $P < 0.05$; Figure 17). On Day 28, Hut + Mat exhibited higher sitting than Hut, Hut + Mirror and Hut + Feeder toy (0.14 ± 0.03, 0.12 ± 0.05, and 0.12 ± 0.05 sitting per 5 minutes respectively, $P < 0.05$; Figure 17).

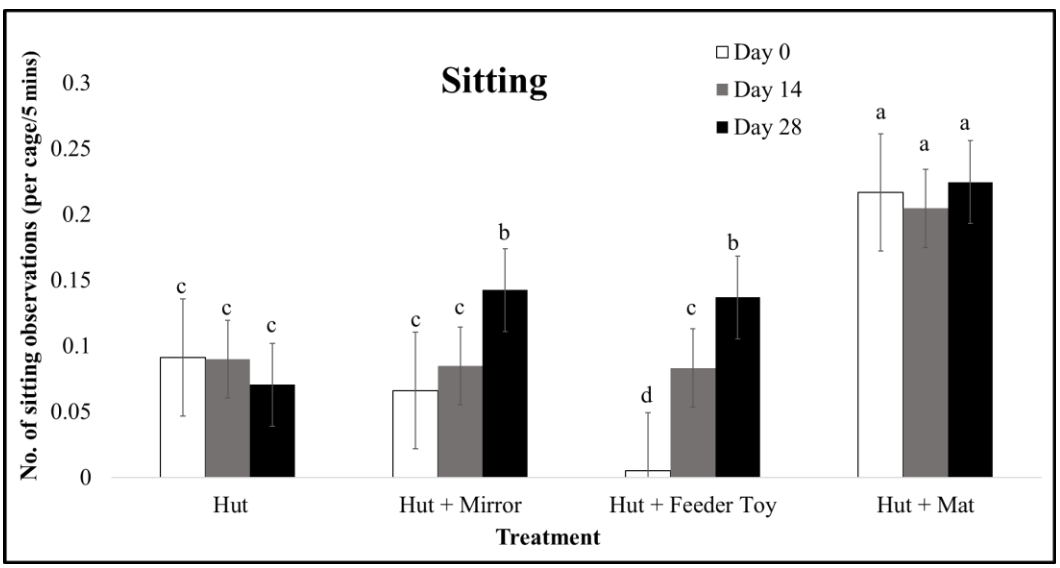

Figure 17*: Number of sitting observations (per cage/5 minutes) in the hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the frequencies of sitting over time ($P < 0.05$).

The treatments did not impact eating, drinking, wing flapping, or preening (P > 0.05, > 0.05, > 0.05, and > 0.05 respectively; Figure 18).

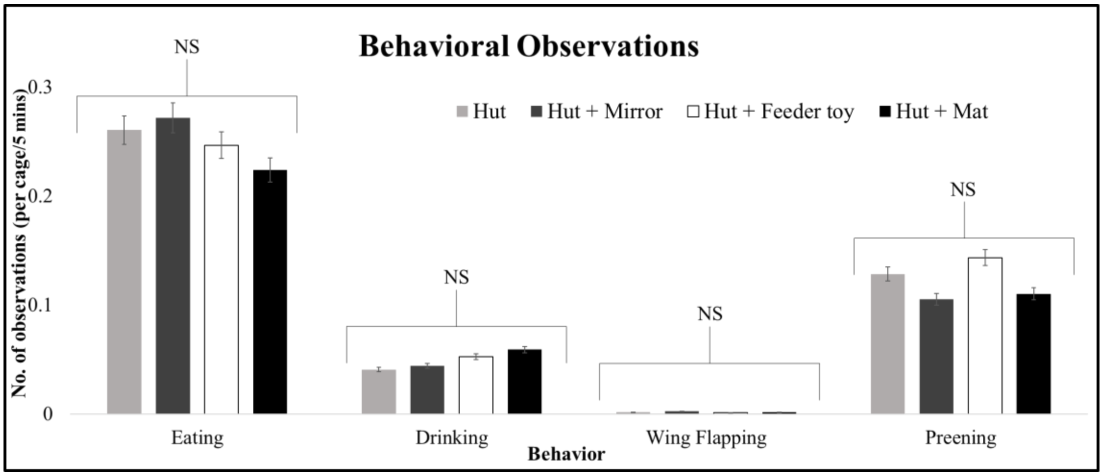

Figure 18: *Number of observations of eating, drinking, wing flapping, and preening (per cage/5 minutes) in the hut, hut + mirror, hut + feeder toy, and hut + mat. Bars indicate LS means±SEM. NS (non-significant) indicates a lack of significant difference between treatments.*

<u>Discussion</u>

This study analyzed the behavioral effects of enrichment(s) exposure in colony-caged Japanese quail by analyzing cage behaviors and enrichment use. Each enrichment exerted diverse behavioral impacts, however exposure to a single enrichment reduced stress-related damaging behaviors and increased resting; while exposure to a combination of enrichments reduced a larger variety of stress-related damaging behaviors more efficiently.

Exposure to a Single Enrichment

Among the protective enrichments, interactions with the Grass remained consistently the highest likely due to its close resemblance to a natural resource and the provision of a stimulatory feedback. Interactions with the Hut were lower than those with the Grass, however, the interactions with the Hut remained consistent over time, indicating its utilization for obtaining shelter. Interactions with the Plastic leaves were as high as Grass on the day of enrichment introduction, however, the interactions reduced over time. The reduced interactions could be accounted for by the texture of the Plastic leaves, which can be hard to tear apart, and therefore may not offer stimulatory feedback as strong as Grass. Among the stimulatory enrichments, interactions with the Mat remained consistently the highest likely due to its flat and soft structure, which could have facilitated standing and sitting while interacting with the enrichment. Interactions with the Mirror did not decrease over time, likely due to the novelty it provided the birds each time they viewed it. Interactions with the Feeder toy were consistent over time as it possibly made the feed obtained by pecking the toy seem like a reward.

Sexual mounting was consistently lower in all the enrichments as compared to the control and did not increase over time in the enrichments. The enrichments such as Hut, Plastic leaves, and Grass, likely created a physical barrier between the birds, reducing the male's ability to mount the female. The hiding space provided by these enrichments could have possibly reduced fear and stress-related mounting, as hiding is the quail's natural response to fearful stimuli (Guyomarch et al., 1986). The enrichments such as Grass, Mirror, Feeder toy, and Mat likely kept the birds occupied as seen through the consistent interactions with the enrichments. Such physical and mental stimulation could have reduced the motivation for excessive sexual mounting, as has been observed previously in quail (Miller and Mench, 2006) and chickens (Bessei, 2006; Riber et al., 2018).

Vigilance behavior, an expression of fearfulness (Rutherford et al., 2003), was consistently lower than the control in the Hut, Plastic leaves, Grass, Feeder toy, and Mat. Our results are in accordance with a previous quail behavior study which shows that provision of a hiding area, such as hanging ropes, plastic leaves and slanted roofs reduces fearfulness (Guesdon et al., 2011). Vigilance in Mirror was higher than in the other enrichments on Day 0 likely because the Mirror broke the structural and social monotony of the cage, which could have simulated the presence of new and more conspecifics. The reduction in the vigilance in Mirror over time indicates its role in causing an effect of an increased group size without the negative interactions associated with a large group, which could have reduced fearfulness. As the birds had a choice of either viewing the Mirror or not viewing it, the Mirror could have provided novelty that was not excessive. Such controllable novelty is known to decrease fearfulness (Jones and Waddington, 1992). Reduction in vigilance by the Mirror echoes previous studies which have noted a similar reduction in fearfulness through exposure to mirrors in quail (Taskin and Karadavut, 2017) and geese (Taskin et al., 2022). Our study did not analyze whether the birds could recognize themselves in the mirror, and therefore the potential impacts that such an ability would have on the vigilance behavior remains unknown. According to the authors' knowledge, quail have not been tested on a mirror test previously; and exploring this study area can provide newer perspectives on the use of a mirror as an enrichment. The across time increase of vigilance behavior in the control birds could plausibly be due to the lack of stimulation in the control, which could have increased the birds' fearfulness (Mason, 1991).

Feather pecking was consistently lower in all the enrichments as compared to the control, and did not increase over time in the enrichments. The Plastic leaves, Grass, Feeder toy, and Mat reduced feather pecking likely by acting as a pecking substrate, which re-directed pecking towards the enrichment. Foraging is a highly motivated behavior in quail (Guyomarch et al., 1986), which was plausibly enabled by the Grass and Mat, and to an extent, by the Plastic leaves; and could have resulted in reduced feather pecking frequencies, as has been observed previously (Wechsler and Huber-Eicher, 1998; Nazar and Marin, 2011). Stress-induced feather pecking (Rodenburg et al., 2013) may have decreased due to the protective enrichments, which have shown to lower damaging behaviors before in quail (Miller and Mench, 2005).

Cage pecking was consistently lower in the Hut and Plastic leaves as compared to the control, and was lower than the other enrichments on Day 0, probably because the structure and the dark green color of the enrichments reduced the access to and the visibility of the cage floor and/or walls. As discussed previously, Grass, Feeder toy, and Mat likely re-directed pecking by acting as a pecking substrate; and Grass and Mat plausibly reduced pecking further by providing a foraging opportunity. Mirror could have reduced cage pecking probably by changing the birds' perception of the cage floor and walls as visible in the Mirror, and thereby re-directing cage pecking at the image of the cage floor visible in the enrichment.

Floor-scratching, which measured the frequency of scratching the cage floor with one or both feet, was consistently lower than the control in Plastic leaves, Grass, and Mat, and did not change over time in the three enrichments. Plastic leaves, Grass, and Mat reduced floor-scratching likely by acting as a fairly even scratching surface as compared to the meshed cage floor. Scratching occurs in conjunction with foraging (Guyomarch et al., 1986). Therefore, enrichments such as Mat and Grass which facilitated foraging, could have re-directed scratching at the enrichment. Scratching is also a pre-cursor to other natural behaviors including dust-bathing (Kruijt, 1964). The use of the Grass for dust-bathing could have further re-directed scratching at the enrichment. Floor-scratching was consistently lower than the control in the Feeder toy because the feeder toy's small openings likely reduced the chances of feed spilling, and thereby the need of scratching the cage floor for obtaining feed. Day 14 onward, floor-scratching in the Hut was lower than the control, plausibly because of the Hut's sheltering effect which increased resting frequencies and could have thereby reduced the time spent floor-scratching.

Walking frequencies were consistently lower in all the enrichments as compared to the control, and did not increase over time in the enrichments likely due to the interactions with the enrichments. Exposure to certain enrichments such as Plastic leaves, which led to lower enrichment interactions than to the Hut and Grass on Day 42, exhibited consistently higher walking frequencies than the Hut and Grass. These results suggest that the Hut and Grass likely reduced walking by providing the birds a concrete resting area. The Hut and the Grass plausibly facilitated positive behaviors such as sitting and dust-bathing respectively, which could have reduced the birds' need to walk.

Standing frequencies were consistently higher in the Grass, Mirror, Feeder toy, and Mat as compared to the control, and did not decrease over time in the enrichments, likely as an effect of the lower walking frequencies seen in these enrichments. However, standing frequencies in Hut and Plastic leaves were comparable to the control on Day 0 and rose gradually until Day 42. The novelty of the green cover provided by the Hut likely led to high floor-scratching as an exploratory behavior (Guyomarch et al., 1986), thereby reducing the time spent standing until the birds became comfortable with the Hut. The Plastic leaves resembled natural leaves in color and shape to an extent but likely lacked the same stimulatory feedback as provided by the Grass. This property of the leaves could have induced the birds to explore the leaves, thereby decreasing the standing frequencies until the use of the enrichment was established by the birds.

Sitting behavior, a common resting behavior in poultry (Blokhuis, 1983), was consistently higher in the Grass and Mat as compared to the control due to their soft texture which plausibly encouraged sitting and their adequate surface area which allowed their simultaneous use by more than one bird. Sitting could have also occurred as a pre-requisite for behaviors such as dust-bathing and egg laying, which we noticed occurring in the Grass. The potential over time decrease in the novelty of the Hut, Mirror, and Feeder toy could have possibly increased their sitting frequencies, making them higher than the control by Day 42. However, the sitting in

Plastic leaves remained consistently lower than that in the Grass, indicating towards the stronger effect of a natural resource on improving resting.

Exposure to a Combination of Enrichments

Interactions with the hut were higher in the Hut as compared to the Hut + Mat combination likely due to the lack of a choice of enrichments in the Hut, which led to the birds utilizing the hut more frequently. Interactions with the hut in Hut + Mirror and Hut + Feeder toy were not notably different than those in the Hut because interactions with the mirror and the feeder toy plausibly did not facilitate resting behaviors, leading to the use of the hut for resting. Interactions with mat were notably higher than those with the hut in the Hut + Mat. Interactions with the mat were also higher than those with the mirror. These results can be attributed to the large range of behaviors which were possible on the mat. Behaviors such as pecking, foraging, and scratching were not possible with the hut. The mat also provided a soft surface to stand, sit or walk on, unlike the hut and the mirror.

Vigilance behavior, an expression of fearfulness (Rutherford et al., 2003), decreased over time in the enrichment combinations likely because of the combined impact of the enrichments which encouraged a larger variety of highly motivated behaviors such as foraging, pecking, and hiding from fearful stimuli. The availability of the hut as an escape from potential fearful conditions could have further reduced the birds' vigilance while interacting with the other enrichment. The combinations provided the birds an opportunity to choose between the two enrichments, which has been seen to reduce fearfulness in quail (Miller and Mench, 2005). In Hut + Mirror,

mirror likely reduced fearfulness by providing the effect of an increased group size without the negative interactions associated with a large group, which could have resulted in the vigilance being significantly lower on Day 28 in the Hut + Mirror as compared to the Hut.

Cage pecking was notably lower on Day 0 and Day 28 in the Hut + Mat as compared to the Hut and Hut + Mirror likely because the mat re-directed cage pecking at itself. The feeder toy in the Hut + Feeder toy combination could have re-directed pecking at itself too, however, the use of the toy was limited by its size. A larger number of birds could simultaneously use the mat as compared to the feeder toy, which could have resulted in reduced cage pecking on Day 28 in the Hut + Mat as compared to that in the Hut + Feeder toy. Similar use of enrichments has been seen in laying hens exposed to two enrichments which could be used by a group of birds simultaneously (Bubier, 1996). Unlike in the Hut, the behavior reduced over time in the Hut + Mirror combination plausibly due to the birds' engagement with the mirror. Viewing the mirror could have resulted in sustained upright positions, which could have reduced the motivation to bend and peck the cage.

Frequencies of certain behaviors such as feather pecking, sexual mounting, and walking, which can signify or lead to negative behaviors, did not vary across treatments. The frequencies of standing increased over time in all the treatments. These results indicate towards the effectiveness of the hut in reducing such behaviors irrespective of the presence of another enrichment, likely by providing the birds an escape from unwanted social interactions and plausibly be acting as a safe haven to perform more positive and natural behaviors.

Sitting frequencies were consistently higher in the Hut + Mat as compared to any of the treatments plausibly due to the physical spread and the surface properties (discussed previously) which could have facilitated its simultaneous utilization for sitting by a group of birds. Stimulatory activities such as pecking the mat or natural behaviors such as egg laying were possible while being seated on the mat, which could have further encouraged the birds to sit on the mat. The mirror in the Hut + Mirror combination and the feeder toy in the Hut + Feeder toy combination did not likely facilitate sitting through their structure; however, the over time increase in sitting in these two treatments can be attributed to the stimulation provided by them. The stimulation involved physical and/or mental engagement, which are energy-consuming activities (Maille and Schradin, 2017), and could have therefore induced sitting as an expression of resting (Blokhuis, 1983).

Limitations

A majority of the enrichments used in this study including Hut, Plastic leaves, Mirror, and Mat are easy-to-use; however, Grass and Feeder toy have some practical limitations. Both the enrichments require routine replacement and are prone to breaching biosecurity. This limitation of the Feeder toy can be overcome through automating the toy. Grass, although not practical in an industrial setting, can prove to be an effective enrichment in backyard quail farms or in free-range commercial farms.

Conclusion

Exposing breeding colonies of Japanese quail to individual protective or stimulatory enrichments reduced unwanted social interactions and stress-related damaging behaviors; while exposure to a combination of protective and stimulatory enrichments resulted in a greater reduction of a larger variety of damaging behaviors. Future analyses will investigate if and how these behavioral changes correlate with other measures of health and welfare, such as the measure of physiological stress through hormonal assays, and production parameters including body weight gain and egg production. Overall, quail exposed to enrichment(s) exhibited more resting and less damaging behaviors. Given that unwanted interactions and stress-related damaging behaviors can lead to injuries, mortalities, and lowered production, the use of optimal enrichments or combinations of such enrichments may lead to increased quail well-being and improved production and economics of the quail industry.

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Chapter 4: Effects of Environmental Enrichments on Production, Physical scores, and Fecal Corticosterone in Colony-Caged Japanese Quail (*Coturnix japonica*)

Abstract

Breeding purpose Japanese quail (*Coturnix japonica*) are often maintained in barren cages with little environmental complexity. These conditions can lead to unwanted social interactions and stress-related behaviors that can cause serious injury, mortality, and reduce productivity. Use of environmental enrichments has shown to improve poultry well-being; however, the exact health and production impacts of enrichments remain largely unknown. The aim of this project was to evaluate the effectiveness of various enrichments on certain health and production parameters of breeding quail colonies (1 male, 2 females/cage). The enrichments studied were designed to reduce unwanted interactions and stress-related behaviors by either providing a shelter (protective enrichments) or by providing mental stimulation (stimulatory enrichments). In Experiment 1, using an incomplete Latin square design, each cage received one protective enrichment (Hut, Plastic leaves, or Grass), or one stimulatory enrichment (Mirror, Feeder toy, or Mat), or no enrichment (control) (average n=17/treatment). In Experiment 2, using an incomplete Latin square design, each cage received one of the following enrichment combinations: Hut + Mirror, Hut + Feeder toy, Hut + Mat, or only Hut (average n=14/treatment). Parameters measured included body weight, Hen Day Egg Production (HDEP), egg weight, physical scores, and fecal corticosterone metabolites (FCM) levels; and were analyzed using a one-way ANOVA or Chi-square test on SAS 9.4. Body weight and egg weight were not impacted by exposure to enrichments in Experiment 1 ($P > 0.05$, >0.05). The HDEP improved upon exposure to Hut ($P=0.0450$) and to Grass ($P=0.032$), and remained unchanged in the other enrichments ($P > 0.05$) in Experiment 1. No changes were seen in the body weight ($P > 0.05$), in HDEP ($P > 0.05$) and in the egg weight ($P > 0.05$) in Experiment 2. In Experiment 1, as compared to the control, the enriched birds exhibited lower feather scores ($P < 0.05$), lower injury scores ($P < 0.05$), and lower footpad scores ($P < 0.05$). In Experiment 2, compared to the control, feather scores were lower in Hut + Mirror ($P=0.028$) and Hut + Feeder toy ($P=0.034$), and decreased over time in Hut + Mat ($P=0.031$). Injury scores were also lower in the Hut + Mirror ($P=0.024$) and Hut + Feeder toy ($P=0.041$) compared to only Hut, and exhibited an over time decrease in Hut + Mat ($P=0.048$). Footpad scores remained unaffected by the treatments in Experiment 2 ($P > 0.05$). Experiment 1 recorded no significant differences across treatments in the FCM levels ($P > 0.05$), however, in Experiment 2, lower FCM levels were seen in Hut + Mat as compared to only Hut ($P=0.048$). In conclusion, two of the three protective enrichments (Hut, Grass) exerted a positive impact on production, while combinations of protective and stimulatory enrichments were more efficient in decreasing physical scores as compared to a single enrichment. The Hut + Mat combination reduced the FCM levels. The use of optimal enrichments or combinations of such enrichments may enhance quail health and production, and improve economics of the quail industry.

Keywords: Japanese quail, Environmental enrichment, Production, Corticosterone, Welfare

Introduction

Japanese quail, compared to other poultry species, exhibit a faster growth rate and maturation, shorter incubation period, are easier to handle and maintain, and can be reared in an urban setting. These characteristics make them an ideal candidate for meat and egg production. The quail population involved in production of meat and eggs represents around 11.8% of all productive birds, and contributes to around 10% of all table eggs number in the world (Conway, 2017) and 0.2% of the global poultry meat produced (Lukanov, 2019). The population of farmed quail is secondary only to chicken, making them an ideal solution to the problem of animal protein shortage in developing countries, and an alternative to chicken in developed nations (Shanawany, 1994). Quail are frequently used as laboratory models for animal science, developmental biology, veterinary and human medicine given their robustness, quick maturation, and good laying ability (Huss et al., 2008; Tserveni-Goussi and Fortomaris, 2011). Unlike that of other poultry species, the quail genome has been entirely sequenced (Kawahara- Miki et al., 2013), which makes them suitable for genetic and epigenetic research, replacing the domestic chicken (Andraszek et al., 2014).

Breeding quail form an important part of the quail industry and housing conditions form a major determinant of their productivity (Cheng et al., 2010; Narinc et al., 2013). In an industrial setting, breeding quail are typically reared in small barren cages, where females and males are grouped in the ratio of 1:3 or 1:4, and share the cage continuously (Narinc et al., 2013). The lack of a stimulatory environment, coupled with continuous close exposure to females, results in unwanted sexual behavior in males, causing injuries to the females who cannot escape (Galef et al., 2006). Such an environment causes unwanted social interactions, like feather pecking, cannibalism, and chasing, which can lower insulation due to feather losses, cause body and footpad injuries and/or death (Herremans et al., 1989; Savory, 1995); ultimately resulting in reduced productivity. Repetitive unavoidable social interactions can result in physiological stress (Hill, 1983), which reduces the reliability of the results obtained from laboratory quail (Edwards et al., 2018) and lowers well-being and productivity (Hill, 1983).

A potential solution to this bird health issue is the use of environmental enrichments. Environmental enrichments are additions to the environment which help increase the occurrence and range of the birds' normal or species-specific behavior, prevent the development of abnormal behavior or reduce its extent, increase the positive exploitation of the environment, and increase the birds' ability to handle behavioral and physiological challenges (Newberry, 1995). Thus, environmental enrichments improve the bird's biological functioning (Newberry, 1995), which in turn can improve health. Enrichment categories that can enhance breeding quail well-being include protective enrichments, designed to provide protection from unwanted social interactions, and stimulatory enrichments, designed to provide stimulation and therefore elicit greater behavioral diversity.

Enrichments, despite their numerous biological benefits, are often perceived to reduce productive performance (Estevez, 2009), plausibly due to the inconsistent effects enrichments have exhibited on various health parameters including production, physical scores, and stress-hormone levels. For instance, body weight increased with the use of sound-based environmental enrichments (Gvaryahu et al., 1989; Woodcock et al., 2004) but decreased with foraging enrichments such as feed scattered on bedding that increased the bird's activity (Jordan et al., 2011). However, body weight remained unchanged in a range of other enrichment studies including perches (LeVan et al., 2000; Pettit-riley and Estevez, 2001), UV light (Lewis et al., 2000) and ropes and vegetable matter (Sherwin et al., 1999). The impacts of egg production, like body weight, seems to vary across enrichments. Exposure to a protective enrichment such as a cover panel improved egg production in one study (Leone and Estevez, 2008), but did not impact egg production in other studies (Dikmen et al., 2016; Fanatico et al., 2016). Amongst physical scores, enrichment use has commonly shown a positive impact on the birds' feather score (Reiter et al., 1997; Nordi et al., 2012; Lindenwald et al., 2021) and injury score (Crowe and Forbes, 1999; Riber and Mench, 2008; Nordi et al., 2012; Lindenwald et al., 2021); however, the effects on footpad score vary from one enrichment to another. For example, footpad scores improved upon exposure to plastic ball (Colton and Fraley, 2014), perches (Groves and Muir, 2013; Yildirim and Taskin, 2017), and vertical panels (Cornetto and Estevez, 2001), but worsened with the use of moisture-generating enrichments such as deep litter (Abdel-Hamid et al., 2020) and (in ducks) water troughs (Schenk et al., 2016). Effects of enrichments on corticosterone, a stress-hormone, appear inconsistent as seen through increased fecal corticosterone levels upon exposure to foraging enrichments rich in fiber (Dawkins et al., 2004; Alm et al., 2014), but decreased levels when exposed to another type of foraging enrichment such as alfalfa hay (Son et al., 2022). Fecal levels of corticosterone remained unchanged after exposure to a protective enrichment such as a nest box (Engel et al., 2019), however, another study exhibited an increase in levels when birds were deprived of a nest box (Alm et al., 2016). Such disparity in the effects of enrichments on the same health parameter points towards the need to explore the potentially varying effects each enrichment category (e.g., protective, stimulatory, foraging) might be exerting on the birds' health and production.

The aim of this project was to evaluate the impacts of three protective enrichments (Hut, Plastic leaves, Grass), three stimulatory enrichments (Mirror, Feeder toy, Mat), and three enrichment combinations (Hut + Mirror, Hut + Feeder toy, Hut + Mat) on certain health and production parameters of breeding quail. Our Hypothesis 1 was that the use of a single enrichment in breeding quail will result in a) improved production, b) better physical scores, and c) lowered fecal corticosterone; reflecting an improved health condition. Hypothesis 2 was dependent on Hypothesis 1, and excluded two protective enrichments, Plastic leaves and Grass, as the enrichments were observed to lack practicality when Hypothesis 1 was tested. Therefore, the only protective enrichment used to test Hypothesis 2, was the Hut. Hypothesis 2 was that the use of a combination of a protective and a stimulatory enrichment will result in a) better physical scores, and b) lowered fecal corticosterone, in comparison to exposure to a single enrichment; thereby proving more beneficial for improving well-being and productivity.

Materials and Methods

The experiment was conducted in the Animal Sciences Wing of the Animal and Avian Sciences (ANSC) department of the University of Maryland, College Park (UMD). The protocol for the experiment was approved by the University of Maryland Institutional Animal Care and Use Committee (#981251-1).

Animals and Housing

A total of 120 in-house strain of Japanese quail (*Coturnix japonica*; Department of Animal and Avian Sciences, University of Maryland, College Park), hatched and raised in the University of Maryland Animal Wing facility were used for this experiment. The birds belonged to a closed population for over 25 years and have been shown to express low levels of aggressive behaviors (Ottinger, 2001) which reduced the mortality rate and enabled long behavioral observations. The birds were reared in brooding pens with litter and supplemental heat until 6 weeks of age, before being transferred into stainless steel colony cages (~71.12 x 25.4 x 22.86 cm) in the ratio of 1 male:2 females. Cage banks were quartered in a way that each cage shared two adjacent sides with other cages (Appendix 1). All birds received commercial feed and water *ad libitum*. A light schedule of 14L:10D was applied throughout the experimental period. The indoor climate was controlled using an automatic ventilation system that monitored and controlled temperature and humidity. The air humidity was 60 ± 2%, and the ambient temperature was kept at 32 ± 0.5°C from day of hatch and gradually decreased to reach and stabilize at 20 ± 0.5°C by day 35. All the subjects were housed in the same room.

Experimental Set-up

The experiment utilized three protective enrichments and three stimulatory enrichments. The protective enrichments formed a physical and/or visual barrier for the birds, and consisted of a) hand-made flat-topped acrylic hut measuring 25.4 x 16.51 x 15.24 cm (Hut) (Appendix 1, Figure 2), b) artificial plastic leaves hanging measuring 22.86 cm in height (Plastic leaves) (Appendix 1, Figure 3), and c) patch of live grass measuring 17.78 x 10.16 x 15.24 cm and placed in a green plastic tray (Grass) (Appendix 1, Figure 4). Grass also provided a foraging opportunity and was replaced every two weeks in order to maintain the protective effect of the enrichment. The stimulatory enrichments were designed to encourage mental stimulation and/or redirect pecking. These consisted of a) 12.7 cm wide octagonal mirror (Mirror) (Appendix 1, Figure 5), b) 15.24 x 3.17 cm hand-made PVC pipe-style feeder toy (Feeder toy) (Appendix 1, Figure 6), and c) heavy-duty fingertipped rubber mat measuring 20.32 x 25.4 cm (Mat) (Appendix 1, Figure 7). Feeder toy contained the birds' regular commercial feed and was refilled every two weeks. The enrichments were secured with zip ties or metallic clips in the cage to avoid displacement.

Exposure to a Single Enrichment (Experiment 1)

The first experiment studied the behavioral effects of exposure to either a protective or a stimulatory enrichment. The treatments consisted of Hut, Plastic leaves, Grass, Mirror, Feeder toy, Mat, and a control (no enrichment). Treatments were randomly assigned to 40 cages (1 male and 2 females/cage), using an incomplete Latin square design, replicated thrice in time (average n=17/treatment).

The experiment was conducted for 18 weeks, with each replicate lasting for 6 weeks. Replicate 1 was birds ages 7-12 weeks, replicate 2 was ages 13-18 weeks, and replicate 3 was ages 19-24 weeks. The birds maintained the same cages throughout the experiment, while treatments were re-assigned for each iteration of the Latin square design. The enrichment was placed on the side of the cage which was not shared with another cage (Appendices 2-7).

Exposure to a Combination of Enrichments (Experiment 2)

The second experiment studied the behavioral effects of exposure to enrichment combinations consisting of one protective and one stimulatory enrichment. Grass and Plastic leaves were not used in Experiment 2 due to their lack of practicality and/or use by the birds observed in Experiment 1. Therefore, the treatments consisted of Hut + Mirror (Appendix 1, Figure 8), Hut + Feeder toy, Hut + Mat (Appendix 1, Figure 9); and only Hut. Treatments were randomly assigned to 29 cages (1 male and 2 females/cage), using an incomplete Latin square design, replicated twice in time (average n=14/treatment). The experiment was conducted for 8 weeks, with each replicate lasting for 4 weeks. Replicate 1 was birds ages 26-29 weeks and replicate 2 was ages 30-33 weeks. The birds maintained the same cages throughout the experiment, while treatments were re-assigned for each iteration of the Latin square design. The Hut was placed on the shared side of the cage, while the other enrichments, if present, were placed on the side of the cage that was not shared with another cage.

Evaluation of Production

Each bird was individually weighed on a digital weighing scale (Adam Equipment CPWplus6 Bench scale). The body weight was measured on the day of introduction of the enrichment (Day 0), two weeks after enrichment introduction (Day 14), and on the last day of enrichment exposure (Day 42) in Experiment 1. In Experiment 2, the measures were conducted on Day 0, Day 14, and on the last day of enrichment exposure (Day 28).

The number of eggs laid were recorded daily and Hen Day Egg Production (HDEP) was calculated as follows:

HDEP (in %) = (Number of eggs laid in cage/Number of hens present in cage) *100

To avoid potential sampling bias in a given day, the weekly HDEP was calculated as the average of the daily HDEP for the seven days for weeks 1, 2 and 6.

Eggs were weighed on the first and the last day of enrichment exposure using a digital scale (Mettler Toledo PM4600 Balance).

Evaluation of Plumage and Integument

The plumage and integument condition were evaluated through physical scores of the feathers, injuries, and footpads. The feather score ranged from 0 to 5, where 0 described a bird with intact feathers, and 5 represented a completely denuded area (Webster and Hurnik, 1990) (Table 2).

***Table 2**: Feather scores scale used for scoring the birds*

Score	**Feather condition**
0	Intact feathers
1	Some feathers scruffy, up to 3 missing feathers
2	More damaged feathers, > 3 feathers missing
3	Bald patch <50% of total area
4	Bald patch >50% of total area
5	Completely denuded area

The assessment of the injury score was carried out by scoring the body injuries on a scale of 0 to 4. An injury score of 0 depicted the absence of injuries or scratches, while a score of 4 represented the presence of one or more wounds measuring >2 cms in diameter (Bilcik and Keeling, 1999) (Table 3).

Table 3*: Injury scores scale used for scoring the birds*

Score	**Injury condition**
0	No injuries or scratches
1	<5 pecks or scratches
2	5 or more pecks or scratches
3	Wound >1 cm in diameter, but <2 cms
4	Wound more than 2 cms in diameter

Footpad score ranged from 0 to 2, with 0 describing the absence of any lesion, and 2 depicting severe lesions (Ekstrand and Algers, 1997) (Table 4).

Table 4*: Footpad scores scale used for scoring the birds*

Score	**Footpad condition**
0	No lesions: mild hyperkeratosis, no discoloration or scars
1	Mild lesions: superficial lesions, erosions, papillae; discoloration of footpad
2	Severe lesions: deep lesions; ulcers and scabs

The physical scores were measured individually for each bird every time the bird was weighed. The assessment was carried out by three people working together to ensure maximum consistency.

Measure of Fecal Corticosterone

Fecal corticosterone analysis was the chosen method for measuring physiological stress to minimize handling stress. Corticosterone is extensively metabolized in the liver and is therefore present as metabolites in the feces. The fecal corticosterone metabolites (FCM) are a short-term stress measure and reflect the stress levels experienced by the bird 4-5 hours prior to sample collection (Goymann et al., 2006). To analyze the effects of the treatments on FCM, bird droppings were sampled between 1000 and 1200 h on the last day of the enrichment exposure. The samples were collected one cage at a time. The male from the cage was transferred to another cage to obtain a pooled sample from the females. Females were chosen for sampling to eliminate any sex related differences. With the two females in the cage, the manure sheet was removed from the base underneath the cage and was replaced with a clear, unused sheet. Upon defecation, the pooled samples were collected in sterile microcentrifuge tubes and frozen at -80 °C until further analysis.

The FCM were extracted from the samples using a methanol extraction technique adapted from Dehnhard et al., 2003. For each cage, 0.5 g of thawed sample was mixed with 5 ml of 80% methanol and homogenized until smooth. The mixture was vortexed at 250 rpm for 30 minutes, followed by a centrifuge at 2500 rpm for 20 minutes, before recovering the supernatant. To the pellet, 5 ml of 80% methanol was added, and the vortex and centrifuge procedures were repeated. The supernatant obtained was mixed with the previous supernatant and the mixture was diluted with 1 ml of phosphate buffer saline.

The FCM levels were measured (as picogram/milliliter) from the extracts by analyzing them in the DetectX Corticosterone Enzyme Immunoassay kit (Arbor Assays, Ann Arbor, MI). All samples were analyzed in duplicate. The sensitivity of the assay was 20.9 pg/mL, with a detection limit of 17.5 pg/mL. The intra- and inter-assay variance was 9.5 and 11.4% respectively. The cross-reactivities of the assay reported by Arbor Assays were corticosterone (100%), aldosterone (0.62%), cortisol (0.38%), progesterone (0.24%), cortisone (<0.08%), and estradiol (<0.08%).

Statistical Analysis

The data were analyzed using generalized linear mixed models in SAS 9.4 (SAS Institute Inc., 2016, Carey, NC), with cage as the experimental. PROC UNIVARIATE was used to assess normality. Square root transformations were performed to normalize the body weight and HDEP, while FCM levels were normalized using log transformation. PROC MIXED was used to analyze body weight, HDEP, egg weight, and FCM levels. The time past after enrichment exposure was accounted for while analyzing the body weight, HDEP, and egg weight by including the day after exposure as a classification variable. Random effects accounted for included those of the replicates, cage location, and the interactions between replicate, cage location, and the treatment. Physical scores, which were non-continuous measures, were analyzed using PROC FREQUENCY and CHI SQUARE.

P-values were adjusted for multiple comparisons using the Bonferroni adjustment and significance was set at adjusted P-value < 0.05.

Results

Effects of Exposure to a Single Enrichment

Body weights did not differ across treatments on Day 0 (P > 0.05; Figure 19). Body weight increased from Day 0 to Day 42 in Hut (9.37 ± 0.04 grams, P=0.039; Figure 19), resulting in a higher body weight in the Hut as compared to the control on Day 42 (5.30 ± 0.04 grams, P=0.031; Figure 19). The measure remained unchanged from Day 0 to Day 42 in Plastic leaves, Grass, Mirror, Feeder toy, and Mat (P > 0.05; Figure 19).

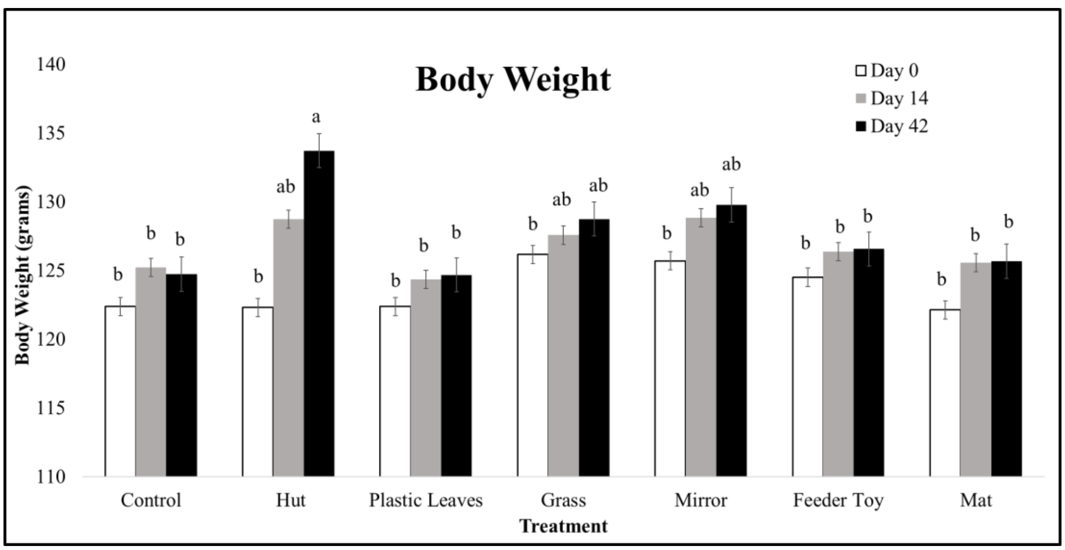

***Figure 19**: Body weight (in grams) in control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the body weights over time (P < 0.05).

The average eggs laid per day per cage in the first, second, and sixth week after treatment exposure were as follows: in Control 1.29, 1.32 and 1.60 eggs respectively; in Hut 1.26, 1.61 and 1.63 eggs respectively; in Plastic leaves 1.31, 1.35 and 1.59 eggs respectively; in Grass 1.30, 1.63 and 1.63 eggs respectively; in Mirror 1.33, 1.34 and 1.58 eggs respectively; in Feeder toy 1.29, 1.36 and 1.60 eggs respectively; and in Mat 1.30, 1.47 and 1.62 eggs respectively. Weekly HDEP (Figure 20) percent were determined by the equation:

HDEP (in %) = (Number of eggs laid in cage/Number of hens present in cage) *100

To avoid potential sampling bias in a given day, the weekly HDEP was calculated as the average of the daily HDEP for the seven days for weeks 1, 2 and 6.

The HDEP increased from Week 1 to Week 2 in the Hut and Grass (16.20 ± 0.08 % and 16.74 ± 0.07 % respectively, $P < 0.05$; Figure 20), and in Week 2, was higher in the Hut and in Grass as compared to the control (12.18 ± 0.05 % and 11.84 ± 0.05 % respectively, $P < 0.05$; Figure 20). The HDEP did not change Week 2 onwards in the Hut and in the Grass ($P > 0.05$; Figure 20).

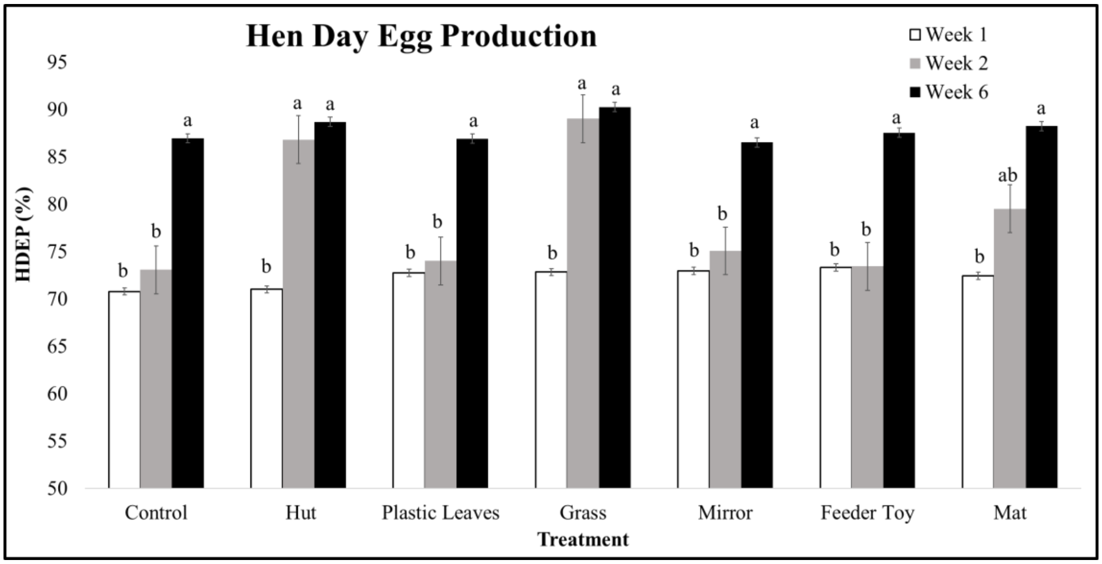

Figure 20*: HDEP (in percentage) in control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the HDEP over time (P < 0.05).

Egg weights did not differ within and between the treatments (P > 0.05, >0.05; Figure 21).

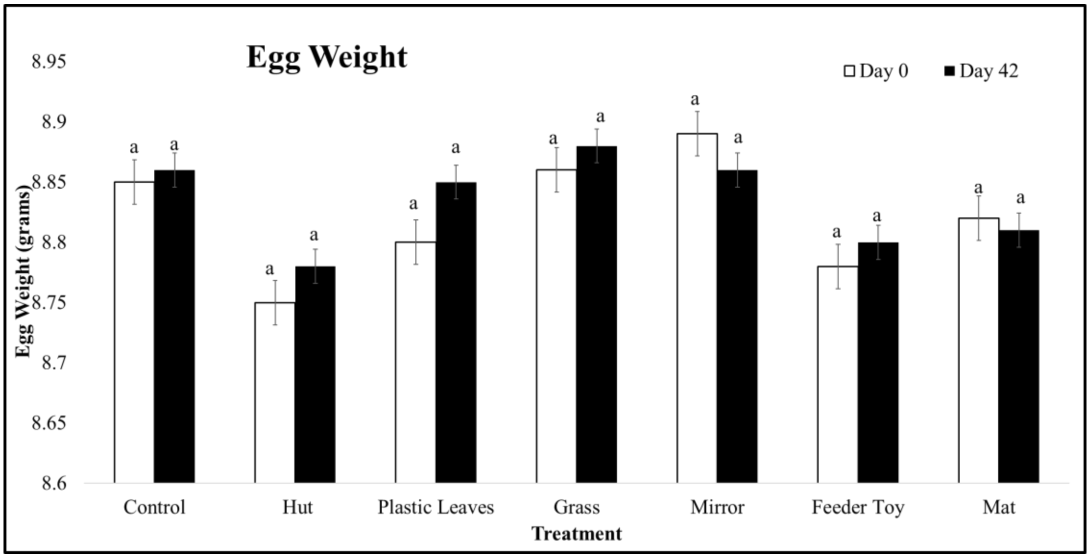

Figure 21*: Egg weight (in grams) in control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the egg weights over time (P < 0.05).

The feather score (lower is better) was higher on Day 0 in the control as compared to Hut, Plastic leaves, and Grass (0.31 ± 0.08, 0.30 ± 0.07, and 0.28 ± 0.07 respectively, P < 0.05; Figure 22). The score did not change from Day 0 to Day 42 in any of the enrichments (P > 0.05; Figure 22). Feather score increased from Day 0 to Day 42 in the control (0.59 ± 0.08, P=0.010; Figure 22), making it higher than those of Hut, Plastic leaves, Grass, Mirror, Feeder toy, and Mat on Day 42 (0.78 ± 0.08, 0.73 ± 0.08, 0.81 ± 0.08, 0.64 ± 0.06, 0.60 ± 0.08, and 0.59 ± 0.08 respectively, P < 0.05; Figure 22).

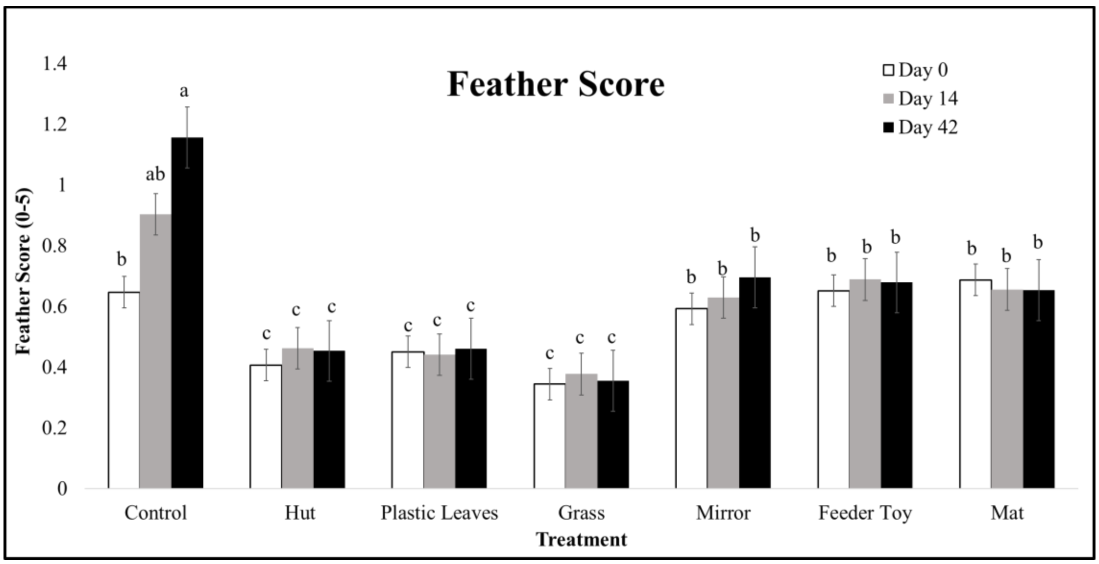

***Figure 22**: Feather score (scale of 0 to 5) with control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the feather scores over time (P < 0.05). A lower feather score implies a better plumage condition.

Injury scores (lower is better) did not vary across the treatments on Day 0 (P > 0.05; Figure 23). The score increased from Day 0 to Day 42 in the control (0.04 ± 0.006, P=0.041; Figure 23), such that on Day 42, it was higher than the Hut, Plastic leaves, Grass, Mirror, Feeder toy, and Mat (0.16 ± 0.004, 0.18 ± 0.006, 0.22 ± 0.006, 0.06 ± 0.004, 0.10 ± 0.006, and 0.21± 0.006 respectively, P < 0.05; Figure 23). Injury score was lower in Grass on Day 14 as compared to Hut and Plastic leaves (0.03 ± 0.006 and 0.02 ± 0.006 respectively, P < 0.05; Figure 23). The score was higher in the Mirror on Day 14 as compared to the Feeder toy and Mat (0.03 ± 0.008 and 0.04 ± 0.006 respectively, P < 0.05; Figure 23). The Mat recorded lower injury scores on Day 42 as compared to the Mirror and the Feeder toy (0.10 ± 0.004 and 0.05 ± 0.006 respectively, P < 0.05; Figure 23).

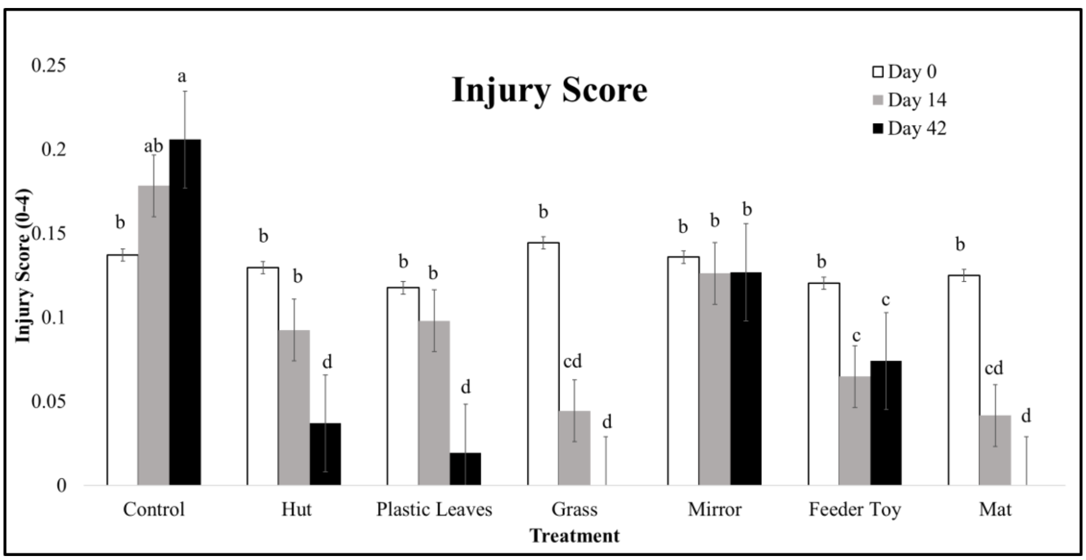

***Figure 23**: Injury score (scale of 0 to 4) with control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the injury scores over time (P < 0.05). A lower injury score implies a more intact skin.

Footpad scores (lower is better) did not vary across the treatments on Day 0 (P > 0.05; Figure 24). The score increased from Day 0 to Day 42 in the control (0.19 ± 0.003, P=0.013; Figure 24), such that on Day 42, it was higher than the Hut, Plastic leaves, Grass, Mirror, Feeder toy, and Mat (0.23 ± 0.001, 0.18 ± 0.003, 0.26 ± 0.003, 0.17 ± 0.006, 0.11 ± 0.003, and 0.22 ± 0.003 respectively, P < 0.05; Figure 24). Footpad score decreased from Day 14 to Day 42 in Hut and in Grass (0.09 ± 0.001 and 0.07 ± 0.003 respectively, P < 0.05; Figure 24). The score was higher on Day 42 in Plastic leaves as compared to Hut and Grass (0.04 ± 0.003 and 0.05 ± 0.003 respectively, P < 0.05; Figure 24). In Mat, the score decreased from Day 0 to Day 42 (0.02 ± 0.006, P=0.047; Figure 24), such that on Day 42, it was lower in the Mat as compared to the Mirror and Feeder toy (0.06 ± 0.003 and 0.11 ± 0.005 respectively, P < 0.05; Figure 24).

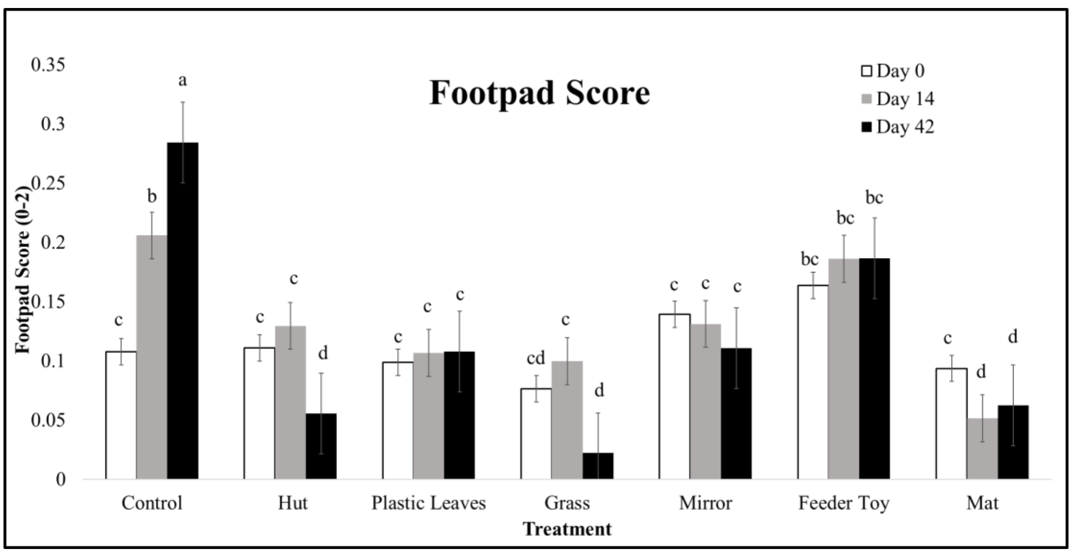

Figure 24*: Footpad score (scale of 0 to 2) with control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the footpad scores over time (P < 0.05).

Fecal corticosterone levels did not vary across treatments (P > 0.05; Figure 25).

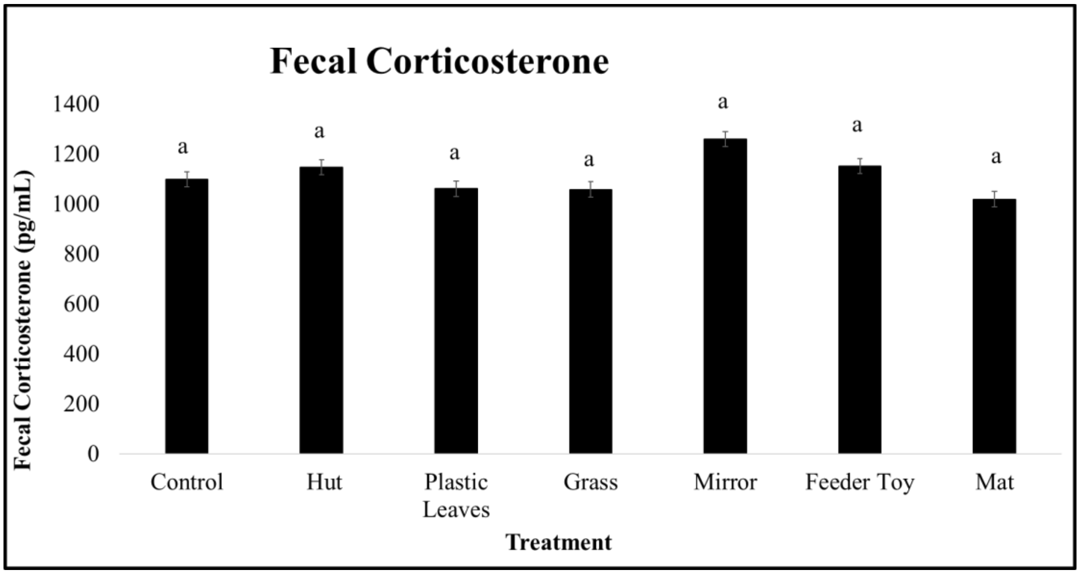

***Figure 25**: Fecal corticosterone (in picogram/milliliter) of the control, hut, plastic leaves, grass, mirror, feeder toy and mat.*
Bars indicate LS means±SEM. Common letters indicate absence of significant differences across treatments (P > 0.05).

Effects of Exposure to a Combination of Enrichments

No differences were seen within and across treatments in the body weights (P > 0.05 and > 0.05 respectively; Figure 26), weekly HDEP (P > 0.05 and > 0.05 respectively; Figure 27), and in the egg weight (P > 0.05 and > 0.05 respectively; Figure 28).

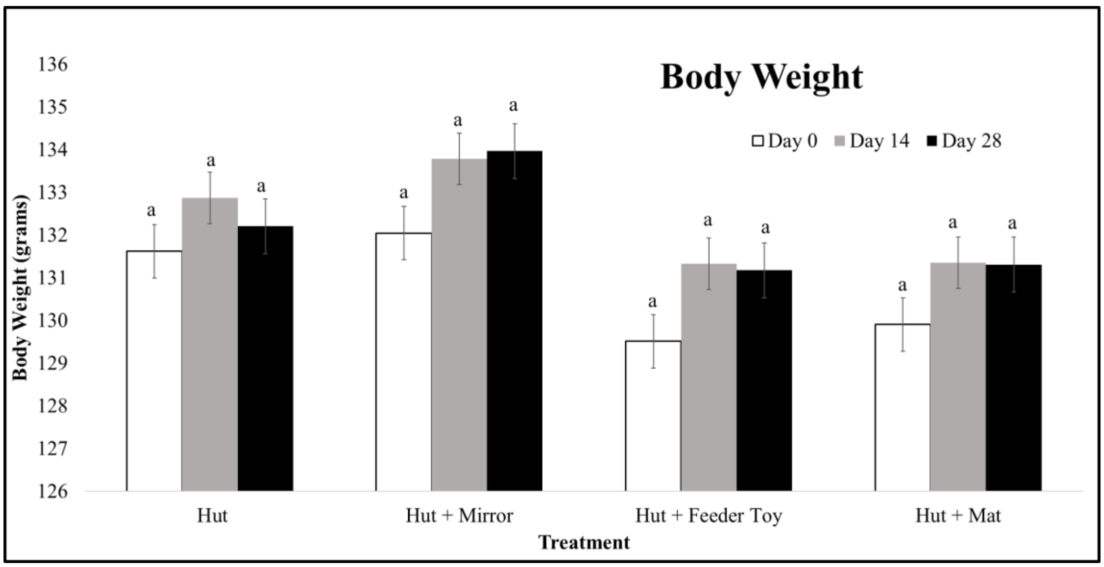

***Figure 26**: Body weight (in grams) with hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Common letters indicate absence of significant differences across treatments (P > 0.05).

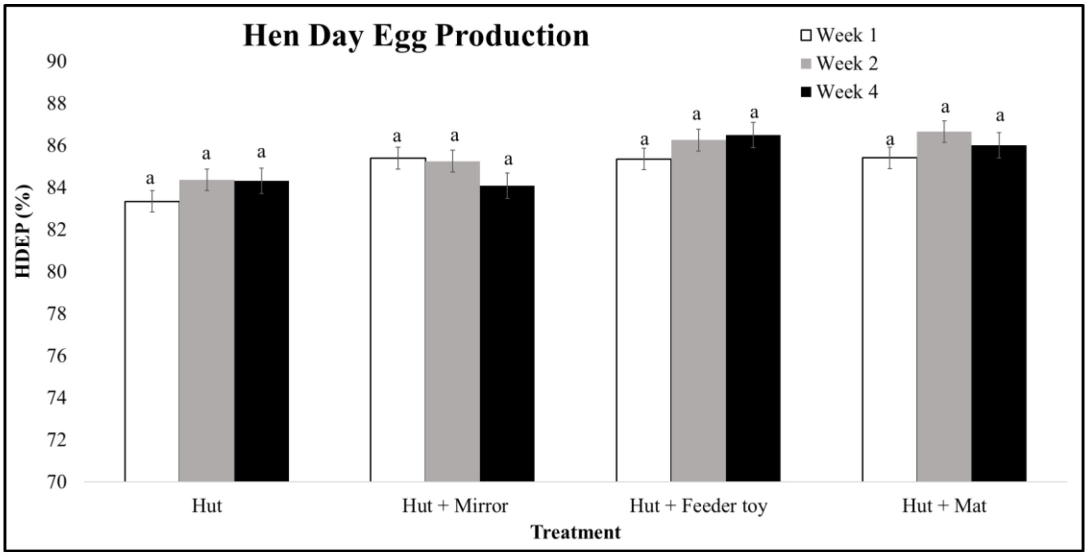

Figure 27: *HDEP (in percentage) with hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Common letters indicate absence of significant differences across treatments (P > 0.05).

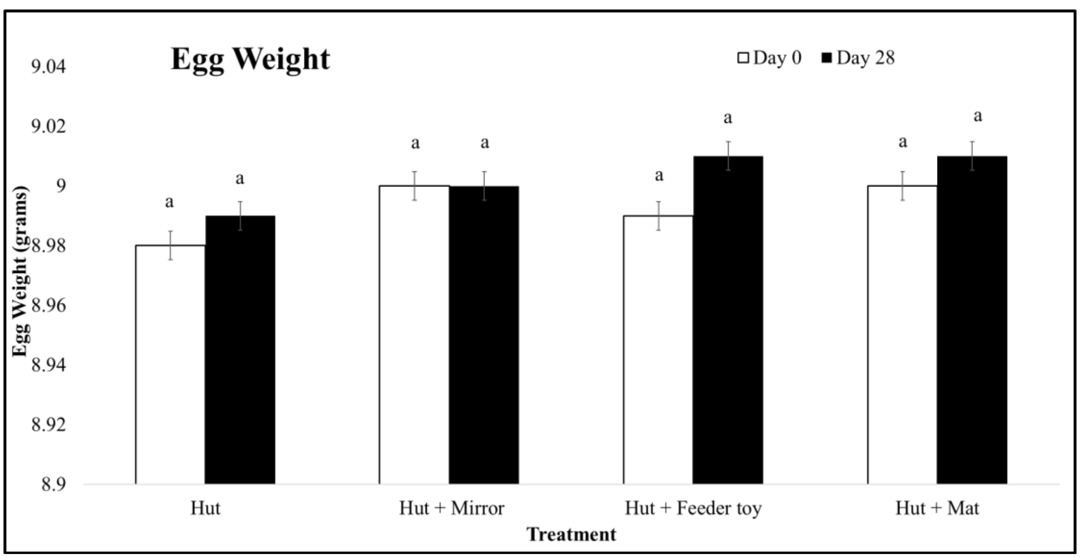

Figure 28: *Egg weight (in grams) with hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Common letters indicate absence of significant differences across treatments (P > 0.05).

Feather score (lower is better) decreased from Day 0 to Day 28 in the Hut + Mat (0.21 ± 0.01, P=0.031; Figure 29), such that on Day 28 the feather score in Hut + Mat was lower than that in Hut (0.10 ± 0.01, P=0.045; Figure 29). The score did not change from Day 0 to Day 28 in the Hut, Hut + Mirror and Hut + Feeder toy (P > 0.05, > 0.05 and > 0.05 respectively; Figure 29).

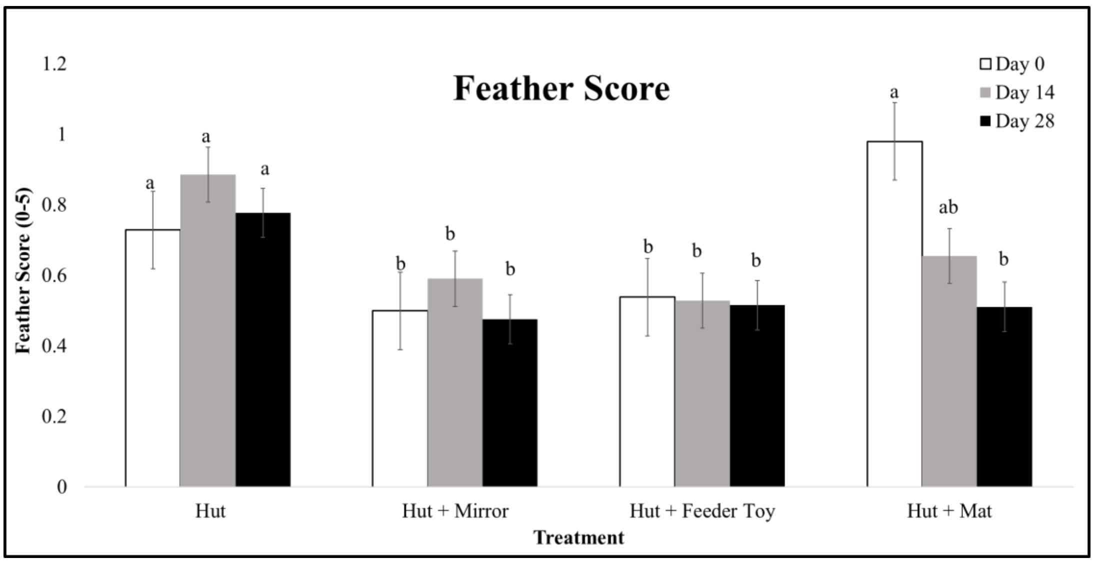

Figure 29*: Feather score (scale of 0 to 5) with hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the feather scores over time (P < 0.05). A lower feather score implies a better plumage condition.

Injury score (lower is better) did not increase from Day 0 to Day 28 in Hut + Mirror and Hut + Feeder toy (P > 0.05 and > 0.05 respectively; Figure 30), and decreased over time in Hut + Mat (0.07 ± 0.009, P=0.037; Figure 30). On Day 28, the score was higher in the Hut as compared to Hut + Mirror, Hut + Feeder toy, and Hut + Mat (0.13 ± 0.007, 0.05 ± 0.007 and 0.06 ± 0.009 respectively, P < 0.05; Figure 30).

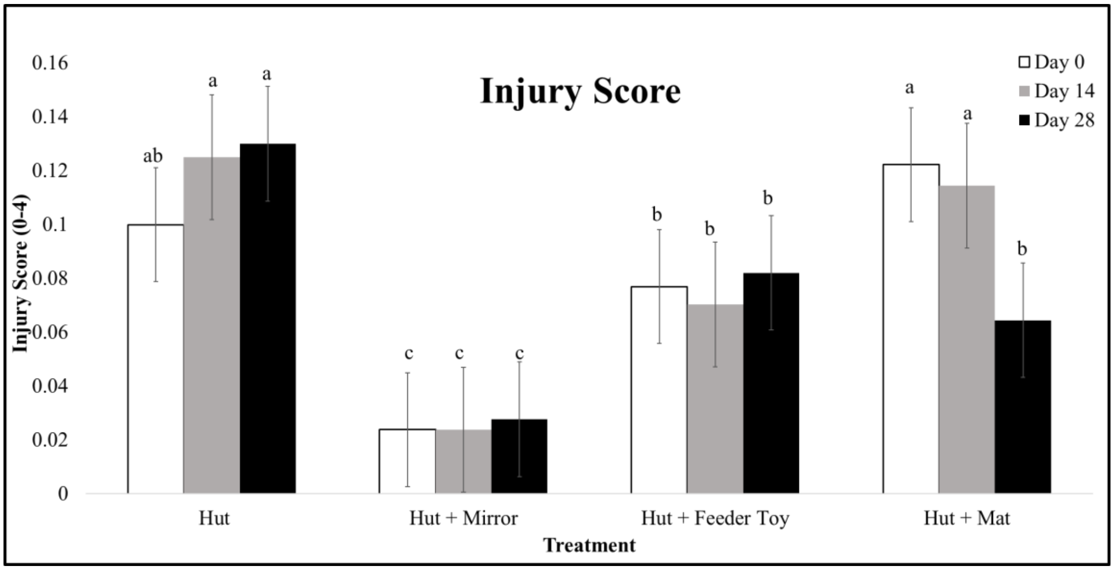

***Figure 30**: Injury score (scale of 0 to 4) with hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences between the injury scores over time (P < 0.05). A lower injury score implies a more intact skin.

Footpad score did not vary across and within treatments (P > 0.05 and > 0.05 respectively; Figure 31).

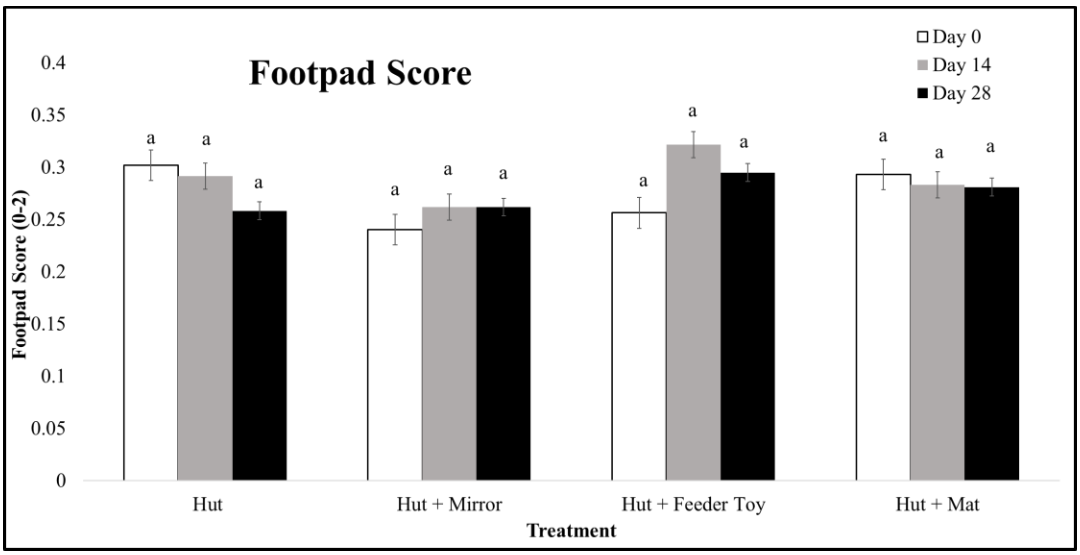

Figure 31*: Footpad score (scale of 0 to 2) with hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Common letters indicate absence of significant differences across treatments (P > 0.05).

FCM, the measure for fecal corticosterone, was lower in Hut + Mat as compared to the Hut (204 ± 4.7 pg/ml, P=0.048; Figure 32). FCM in Hut +Mirror tended to be low as compared to the Hut (M=166 ± 5.2 pg/ml, P=0.060; Figure 32).

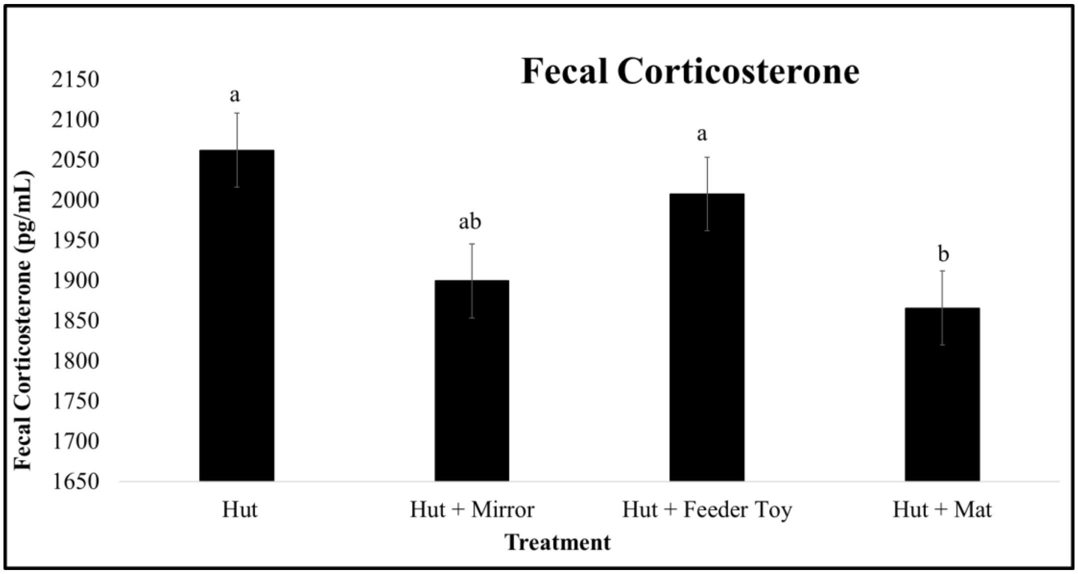

Figure 32*: Fecal corticosterone (in picogram/milliliter) of the hut, hut + mirror, hut + feeder toy, and hut + mat.*
Bars indicate LS means±SEM. Different letters indicate significant differences across treatments (P < 0.05).

<u>*Discussion*</u>

This study analyzed the health and production effects of enrichment(s) exposure in colony-caged Japanese quail by analyzing the birds' production, physical scores, and fecal corticosterone levels. Each enrichment category (protective, stimulatory) exerted diverse health impacts, however, two out of the three protective enrichments exerted a notable impact on production, while combinations of protective and stimulatory enrichments were more efficient in decreasing physical scores and corticosterone levels compared to a single enrichment.

Exposure to a Single Enrichment

Body weight increased from Day 0 to Day 42 in the Hut, making it higher than the control on Day 42. This weight gain can be attributed to the green cover provided by the enrichment, which could have simulated a natural hiding environment that could be used to escape energy-consuming unwanted social interactions. The relative darkness inside the hut could have facilitated resting (Blokhuis, 1983) and reduced energy expenditure, leading to the weight gain. Body weight did not change significantly over time in the Plastic leaves, Grass, Mirror, Feeder toy, and Mat, because unlike the Hut, each of them likely provided the birds stimulatory feedback, leading to higher activity levels. The body weight gain upon exposure to the Hut was unlike that observed in previous quail studies where body weight remained unchanged upon exposure to a protective enrichment (Miller & Mench, 2006; Laurence et al., 2015; Taskin and Karadavut, 2017). Such result discrepancies can be attributed to the fact that the previous studies did not involve mixed-sex colonies of quail and therefore lesser agonistic energy-consuming behaviors were displayed than those in the mixed-sex quail colonies.

The HDEP increased from Week 1 to Week 6 in all the treatments, including control. However, the HDEP increased faster in the Hut and in the Grass plausibly because the two enrichments acted as nesting sites. The sheltering effect of the enrichments could have reduced the risk of egg predation (Mainwaring et al., 2014), facilitating egg laying in the enrichment. Our results are supported by a quail study which shows that enrichments that provide cover have been preferred as egg laying sites by quail before (Buchwalder and Wechsler, 1997). The sheltering effect could have also induced resting, and therefore encouraged egg laying. Quail are a ground-nesting species that commonly lay eggs in grasslands (Orcutt and Orcutt, 1976). In addition to shelter, the Grass plausibly acted as an ideal egg laying substrate, leading to the faster HDEP increase. Our results partially align with those of Leone and Estevez (2008), who showed an increased egg quantity upon exposure to cover panels in broiler breeders; and those of Houdelier et al. (2018) who exhibited an improved body weight in chicks of structurally enriched quail hens.

Feather scores were consistently lower in the Hut, Plastic leaves, and Grass as compared to the control plausibly due to the protective nature of the enrichments. The three enrichments acted as a physical and/or visual barrier between the birds, leading to a faster reduction in unwanted social interactions and stress-related damaging behaviors as compared to the stimulatory enrichments. Although higher than those in the protective enrichments, the feather scores in the stimulatory enrichments did not increase from Day 0 to Day 42, and were lower than those in the control on Day 42. The stimulatory enrichments facilitated consistent interactions with the enrichment which likely reduced the motivation to indulge in behaviors and interactions that could cause physical harm. A majority of our enrichments such as Plastic leaves, Grass, Feeder toy, and Mat served as foraging and/or pecking sites and likely re-directed pecking towards the enrichment, which could have further reduced feather pecking and helped maintain the feathers. A positive effect on the feather condition has been observed previously with exposure to enrichments which encourage foraging and re-direct pecking including saw-dust bedding and sand-bath (Nordi et al., 2012) and gravel trays and artificial grass (Miller and Mench, 2006).

Injury scores decreased over time in the three protective enrichments likely because of the enrichments served as a shelter. Previous research has shown that provision of a cover can reduce injuries in quail, especially those caused due to flying attempts (Buchwalder and Wechsler, 1997). The scores decreased from Day 0 to Day 14 in the Grass unlike in Hut and Plastic leaves where it remained significantly unchanged until Day 14. This faster decline in the injury scores in Grass is likely due to the stimulatory feedback provided by the Grass in addition to acting as a protective enrichment. The Grass, resembling a natural resource, encouraged several natural behaviors such as dust-bathing and egg laying, which could have decreased the birds' motivation to perform behaviors and interactions which could lead to injuries. Unlike the other protective enrichments, Grass provided the birds a foraging opportunity which could have re-directed pecking at the Grass, and reduced pecking-related injuries. The Mirror recorded lower injury scores than the control on Day 42, however, the scores in Mirror did not reduce significantly over time as those in Feeder toy and in Mat. The Mirror was designed to provide a different kind of stimulatory feedback as compared to the other stimulatory enrichments, and it likely did not encourage pecking at itself. Re-directed pecking at the Feeder toy and the Mat could have reduced injurious feather pecking in them. The Mat's structure helped sustain the birds' stimulation, which could have decreased their incentive to engage in injurious behaviors and further reduced the scores. The effects of Mat on lowering injuries reflects those of a turkey study that showed lower injury scores upon exposing the birds to structural enrichments that provide a sitting area (Lindenwald et al., 2021).

The footpad scores were lower in birds exposed to any of the enrichments as compared to the control on Day 42 possibly due to the stereotypic pacing resulting from a lack of stimulation in the control (Mason, 1991). The birds in the control walked significantly more and sat and stood significantly lesser than those with the enrichments (Chapter 3), which could have deteriorated the footpad health. Footpad scores decreased from Day 0 to Day 42 in the Hut and Grass likely due to their sheltering effect which facilitated resting, thereby reducing walking. The Hut's provision of a dark and green cover, and the Grass' close resemblance to a natural resource could have reduced the birds' need to perform stress-related pacing, and further improved the footpad scores. Our results are in accordance with a previous quail study which has shown low footpad scores upon exposure to protective enrichments such as a cover and hay bales (Ohara et al., 2015). Grass and the Mat offered the birds a softer surface than the control, which could have helped maintain their footpads, as has been observed previously in laying hens (Pickel et al., 2011). The two enrichments also provided a scratching opportunity, which could have impacted the footpads, as has been seen before in laying hens (Dikmen et al., 2016). However, exposure to moisture is known to worsen footpad health in poultry (Shepherd and Fairchild, 2010), and therefore further studies should assess the long-term footpad condition effects of exposure to Grass.

Exposure to a Combination of Enrichments

The production measures did not differ significantly across the treatments plausibly due to the presence of the hut in every treatment. The hut likely provided shelter for resting and an escape from energy-consuming unwanted social interactions; leading to more standing and lesser walking (Chapter 3), and the facilitation of egg laying, irrespective of the presence of the stimulatory enrichment.

Feather scores decreased from Day 0 to Day 28 in the Hut + Mat and was lower in all the enrichment combinations on Day 28 as compared to only Hut. The hut likely acted as a barrier between birds, reducing negative interactions that could cause physical harm. The presence of a stimulatory enrichment in addition to a hut plausibly led to a further reduction in harmful interactions through sustained engagement with the stimulatory enrichment, which reduced the motivation to perform harmful interactions. The impact of the enrichment combinations aligns with a previous quail enrichment study where better feather scores were observed upon exposure to a combination of protective enrichments such as artificial leaves and branches and stimulatory enrichments such as rocks (Miller and Mench, 2006).

Injury scores reduced over time in the Hut + Mat, as the mat likely re-directed pecking behavior towards itself. Re-directed pecking was plausibly more consistent in the Hut + Mat as compared to the Hut + Mirror and the Hut + Feeder toy; while Hut did not facilitate pecking at itself. Our results partially align with those of Nordi et al., 2012, where exposure to protective enrichments such as nests, along with stimulatory enrichments that can be pecked such as sand-box, reduced injuries in egg-laying Japanese quail.

As compared to the Hut, FCM levels in Hut + Mat were significantly lower and those in Hut + Mirror exhibited a trend of being lower. Lowering of fecal corticosterone upon enrichment exposure has not been observed in quail before. The presence of a mirror or a mat in addition to the hut likely de-stressed the birds by providing them with a choice, as has been observed in chickens previously (Pettit-Riley et al., 2002; Nicol et al., 2009). The feeder toy did not facilitate the simultaneous use of the enrichment by more than two birds, which could have reduced the choice that the Hut + Feeder toy combination offered the birds. The Hut + Mat combination facilitated highly motivated behaviors such as foraging, scratching, and hiding from fearful stimuli (Guyomarch et al., 1986), and reduced stress likely by allowing the expression of a larger variety of natural behaviors (Bracke and Hopster, 2006). Our results are in partial accordance with a layer enrichment study which exhibited reduced FCM levels when hens were exposed to a combination of a protective and stimulatory (hay bales and pumice stone) (Son et al., 2022).

Limitations

A majority of the enrichments used in this study including Hut, Plastic leaves, Mirror, and Mat are easy-to-use; however, Grass and Feeder toy have some practical limitations. Both the enrichments require routine replacement and are prone to breaching biosecurity. This limitation of the Feeder toy can be overcome through automating the toy. Grass, although not practical in an industrial setting, can prove to be an effective enrichment in backyard quail farms.

Conclusion

Two of the three protective enrichments exerted a notable positive impact on production, while combinations of protective and stimulatory enrichments were more efficient in decreasing physical scores and corticosterone levels compared to a single enrichment. Future research could explore the health effects of long-term enrichment exposure and analyze how the exclusion and re-introduction of enrichments can impact breeding quail health. Analyzing the inter-generational stress effects of enrichment exposure through measuring yolk corticosterone can be a potential research avenue. Future studies can also investigate the effects of these enrichments on other broiler species such as chicken broilers which are reared under similar conditions. Overall, quail exposed to enrichment(s) exhibited better physical scores, better production rate in Hut and Grass, and lower FCM levels in Hut + Mat. Given that higher physical scores, higher FCM levels, and low production are an indicative of stress and poor health, the use of optimal enrichment(s) can offer a win-win situation by improving quail well-being and by impacting the industry economics positively.

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Chapter 5: Discussion

The aim of this study was to analyze the impact of two categories of environmental enrichments on breeding quail well-being by exposing the birds to one protective enrichment (Hut, Plastic leaves, or Grass), one stimulatory enrichment (Mirror, Feeder toy, or Mat), or a combination of protective and stimulatory enrichments (Hut + Mirror, Hut + Feeder toy, or Hut + Mat). Investigation of the enrichment use and behavioral frequencies showed that exposure to either one of the enrichments reduced stress-related damaging behaviors and increased resting; while exposure to a combination of enrichments reduced a larger variety of stress-related damaging behaviors more efficiently. Measuring the birds' production, physical scores, and fecal corticosterone levels revealed that two of the three protective enrichments exerted a notable impact on production, while a combination of protective and stimulatory enrichments were more efficient in decreasing physical scores and corticosterone levels compared to a single enrichment. These results indicate that both protective and stimulatory enrichments are individually efficient at improving breeding quail well-being as compared to the absence of an enrichment, however, the well-being effects of exposure to a combination of optimal enrichments are higher than those of exposure to a single enrichment in breeding quail.

Well-being of Breeding Quail

Breeding quail form an important part of the quail industry; however, their well-being is quite compromised under commercial conditions (Mench, 2002; Hristov et al., de Jong and Gunnink, 2019). Breeding quail are typically reared in small barren cages, where females and males are grouped in the ratio of 1:3 or 1:4, and share the cage continuously (Narinc et al., 2013). Such barren housing conditions often result in unwanted social and sexual interactions (Edens, 1987) and self-damaging behaviors (Blokhuis et al., 1991), which have been seen to cause stress and lower productivity (Hill, 1983), decrease progeny well-being (Eriksen et al., 2013), and in a biomedical setting, reduce the reliability of the results obtained from the birds (Edwards et al., 2018).

A solution to this well-being issue is the use of environmental enrichments, which can improve biological functioning by reducing stress (Newberry, 1995). To be effective, enrichments should meet the need of the species and do so in accordance with the rearing purpose of the species. For breeding quail, this would mean using enrichments which would help reduce unwanted interactions and stress-related damaging behaviors. To study the effect of two enrichment categories on the well-being of breeding quail, in our study, we exposed breeding quail to a protective enrichment, which would provide the birds a shelter, and/or a stimulatory enrichment, which would provide mental stimulation. We hypothesized that the use of a single enrichment in breeding quail will result in a) decreased unwanted social and sexual interactions, b) reduced stress-related damaging behaviors, and c) increased positive behaviors, ultimately improving their well-being and productivity. Our second hypothesis was that the use of a combination of a protective and a stimulatory enrichment will result in a) reduction of a larger variety of unwanted interactions and damaging behaviors, and b) an increase in more positive behaviors, in comparison to exposure to a single enrichment; thereby proving more beneficial for improving well-being and productivity.

Analyzing the Impacts of Enrichments

Protective Enrichments

The protective enrichments notably improved more than one well-being parameter. Behavioral repertoire improved with all three enrichments, namely Hut, Plastic leaves, and Grass, as evident through the decreased frequencies of various stress-related damaging behaviors and improved resting behaviors. Hiding is the quail's natural response to fearful stimuli (Guyomarch et al., 1986) and the protective enrichments likely facilitated this response by providing shelter. Hiding plausibly aided an avoidance of unwanted social interactions and resultantly reduced stress-related damaging behaviors. The Hut and the Grass impacted the birds' production positively. The Hut improved the HDEP and exhibited a tendency of improved body weight possibly by acting as a physical protective barrier from damaging social interactions. The Grass provided a visual barrier in addition to a physical one, which could have created a safe haven for the birds and resultantly improved their production. Physical scores such as feather score and injury score were seen to improve upon exposure to either of the enrichments plausibly due to the enrichments' sheltering effect. Availability of a retreat likely protected the birds from unwanted social interactions such as aggressive pecking and sexual mounting, which can impact the feather and skin conditions. The enrichments impacted the footpad score positively likely due to their reducing fear through sheltering and thereby decreasing damaging behavior such as pacing.

Overall, each of the three enrichments improved the birds’ well-being, but the Hut and the Grass exhibited greater well-being benefits than the Plastic leaves. Compared to the Plastic leaves, the Hut provided a well-structured hiding area and the Grass resembled a natural resource more closely. The area available for standing or sitting under/on the enrichment was higher in the Hut and the Grass as compared to the Plastic leaves. Such properties of the Hut and the Grass plausibly provided the birds an opportunity to exhibit more natural behaviors such as resting under a shelter, and pecking grass and mud. The birds also used the two enrichments to lay eggs and the Grass to dust-bath, which we noticed as an observation. The Plastic leaves have a coarse surface which can accumulate feed particles and feces. This makes the enrichment prone to food safety issues if not washed on a regular basis. The Grass requires regular changing and can lead to biosecurity issues due to dispersing of the mud. However, the Grass can be used efficiently in a backyard farm setting. The Hut is practical for a commercial setting as it overcomes the shortcomings of the Plastic leaves and the Grass. Given the Hut’s structure, it cannot accumulate feed particles or feces, and can be cleaned while being kept in the cage. The enrichment can indeed be pre-fixed within the cage, making its use even more convenient.

Stimulatory Enrichments

The stimulatory enrichments had a significantly positive impact on various behavioral and physiological parameters. The behaviors exhibited by the birds improved upon exposure to either of the enrichments, namely Mirror, Feeder toy, and Mat, as seen through reduced stress-related damaging behaviors and improved resting frequencies. These effects can be attributed to the enrichments' keeping the birds occupied by providing mental stimulation and engagement, and thereby decreasing the need to exhibit damaging behaviors. In the production measures, the enrichments did not exhibit an impact on the body weight or egg parameters plausibly due to an interactive engagement with the enrichment including energy-consuming activities like pecking and scratching, which could have prevented a notable increase in production. Physical scores such as feather score and injury score were better in the enrichments compared to the control likely because they kept the birds occupied, resulting in reduced unwanted social interaction frequencies. Footpad scores improved upon enrichment exposure plausibly due to the interactions with the enrichments which increased the time spent standing or sitting near the enrichment, and thereby reduced the time spent walking or pacing on the stainless-steel surface.

The stimulatory enrichments positively impacted the well-being measures, however, the effect an enrichment exerted on a measure varied from one enrichment to another. The area and the texture of the Mat's surface was likely responsible for causing the highest frequencies of sitting and those of interaction with the enrichment, as compared to the Mirror and the Feeder toy. The surface likely re-directed floor scratching to the Mat, resulting in the greatest reduction of floor scratching and least footpad scores in the Mat compared to the other stimulatory enrichments. Although the injury scores were lower in all the enrichments as compared to the control, they reduced overtime in the Feeder toy and the Mat, unlike in the Mirror. The lower injury scores in the Feeder toy and the Mat can be attributed to the ability of these enrichments to redirect pecking at themselves and to plausibly keep the birds engaged for a longer time than the Mirror. However, enriching with the Mirror is more practical under a commercial setting as compared to the Mat and the Feeder toy. The Mat, similar to the Plastic leaves, has a surface which can accumulate feed particles and feces, and make it prone to biosecurity issues unless washed regularly. Nonetheless, unlike the Plastic leaves which can wear out due to water pressure, the Mat can be cleaned while being kept in the cage. The Feeder toy requires regular re-filling and would require automation to be practical in a commercial setting. However, the enrichment has the potential to be useful in a backyard farm setting or for quail reared as pets.

Enrichment Combinations

Combinations of protective and stimulatory enrichments reduced a larger variety of stress-related damaging behaviors more efficiently, and improved physiological parameters including fecal corticosterone levels as compared to exposure to a single enrichment. The enhancement in reducing damaging behaviors observed in the enrichment combinations was likely because the combinations provided the birds benefits of both enrichment categories, resulting in a protective and a stimulatory effect. The positive impacts of the enrichments on the physical scores such as feather score and injury score can be attributed to the enrichments' impact on reducing stress-related damaging behaviors which can result in feather loss and injuries. The tendency of enrichment combinations to reduce fecal corticosterone levels was unlike that seen in exposure to a single enrichment, and indicates that stress can reduce significantly when the birds are exposed to an environmental complexity which offers both, a shelter and mental stimulation.

Overall, the enrichment combinations had a greater positive impact on the birds' well-being as compared to a single enrichment. The Hut + Mat combination seemed to exert the greatest impact likely because it provided the birds an opportunity to perform two highly motivated behaviors, namely pecking and seeking shelter. Although the Hut + Feeder toy also provided the opportunity to perform these behaviors, the mat in the Hut + Mat combination plausibly encouraged scratching in addition to pecking, while also providing the birds a soft resting surface. A resting surface along with a hiding avenue to rest, likely increased the sitting frequencies and lowered the fecal corticosterone in the Hut + Mat combination as compared to the other two combinations. The study did not analyze certain aspects of the enrichment combination, which can be evaluated through future studies. For example, were the birds able to view the mirror while under the hut? If yes, how did it change the nature of what they viewed in the mirror? How does placing the hut on top of the mat impacts the well-being measures? How does such an arrangement impact utilization of the hut for egg laying? The Hut + Mirror combination is easy to maintain, and can be used in a range of settings including commercial, backyard, and for pet quail. The Hut + Mat combination can be feasible in a commercial setting if the enrichments are pre-fixed. Automating the toy in the Hut + Feeder toy combination can enable its use in commercial farms; however, the non-automated toy with the hut can be used for backyard and pet birds.

Conclusion and Future Directions

Our results show that using enrichments that meet the species' needs can facilitate positive behaviors that enhance well-being; and that the use of a combination of such optimal enrichments can improve the birds' well-being further. The research methods analyzed the well-being impacts of exposure to a specific enrichment, followed by those of exposure to an enrichment combination, thereby contributing to the literature of the well-being effects of various enrichment categories in breeding quail. The majority of the enrichments tested in the study have good practicality for a commercial breeding quail setting. The enrichments that lack a practical value can be made practical through various modifications and/or be conveniently utilized in backyard farm or for pet quail.

Our experiment had some limitations, which can be addressed in future research studies. We tested the well-being effects of the enrichments by exposing breeding quail to the enrichments for either 6 weeks (Experiment 1, single enrichment exposure) or for 4 weeks (Experiment 2, exposure to enrichment combination) and observed improvements in well-being parameters including behavior, production, and physiological parameters. However, the effects of the enrichment varied from one enrichment to another, and not all enrichments improved every parameter. Previous studies have shown that quail exposed to a set of stimulatory enrichments for a period of 10 weeks exhibited reduced fearfulness (Miller and Mench, 2005) and production improvements have been noticed in layers exposed to protective enrichments throughout the production period (Leone and Estevez, 2008). Therefore, future studies should investigate the effects of long-term enrichment exposure on quail well-being. Removal of long-term enrichments has shown to increase stress in Clark's nutcrackers as measured through feather corticosterone levels (Fairhurst et al., 2011), and research on the effects of enrichment removal should be conducted in quail to better understand the enrichments' role in their well-being. Several quail enrichment studies analyze the well-being impacts of a combination of more than two enrichments (Miller and Mench, 2005; Nazar and Marin, 2011; Nordi et al., 2012; Taskin and Karadavut, 2017), and a similar experiment could be designed to explore the impacts of exposing birds to more than two enrichments simultaneously and contrasting them with barren housing conditions. The study can potentially be designed to account for the sex differences in behaviors, to obtain a deeper understanding of the sex-dependent effects of enrichments in breeding quail. Maternal corticosterone has been observed to be transferred to the yolk and impact offspring growth in Japanese quail (Hayward and Wingfield, 2004); and exposing quail hens to enrichments has shown to improve the resilience of the progeny (Guesdon et al., 2011). Therefore, a research avenue exists in analyzing the well-being effects our enrichments have across generations. Our study measured the levels of fecal corticosterone only at the end of the enrichment exposure period. Obtaining a baseline sample in addition to the one collected at the end of exposure could have helped obtain a broader perspective of the enrichments' effects (Blas, 2015), and should be considered while designing future studies. Enrichments have been seen to impact other stress indicators such as adrenaline and noradrenaline in layers (Field et al., 2007; Moe et al., 2010) and immunity indicators such as heterophils-to-lymphocyte ratio in quail (Nazar and Marin, 2011; Nordi et al., 2012). Therefore, testing the impacts of our enrichments on such parameters can provide interesting insights in quail well-being. The quail utilized for this study were in-bred and therefore expressed limited genetic variations. The birds belonged to a closed population for over 25 years and have been shown to express low levels of aggressive behaviors (Ottinger, 2001) which reduced the mortality rates, making them good candidates for long-term observations. Future research that concentrates on studying the well-being effects of enrichments on-farm can help expand our knowledge of using enrichments in a practical setting. On-farm studies can further advance our knowledge regarding optimal enrichment materials (e.g., effects of using a wooden hut instead of an acrylic hut), optimal size of the enrichment (e.g., a feeder toy long enough to allow simultaneous use by a group of birds), and designing in-built enrichments for colony cages.

Appendix

Appendix 1

***Figure 1**: Stainless steel colony cages used for quail housing*

Figure 2*: Quail interacting with the Hut enrichment*

Figure 3*: Quail interacting with the Plastic leaves enrichment*

Figure 4*: Quail interacting with the Grass enrichment*

Figure 5*: Quail interacting with the Mirror enrichment*

Figure 6: *Quail interacting with the Feeder toy enrichment*

Figure 7*: Quail interacting with the Mat enrichment*

Figure 8*: Quail interacting with the Hut + Mirror enrichment combination*

Figure 9*: Quail interacting with the Hut + Mat enrichment combination*

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