Norwegian University
of Life Sciences

Master's Thesis 2017   60 ECTS
Faculty of Life Sciences, Department of Animal and Aquacultural Sciences

Assessment of Boxes as a Source of Environmental Enrichment for Broiler Chickens

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Animal Science, Ethology

Abstract

Additions to the environment (e.g. by provision of resources such as different structures and litter materials) could improve both the physical and psychological well- being of commercial broiler chickens. This study aimed to evaluate the value of cardboard boxes as an environmental enrichment for commercially kept broiler chickens. Behaviour observations were conducted on two flocks of fast growing broilers in Farm 1 and one flock of slower growing broilers in Farm 2, at three different ages during the production cycle (3, 18 and 28 days, with additional observations in Farm 2 at 49 days). The houses were divided in quarters (Farm 1) or in half (Farm 2), and for each pair of adjacent pens, one was assigned to the experimental treatment containing cardboard boxes, and one to the control treatment.

Instantaneous scans were performed to record the number of chickens standing (i.e. active) and lying (i.e. resting) on top of boxes (if present) and on the floor within observation patches. Three 10-s scans were then conducted to record "positive welfare" behavioural events based on ethogram description, and a graded scale was used for each behaviour where 0= no events observed in 10-s, 1= rare (one event observed in 10 s), 2= occasional (2-3 events observed in 10 s), 3= frequent (> 3 events observed in 10 s, involving > 3 birds). The chickens' use of the box was assessed by counting the number of birds on top, beside and under 8 boxes per experimental pen. A novel object test was conducted to assess approach versus avoidance tendencies, and finally gait scores to assess lameness.

Providing birds with cardboard boxes resulted in more playful behaviour such as running at 3 days of age (P=0.004) and jumping at 18 days of age (P=0.003) than in control pens, and more resting in patches containing a box than patches without a box in experimental pens (P=0.043). Lameness or response to novel object was not affected by provision of boxes. The enrichment value of cardboard boxes was not high at all ages, but one could see that birds like to use the boxes in different ways at different ages. The results of this study indicate that cardboard boxes as a source of environmental enrichment for broiler chickens are easy to implement and have positive influence on the behaviour and welfare.

Sammendrag

Tilskudd i omgivelsene (f.eks. ved å tilby ulike strukturer og strømaterialer) kan bidra til å forbedre både den fysiske og fysiologiske velvære til kommersielle broilerkyllinger. Formålet med dette studiet var å evaluere verdien av pappesker som en miljøberikelse for kommersielle broilerkyllinger. Atferds observasjoner ble utført på to flokker av hurtigvoksende kyllinger på gård 1, og en flokk med saktevoksende kyllinger på gård 2, på tre ulike aldre gjennom innsettet (3, 18 og 28 dager, i tillegg til en observasjon på gård 2 ved 49 dagers alder). Hvert hus ble delt opp i fire (gård 1) eller to (gård 2), og for hvert bingepar ble en tildelt eksperimentell behandling med pappesker, og en kontroll behandling.

Øyeblikksmålinger ble utført for å registrere antall kyllinger som sto oppreist (aktive) og som lå (hvilte), befant seg på toppen av boksen (hvis den var til stede) og som befant seg på gulvet innad i observasjonsområdet. Tre 10-s målinger ble deretter utført for å registrere atferd som indikerer positiv velferd basert på beskrivelsen i etogrammet. En gradert skala ble brukt for hver atferd hvor 0= ingen atferd observert på 10-s, 1= sjelden (1 atferd observert på 10-s), 2= sporadisk (2-3 atferder observert på 10-s), 3= hyppig (>3 atferder observert på 10-s, med >3 fugler involvert). Kyllingens bruk av boksen ble vurdert ved å telle antall fugler på toppen, ved siden av og under 8 bokser per eksperimentelle binge. Et ukjent objekt ble plassert i bingen for å vurdere tilnærming og unngåelsestendenser, og til slutt ble halthet vurdert ved å score fuglenes gangart.

Å gi kyllingene tilgang til pappesker resulterte i mer lekeatferd slik som løping ved tre dagers alder (*P*=0.004) og hopping ved 18 dagers alder (*P*=0.003) i eksperimentelle binger. Mer hvile ble utført i observasjonsområder med boks i eksperimentelle binger (*P*=0.043). Halthet eller respons til ukjent objekt ble ikke påvirket av tilstedeværelsen av bokser. Miljøberikelsesverdien av pappeskene var ikke så høy som forventet, men det var ingen tvil om at kyllingene likte å bruke dem på varierende måter ved ulik alder. Resultatene av dette studiet indikerer at pappesker som en kilde til miljøberikelse for broilerkyllinger er lett å implementere og har positiv påvirkning på atferd og velferd.

Acknowledgements

Animal behaviour and welfare have always been of great interest for me and this is what eventually led me to NMBU. It has been two educational years and I am thankful for all the knowledge I received, both in classes and while working with pigs, goats and sheep at the SHF farm. I am grateful for each and everyone who has encouraged and helped me throughout the thesis process and wish to express my gratitude to the following:

I want to thank my dedicated supervisor Ruth C. Newberry for consistent and thorough feedback along the entire process, as well as good help with statistical analyses. Also, thank you for joining me in the chicken house and helping with practical work and behaviour observations.

Thank you to my hands-on co-supervisor Guro Vasdal for all your help with practical solutions, teaching me how to gait score and for your comments and suggestions on earlier drafts of the thesis.

To Stian Karlsen, thank you for your enthusiasm and involvement, it's been great to have someone to discuss with and ask for advice in Excel.

Thank you Jan Are Jacobsen for helping me in ArchiCAD with drawings of the chicken pens.

Karoline Kvamme, thank you for proof reading before final hand in.

A special thanks to both chicken farmers who willingly opened their doors and were supportive of me all the way. Without you this study would not have been possible.

And thanks to my faithful dog Izana, for all our walks and time spent snuggling on the couch.

Ås, May 2017

Christine Sagedal

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1 Introduction

1.1 Background

The production of broiler chicken for meat consumption has increased rapidly in the last 50 years. In 1961 the total number of chickens worldwide was less than 4 billion (Nicol, 2015) whereas, in 2013, the number of chickens slaughtered for meat had increased to nearly 60 billion (Wilhelmsson, 2016). Europe produces over 6 billion birds of the total production, while Norway accounts for a relatively small proportion with 63 406 246 chickens produced in 2015 (Animalia, 2016). The large consumption of broiler meat is predicted to increase (Nicol, 2015; Wilhelmsson, 2016). This raises concerns, as the living conditions of intensively bred chickens potentially limit animal welfare. Commercial broiler chickens are typically reared in large indoor floor systems, with high stocking densities (Newberry, 1999; Bokkers and Koene, 2003; Febrer et al., 2006; de Jonge and van Trijp, 2013). In Norway the maximum density allowed is 36 kg/m^2 (Landbruks- og matdepartementet, 2001), while in EU the maximum density is 42 kg/m^2 (European Commission, 2000). The housing environments are lacking in structural complexity (e.g. cover panels, perches) (Norring et al., 2016), and the absence of varied environmental stimuli has been identified as an animal welfare concern in several studies and reports (European Commission, 2000; Jones et al., 2000; Bailie et al., 2013). The public has also expressed concern regarding the environment in which chickens are housed, with emphasis on high stocking densities and limited opportunities to engage in natural behaviour (de Jonge and van Trijp, 2013). Dawkins (1989) studied semi wild junglefowl and found that they spend most of the active part of the day foraging, and the remaining time grooming, sitting or standing. There are few qualitative behavioural differences between domestic fowl and their ancestors (European Commission, 2000; Appleby et al., 2004), but changes in broiler genetics over the last five decades have resulted in a quantitative alteration of activity patterns.

1.2 Welfare consequences in modern broiler production

Selection for high growth rate and increased feed conversion ratio, as well as management and nutritional improvements, has enabled the growing expansion of broiler production (Cooper and Wrathall, 2010; Nicol, 2015). Within 5 weeks broiler chickens reach a live weight of about 2.2 kg (Aviagen, 2014). Compared to their ancestor, the red junglefowl (*Gallus gallus),* commercial broilers grow to more than four times the body mass (Schutz and Jensen, 2001).

Rapidly increasing bodyweights is believed to be one of the main factors contributing to behavioural and welfare problems in broiler production (Weeks et al., 2000; Garner et al., 2002; Bessei, 2006; Shim et al., 2012). These welfare challenges include skeletal disorders, lameness, contact dermatitis, ascites, sudden death syndrome and reduced mobility (Weeks et al., 2000; Arnould et al., 2011), as well as birds showing low prevalence of natural behaviour such as ground pecking (Weeks et al., 2000). The metabolic demands for rapid growth seem to reduce the prevalence of energy demanding behaviours such as physical activity and social interactions (Weeks et al., 2000; Schutz and Jensen, 2001). Broiler chickens are highly inactive, spending between 70 and 80% of their time resting, depending on age and rearing conditions (Weeks et al., 2000; Bizeray et al., 2000). Reduced activity as a result of fast growth has been related to lameness and difficulties in walking and rapid locomotion (Kestin et al., 2001; Bailie et al., 2013). Lame birds have been found to change their posture to sitting instead of standing while eating where possible (Weeks et al., 1998; Mench et al., 2001). Leg weakness in broilers is a major problem for both economy and welfare. The main causes for weakness is considered to be infectious, developmental or degenerative (European Commission, 2000).

*1.3 **Environmental enrichment***

Enrichment of the environment (e.g. by provision of resources such as different structures and litter materials) could improve both the physical and psychological well- being of broilers. Various environmental enrichment items have been reported to reduce fear and aggression (Jones, 1996; Gvaryahu et al., 1994), increase activity (Bokkers and Koene, 2003; Bailie et al., 2013), and decrease abnormal behaviour (Meehan et al., 2003). Increasing the level of activity may contribute to reducing leg problems in broilers. However, providing birds with strings to peck at to encourage movement was found to have little effect on leg health (Arnould et al., 2004). Therefore, it is important to determine whether additions to the environment have a demonstrable effect on improving welfare rather than simply assuming that they are enriching (Newberry, 1995).

Providing broilers with the opportunity to perch has been suggested in order to increase the level of physical exercise, which may positively influence leg health and has the potential to improve welfare (Bizeray et al., 2002; Bailie and O`Connell, 2015). However, most studies done on perching indicate that they are used to little extent (Su et al., 2000; Petit- Riley and Estevez 2001; Rodriguez-Aurrekoetxea et al., 2015) with a usage peaking at 4-5 weeks of age and decreasing thereafter (Ventura et al., 2012; Bailie and O`Connell 2015). Other elevated structures which are easier for heavy birds to access and rest on, such as platforms, might be more appropriate for broilers. The few studies published on this subject report that use of platforms exceeds that of perches (Oester and Wiedmer, 2005; Norring et al., 2016), indicating that platforms might have a more optimal design for use by broilers. In an attempt to increase activity, Bailie et al. (2013) found positive effects on walking ability, and increased latency to lay down, in birds who had access to straw bales and natural light. Reduction in resting and sitting was also found in a similar experiment on birds with access to only straw bales (Kells et al., 2001). Therefore, it is possible that providing chickens with low elevated structures such as boxes may help to stimulate activity, with beneficial effects on the development of the musculoskeletal and cardiovascular systems. However, chickens require practice to learn to navigate successfully in threedimensional space and they learn to judge their take-offs and landings most effectively when given access to elevated structures during the first four weeks after hatching (Gunnarson, 1999).

The opportunity to have a safe and uninterrupted resting place is important, and broiler chickens will choose to rest against walls or in corners, particularly at higher stocking densities, to avoid disturbance from other birds (Newberry and Hall, 1990; Buijs et al., 2010). Also, because of their evolutionary history as descendants from junglefowl that sought safety from predators by taking cover in vegetation, chickens may feel safer when they are close to vertical structures. This may be true especially during the age period when they would naturally be brooded on the ground, prior to the growth of flight feathers that would have enabled their ancestors to fly up into trees. Newberry and Shackleton (1997) observed that domestic chickens preferred to rest and preen close to vertical cover rather than in open areas of the house. Their findings suggest that chickens may prefer to be close to cover when engaged in activities on the ground such as resting, that reduce their ability to detect approaching danger. Placing "vertical cover" panels in the center of the pen attracted broiler chicks away from the pen walls and corners, resulting in a more even distribution of the birds throughout the floor space (Cornetto and Estevez, 2001). A more even distribution of birds may help to avoid a build-up of wet, caked litter in heavily used areas, thereby reducing exposure to ammonia and improving foot and respiratory health. Better litter conditions may also stimulate exploratory behavior directed towards the litter (i.e. ground pecking and scratching, also referred to as foraging behavior) and dust bathing behaviour. Providing boxes in the house may be a simple way of gaining such benefits because the sides of the boxes would serve as vertical cover. Furthermore, the opportunity to rest on top of the boxes may also provide an increased sense of security because birds can better detect and avoid approaching danger.

After hatching under natural conditions, chicks stay in close proximity to their mother and spend a large amount of time resting under her wing. Dark brooders have been applied in commercial settings to simulate this aspects of maternal care (Edgar et al., 2015). Experiments done with dark brooders as a substitute for the broody hen have resulted in reduced mortality of layer hen chicks, mainly because a dark environment when resting reduces the risk of feather pecking and cannibalism by active birds (Jensen et al., 2006, Riber et al., 2007). Although these problems are not common in broilers, boxes with openings along the sides could have beneficial "dark brooder" effects for broilers. These enclosures could provide a warmer, less draughty microenvironment, which may be attractive for resting chickens. Given that broilers are less active when the light intensity is low (Newberry et al., 1986), and appear to avoid brightly lit areas except when highly active (Newberry et al., 1985), providing boxes that allow resting in relative darkness may improve the quality of sleep and result in calmer, less stressed birds. Fear is likely to inhibit play (e.g. Spinka et al., 2001), exploration and dust bathing behaviour in chickens. Perching space on the top of boxes, vertical cover from the sides of boxes, and a dark, cosy retreat underneath boxes may have a fear reducing effect resulting in higher expression of these behaviours.

1.4 ***Objective, hypothesis and predictions***

If the provision of boxes has functional relevance to broiler chickens, with positive effects indicated by use of the boxes to express natural behaviours, increased expression of behaviours with positive emotional valence, and reduction of leg problems that impair walking ability. Then boxes can be considered an appropriate source of environmental enrichment that is practical and easy to implement. Therefore, the objective of this study was to determine the enrichment value of cardboard boxes on the behaviour and welfare of commercially kept broiler chickens.

My working hypothesis was that boxes would have a multifunctional enriching effect by providing the chickens with the opportunity to use them as (1) elevated structures upon which to rest while monitoring their surroundings and with which to gain competence in navigating in three dimensions, (2) vertical cover that provides a sense of security when engaged in different behaviours on the ground, and (3) relatively warm, protected, dark shelters that simulate the comfort derived from being brooded by a mother hen. From this hypothesis, I derived the following testable predictions.

Prediction 1: Because of multiple possible uses of the boxes, observation patches containing a box attract more birds than patches without a box, resulting in higher counts of birds active and resting in "box patches". This effect is stronger in younger than in older birds because younger birds are smaller and, therefore, their bodies take up less of the available floor space, resulting in more unoccupied space in which to express location preferences.

Prediction 2: Because boxes add elevated surfaces to the house that require vigorous movements to access, birds are more likely to engage in wing flapping and jumping in patches containing a box than in patches without a box. These behaviours are more likely to occur in younger than older birds because younger birds require more practice to successfully negotiate box take-offs and landings. Also, because younger birds are smaller, the top of the box is relatively higher and, therefore, requires more vigorous movement to access. Furthermore, when birds are younger, their lighter weight makes it easier to move on and off the box.

Prediction 3: Because the sides of boxes provide vertical cover, birds are more likely to engage in resting behaviour (i.e. lying) on the floor when located in patches with as opposed to without a box. This effect of the boxes is stronger in central patches than in patches adjacent to a wall because central patches lack other sources of vertical cover. It is also stronger in younger than older birds because younger birds are less experienced, making them more vulnerable to danger.

Prediction 4: Because boxes provide additional protected locations for resting, birds resting in patches containing a box are less likely to be disturbed by active birds than birds resting in patches without a box. This effect of the boxes is stronger in central patches than in patches adjacent to a wall because central patches lack the protection provided by the wall. It is also stronger in younger than older birds because younger birds are more easily disturbed by active birds

Prediction 5: The presence of boxes results in a more even distribution of active and resting birds between central patches and patches adjacent to walls in pens with than without boxes.

Prediction 6: Because a more even distribution of birds (Prediction 5) should result in better litter quality, levels of dust bathing behaviour, and exploratory behaviour directed towards the litter (i.e. ground pecking and scratching), are higher in pens with than without boxes, especially in younger birds that have less weight to encumber active movement.

Prediction 7: Because boxes offer safe shelter and retreat (on top, beside and underneath), resulting in more secure and confident birds that are less fearful, birds in pens with access to boxes show higher levels of various playful-appearing behaviours (i.e. worm running, play fighting, wing flapping, jumping, running), than birds in pens without boxes, especially when younger, when active movement is less encumbered by high bodyweight and occupied floor space.

Prediction 8: Due to higher confidence (Prediction 7), the latency to approach novel objects is shorter in pens with than without access to boxes, resulting in higher counts of birds near novel objects. This effect is stronger in younger birds that are more vulnerable to danger.

Prediction 9: Because the presence of boxes results in more vigorous activities (Predictions 2, 6, 7), birds in pens with access to boxes throughout the rearing period are less likely to develop leg problems and, therefore, have lower gait scores (i.e. greater ease of walking) near the end of the production cycle than birds in pens without boxes.

Prediction 10: Because chickens, under natural conditions spend more time being brooded by their mother when newly hatched than when older (Edgar et al., 2015), and because there is limited space under boxes that are designed to be low enough to attract perching by broilers, younger chickens are more likely to be found underneath boxes than older chickens.

Prediction 11: Due to increased body size, resulting in less unoccupied space, and increased body weight, making movement more difficult, chickens will be less active as they approach slaughter weight. Therefore, they will show increased resting behaviour, be less likely to be found on top of boxes, jump on and off boxes less frequently, and perform less dust bathing and playful and ground pecking and scratching behaviours at 28 days of age than when they are younger.

2 Methods

2.1 ***Animals, housing and management***

The experiment was carried out from November 2016 to March 2017 on two commercial broilers farms in Norway. Farm 1 was located in Akershus County. Observations were made on two successive flocks in one house with 19,300 (Flock 1) and 16,500 (Flock 2) fast-growing chickens reared conventionally (Ross 308). This house (60 m x 20 m) was divided in quarters with one meter high wire netting attached with plastic electrical ties along the central drinker line and across the middle to form four pens. Each of the four pen areas was 300 $m^{2,}$ containing 4825 (Flock 1) or 4125 (Flock 2) chickens, with a density of 16.7 or 13.7 birds/m^2, respectively. Ten rows along the house were formed by 5 water lines and 4 feeder lines (see Figure 1). Concrete blocks and wooden posts were set up to connect the netting to the walls. A 40 cm deep by 30 cm wide section of netting was then cut out from the top of the netting close to wall in each pen to make a lower gateway for people to step over between pens. To prevent the chicks from getting their heads stuck in the netting, for Flock 1, 20 cm high strip of duct tape was attached along the base of the netting on both sides. For Flock 2, the duct tape was replaced with rolls of corrugated cardboard.

Farm 2, located in Telemark County, rears slower growing broilers (Ross Rowan, obtained from the same hatchery as the Ross 308 flocks). Observations were made in one house on a flock of 1500 birds (Flock 3). The house (23 m x 10 m) was divided in half with the same method as at Farm 1. Instead of cutting the netting to make a gate, a 2m wide wooden fence was attached to the netting with plastic electrical ties. The two pens were 92 and 138 m^2, each holding 750 birds with a density of 5 and 8 birds/m^2, respectively. Six rows were formed by three water and two feeder lines (see Figure 2). Newly hatched chickens of both sexes were randomly assigned between pens in the houses at both farms.

Food and water were provided ad libitum. In order to make food easily available for chicks, farmers provided feed on paper laid along two (Farm 1) or one (Farm 2) rows per pen in addition to feed in the feed pans during the first few days. The fast-growing birds at Farm 1 started with a diet consisting of high levels of protein and energy, and after nine days they were switched to a different diet with lower levels which was provided until slaughter age. Farm 2 birds received only one type of feed with relatively low protein through the whole rearing period. At both farms, the floor of each pen was covered with a 3-cm-deep layer of wood shavings. The slower growing birds at Farm 2 were also given peat once a week and had access to a ball of straw hanging from the celling from three weeks of age. At both farms, the house was heated with hot water pipes and underfloor heating. In addition, both houses had heating fans. The temperature was 33/34^0 C during the first week, and gradually lowered to 22/23^0 C when the birds were four weeks old and then held at this level until slaughter. Details about the two houses are summarized in Table 1.

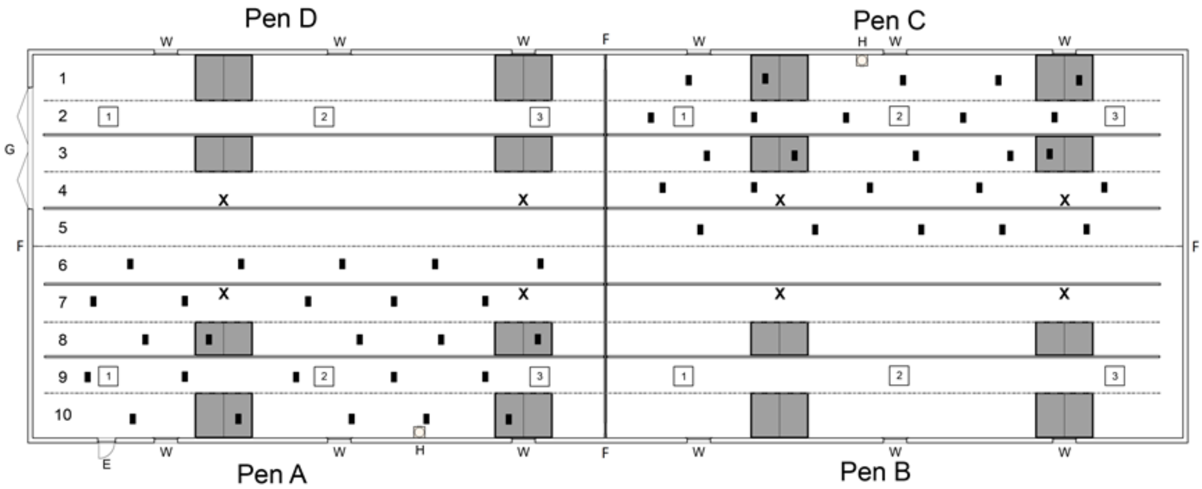


Figure 1: Plan of the four pens in the large house (House 1) at Farm 1, indicating the locations of the observations points (X), observations patches (grey area), boxes (black squares), and novel object locations (squares with number 1-3), fences between pens (F), heating fans (H), garage door (G), windows allowing entrance of natural light (W), and main entrance (E). Rows are marked from 1-10, divided by feeder lines (solid lines) and drinker lines (dotted lines).

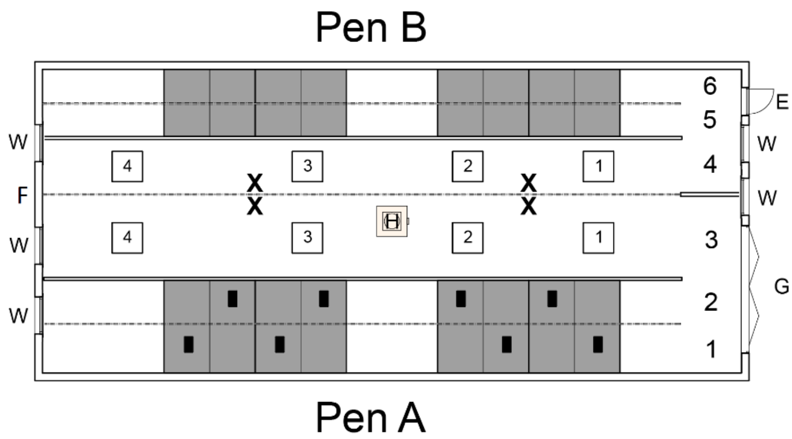


Figure 2: Plan of the two pens in the small house (House 2) at Farm 2, indicating the locations of the observations points (X), observation patches (grey areas), boxes (black squares), novel object locations (squares with number 1-4), fences between pens (F), heating fan (H), garage door (G), windows allowing entrance of natural light (W), and main entrance (E). Rows are marked from 1-6, divided by feeder lines (solid lines) and drinker lines (dotted lines)

Table 1: Summary of differences between the houses at the two different farms

	House 1				House 2	
Farm	1				2	
Pen Id	A	B	C	D	A	B
Pen size	30 x 10 m				23 x 6 m	23 x 4 m
Flock number	1 and 2				3	
Treatment	Experiment	Control	Experiment	Control	Experiment	Control
Number of boxes per pen flock 1	25	0	25	0	8	0
Number of boxes per pen flock 2	20	0	20	0		
Strain of birds	Ross 308, Aviagen, Åstop, SE				Ross Rowan, Aviagen, Åstop, SE	
Number of birds flock 1	19.300 (Flock 1) and 16.500 (Flock 2)				750 (Flock 3)	
Age at slaughter	32 (Flock 1) 34 (Flock 2)				52-60	
Number of birds per pan feeder	65 (Flock 1) and 56 (Flock 2)				62,5	
Type of feeding system	Landmeco, Ølgod, DK				Landmeco, Ølgod, DK	
Number of birds per water nipple	15 (Flock 1) and 13 (Flock 2)				6	
Type of drinking system	Landmeco, Ølgod, DK				Landmeco, Ølgod, DK	
Type of artificial lights	Fluorescent tube				Fluorescent tube	
Photoperiod over different age ranges	24 hours light the first day, then gradually reduced in 1-hour steps until 18 hours of light and 6 hours of dark by 6 days.				24 hours light the first 4 days, then 15 hours light and 9 hours dark. Lights off 2100 hours to 0600 hours.	
Average light intensity at bird level	25-35 lux				Not recorded.	
Duration of dawn and dusk	Lights gradually raised over 20 minutes (morning), and dimmed over 20 minutes until dark (night)				No dawn and dusk programme for lights.	
Room temperature reduction (1-28 days)	34^0-23^0C				32^0C- 22 ^{0}C	
Amount of litter per house	Not recorded				Not recorded	
Amount of peat per house per week	0				360 L	
Amount of straw per house	0				25-30 kg	
Number and type of weighing scale in house	1 (SKOV, Roslev, DK)	0	0	0	0	0
Type of ventilation system	SKOV LPV, DOL539.Hfd, Roslev, DK				Turbovent, Hyderabad, IN	
Protein level in feed 1 and feed 2	23,1 % and 19,7%				18,4%	
Energy level in feed 1 and feed 2	11,1 % and 9,8%				10,4 %	

2.2 *Experimental design*

For each pair of adjacent pens, one pen was assigned to the experimental treatment, containing cardboard boxes with popholes (Figure 3) and the other pen assigned to the control treatment. The location of treatments was balanced across replicate pairs of pens. In the fast-growing birds at Farm 1, experimental pens had 1 box per 200 chickens, for a total of 25 boxes (5/row) in Flock 1 or 20 boxes (4/row) in Flock 2. In the slow-growing flock at Farm 2, experimental pens had 1 box per 100 chickens, for a total of 8 boxes. Boxes were evenly distributed along the length of the experimental pens.

Behavior observations in the large house at Farm 1 were conducted in 8 observations patches per pen (four observation patches in the row bounded by the wall and the first drinker line, and four in a central row bounded by a feeder and drinker line), each about 1,5 m wide and holding roughly 50 birds (Flock 1) if all birds in the flock were equally distributed throughout the pen. Pairs of observation patches were located adjacent to each other. One of these patches was assigned to be a "box" patch (containing a box in the experimental pens), and the other one, a "no box" patch, with patches in the equivalent locations in control pens. In one half of each pen, two pairs of adjacent patches were aligned with a windowless area of wall (forming a block of four patches, with one pair in the wall row and one pair in a more central row). In the other half of the pen, a second block of four patches was aligned with a window (see Figure 1). Each observation patch was defined by marking the wall, feeder and drinker lines with colored tape.

A similar arrangement of adjacent "box" and "no-box" patches was set up in the small house at Farm 2, initially with eight observation patches (two blocks of four patches) in each pen, with the patch width designed to hold about 50 birds (Flock 3) if the birds were equally distributed. However, due to the low stocking density, the patches were about 5.1 m wide, making it hard to conduct scans of the whole patch simultaneously. Therefore, registrations of behaviour of birds older than 3 days were conducted at 16 1.5-m wide observation patches in the pen, eight patches in the wall row and eight patches in the central row, arranged in adjacent "box" and "no-box" pairs (see Figure 2). Because the windows in this house were at the ends rather than along the sides, none of the patches were close to windows. For comparability to Farm 1, the set of eight patches in one half of the pen was considered one block, and the set in the other half of the pen was considered the second block.

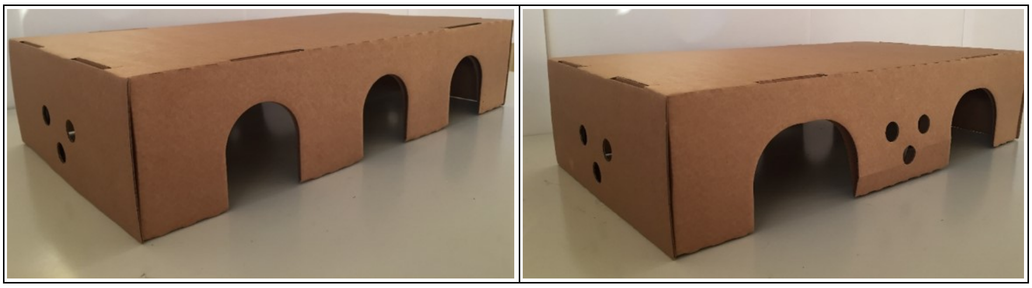


Figure 3: Cardboard box (50 cm x 27 cm x 12 cm), viewed from different sides. Produced by Smurfitt Kappa Nordpapp, Hønefoss, NO.

2.3 *Behaviour observations*

Chickens were observed 3, 18 and 28 days of age, and the slow-growing chickens at Farm 2 were observed again at 49 days of age. On each observation day, three different types of behavioural data were collected: behavior scans, box use, and a novel object test. Before commencing observations, the observer walked slowly through the same route in each pen (standardizing observer exposure), checking and adjusting any boxes that were out of place and removing dust from observation patch markers. Two rounds of behavioural scans were then conducted in each pen by direct observation. In each round through each pen, the observer walked slowly along the row beside the fence dividing the pens. When aligned with the first block of observations patches, the observer turned and moved to an observation point about 3 m from the observation patches and stopped to conduct a set of scans before moving along the fence to the next block of observations patches, repeating this process until scans had been completed for all observations patches. The observer followed a pre-determined order for observing the pairs of patches in each block, with the order reversed across observation rounds. When observing each patch, first, instantaneous scans were performed to record the number of chickens standing (i.e. active) and lying (i.e. resting) on top of the box (if present) and on the floor within the patch, defined by the location of the bird`s head. Then three 10-s scans were conducted to record behavioural events based on ethogram description (Table 2). A graded scale was used for each behavior where 0= no events observed in 10-s, 1= rare (one event observed in 10 s), 2= occasional (2-3 events observed in 10 s), 3= frequent (> 3 events observed in 10 s, involving > 3 birds).

Next, box use was assessed by walking slowly along the wall row and central row containing observations patches (in all pens to balance exposure to the observer) while counting the number of birds on top, beside and under 8 boxes per experimental pen (4 boxes per row).

A novel object test was then conducted to assess approach versus avoidance tendencies. A novel cat toy (from a bag containing eight assorted toys, Kayoba, Item no. 796-245, China) was taped in the center of a square piece of paper (1 m x 1 m), using paper material familiar to the chicks (the type used when feeding them in the first few days). The paper was divided into two square zones, outer and inner, each 0,5 m wide using a black non-toxic permanent marker. The paper was placed on the floor in one of three (Farm 1) or four (Farm 2) pre-determined locations in each pen (see Figures 1 and 2). One location and one toy type was used per observation age (Figure 4). The number of chickens in each zone was recorded using instantaneous scans at 10-s intervals for 5 min.

Finally, a gait score scale was used to assess lameness at 28 days of age, and again at 49 days in the slow growing flock. Fifty chickens per pen (Farm 1) and 30 chickens per pen (Farm 2) were scored on a scale from 0-5, where 0= None, 1= Detectable, but unidentifiable abnormality, 2= Identifiable abnormality that has little impact on overall function, 3= Identifiable abnormality which impairs function, 4= Severe impairment of function, but still capable of walking, 5= Complete lameness, unable to walk (adapted from Kestin et al., 1992).

At each farm, daily mortality was recorded per pen.

Toy 1: Fluffy ball with glitter	**Toy 2:** Transparent ball with bell inside	**Toy 3:** Wheels with bell inside	**Toy 4:** Fabric ball
			
Location 1, day 3	*Location 2, day 18*	*Location 3, day 28*	*Location 4, day 49*
3,5 x 3,5 x 3cm	*3 x 4 x 4cm*	*4,2 x 4 x 4cm*	*3 x 6 x 3cm*

Figure 4: Novel objects used at different ages and different locations

Table 2: Ethogram with description of the different behaviours that was recorded

Behaviour	Description
State	*Mutually exclusive posture during instantaneous scans (Stand + Lie = Number of birds in the observation patch, feeding and drinking birds were excluded)*
Active	Upright posture with weight supported on one or both feet, with keel out of contact with the substrate
Resting	Recumbent posture, with keel in contact with the substrate
Location	*Mutually exclusive location during instantaneous scans of the number of birds standing and lying*
On top	Head and body on top of the box (only possible in box patches)
On floor	Body is not on top of the box (i.e. all visible birds that are not on top of a box)
Event	*All events performed at least once by one or more birds during in each 10-s 1-0 scan period; multiple events possible in each scan*
Run	While in an upright posture with head raised, bird takes rapid steps forward with one foot after the other, with both feet briefly lifted off the substrate during each stride, resulting in moving the body rapidly from one location to another, wings may be raised away from body; Not carrying object in beak
Worm run	While running, bird carries a small object in the beak, such as a wood shaving, piece of paper or a feather.
Jump	Both feet jump away from the substrate simultaneously, so that there is no part of the body in contact with the substrate; may jump up off the substrate (e.g. onto a box, or towards or away from another bird) or down from an elevated location (e.g. off a box)
Wing flap	While in an upright posture, bird raises and lower both wings simultaneously, usually several times in rapid succession; wings are extended; if occurring while running, both running and wing flapping are recorded
Ground peck	While in an upright posture, bird repeatedly strikes the beak down towards the substrate, may be stepping forward slowly with head low
Ground scratch	While in an upright posture, bird rakes the substrate with the toes and claws using a rapid backward kicking movement of the leg and foot, alternating between feet
Vertical wing shake	While dust bathing in a recumbent position with fluffed feathers, bird simultaneously and rapidly lifts the wings up and down multiple times; wings are held close to the body, scooping loose substrate material up into the feathers
Disturbed	Recumbent bird stands up as a result of contact by an active bird
Play fight	Two birds run towards each other and stop and stare at each other, birds can also jump with feet towards each other.
Box use	*Mutually exclusive location during instantaneous scans of the number of birds using boxes in experimental pens*
On top	Head and body located on top of the box (observed from a distance)
Under	Head located under the box (observed after slowly lifting the pophole side of the box)
Beside	Head located within one bird length of any side of the box, outside the box (observed from a distance)
Proximity	*Proximity to a small novel object (cat toy) placed in the center of a 1-m diameter brown paper arena during instantaneous scans every 10 s*
Outer zone	Both feet located 0.5 m – 1.0 m away from the object
Inner zone	Both feet located within 0.5 m of the object

2.4 Statistical analysis

The effects of providing boxes were analysed in SAS Version 9.4 (SAS Institute Inc., NC, USA) using the GLIMMIX procedure for generalised linear mixed models. To analyse the behaviour scan data, two models were used. Model 1 (Table A1) compared behaviour between observation patches with or without boxes within experimental pens only. Factors in the model included observation patch (box vs no box), location (wall row vs central row), age (3, 18 and 28 days) and their 2-way interactions, and flock (1-3). The number of birds expected in the observation patch if birds were uniformly distributed throughout the pen (based on pen stocking density and observation patch area) was included as a covariate to control for differences in pen size, flock size and observation patch area. Model 2 (Table A2) compared behaviour between pens assigned to the experimental (with boxes) and control (without boxes) treatments. This model included treatment (experimental vs control), location, age and their 2-way interactions, flock and number expected. Prior to analysis, data for each age were averaged across scans and observation rounds within pen row and observation patch type and, for Model 2, also across observation patch type within row. From the standing and lying counts made during the instantaneous scan samples, two aggregated variables were included among the variables for analysis, active (sum of birds standing on the floor and on the top of the box if present), and resting (sum of birds lying on the floor and on top of the box if present). A third model (Model 3) (Table A3) was used to investigate the effect of age (3, 18 and 28 days) on average pen counts of birds on top, beside and under boxes during instantaneous scans of 8 boxes per experimental pen. Model 4 (Table A4) evaluated the effects of treatment, age, the interaction of treatment with age, and flock on latency (s) to enter the outer and inner zone of the novel object arena, and average counts of birds in the inner and outer zone, during the 5-min novel object tests. The effects of treatment and flock on average gait score per pen at 28 days were explored in Model 5 (Table A5). In Models 3 to 5, pen stocking density was included as a covariate because it varied between pens.

In all models, pen within flock was the experimental unit. Averaged count data and latencies were analysed according to a lognormal distribution, and averaged score data with a T central distribution. A constant of 1 was added to count data prior to analysis of variables containing zeros. Residuals resulting from repeated observations of the same pen across ages were treated as a random effect with first-order autoregressive structure. Gait score residuals (from one age only) were modelled with a variance components structure. Data collected from Flock 3 (slow-growing breed) at 49 days were excluded from statistical analyses due to lack of replication, as Flocks 1 and 2 (fast-growing breed) were slaughtered prior to this age. Pairwise means comparisons were based on differences in least squares means with the Tukey adjustment for multiple comparisons. Statistical significance was assigned to results with $P<0.05$.

3 Results

3.1 Effect of boxes on resting and activity in experimental pens (Prediction 1)

There was no significant effect of patch type (*P*=0.852) or age (*P*=0.568) on the number of chickens that were active in observation patches, and the interaction between patch type and age was not significant (*P*= 0.383; Figure 5a, Table A6). Chickens engaged in more resting in patches containing a box than in those without a box (*P*=0.043), and tended to show increased resting with age (*P*=0.083), whereas the interaction of patch type with age was not significant (*P* =0.371; Figure 5b, Table A6).

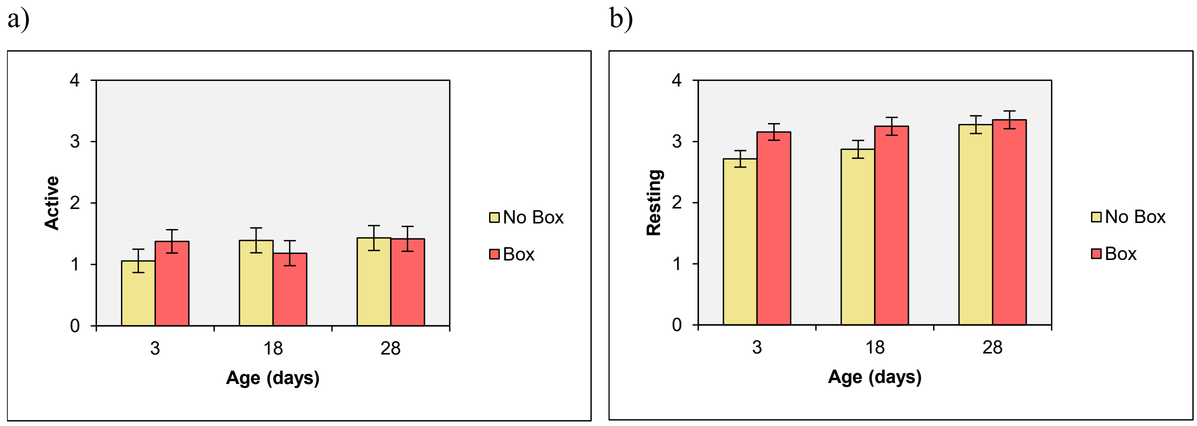


Figure 5: Effect of presence (Box) or absence (No box) of a box in observation patches within box enriched pens on least squares mean number of birds (a) active and (b) resting (Patch type, $P<0.05$) in the patch (log n ± SE).

3.2 Effect of boxes on wing flapping and jumping in experimental pens (Prediction 2)

The effect of patch type (box present or absent) in observation patches on wing flapping was not significant (*P*=0.713). Chickens tended to show increased frequency of wing flapping with age (*P*=0.068), but there was no effect of the interaction between patch type and age (*P*= 0.830; Figure 6a, Table A6). Chickens engaged in more jumping in patches containing a box than in those without a box (*P*=0.011). There was no main effect of age on frequency of jumping (*P*= 0.107), but there was a significant interaction between patch type and age, with most jumping occurring at 18 days in patches with boxes (*P*= 0.020; Figure 6b, Table A6).

a) b)

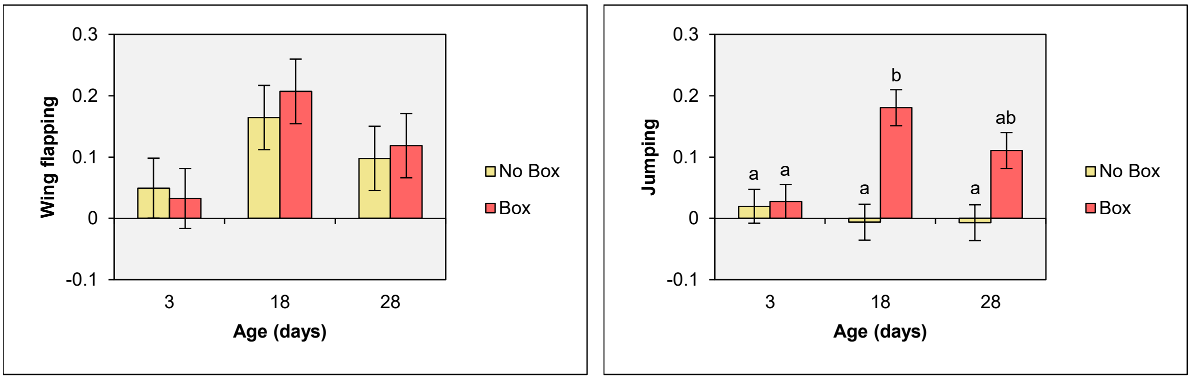


Figure 6: Effect of presence (Box) or absence (No box) of a box in observation patches within box enriched pens on least squares mean score (0-3) for birds (a) wing flapping and (b) jumping (Patch type, P< 0.05) in the patch (n ± SE). Different letters indicate significant differences in pairwise means for the interaction effect (Patch type x Age, Tukey, P<0.05).

3.3 Effect of boxes on birds lying on the floor in experimental pens (Prediction 3)

More chickens were counted lying on the floor in wall patches versus central patches (*P*=0.040), whereas there was no effect of patch type (*P*=0.118), and no significant effect of the interaction between patch type and patch location on birds lying (*P*=0.637; Figure 7a, Table A7). Age did not influence the number of chickens lying on the floor (*P*=0.115), and the interaction of patch type with age was not significant (*P*=0.218; Figure 7b, Table A6).

a) b)

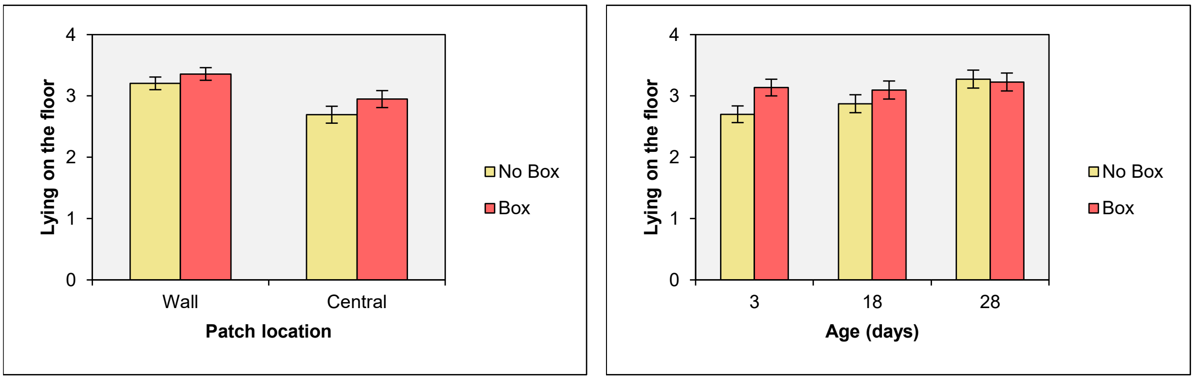


Figure 7: Effect of presence (Box) or absence (No box) of boxes in observations patches within box enriched pens on least squares mean number (log n ± SE) of birds lying on the floor (a) in wall versus central patches (Patch location, P<0.05), and (b) at each age (days).

3.4 Effect of boxes on disturbed birds in experimental pens (Prediction 4)

There was an effect of patch location on disturbances (*P*= 0.046), with more chickens disturbed in wall patches than in central patches. There was however no patch type effect (*P*=0.370) or interaction between patch location and observation patch type (*P*= 0.386; Figure 8a, Table A7). Nor was there any effect of age (*P*=0.960) or interaction between age and patch type on birds disturbed (*P*= 0.186; Figure 8b, Table A6).

a) b)

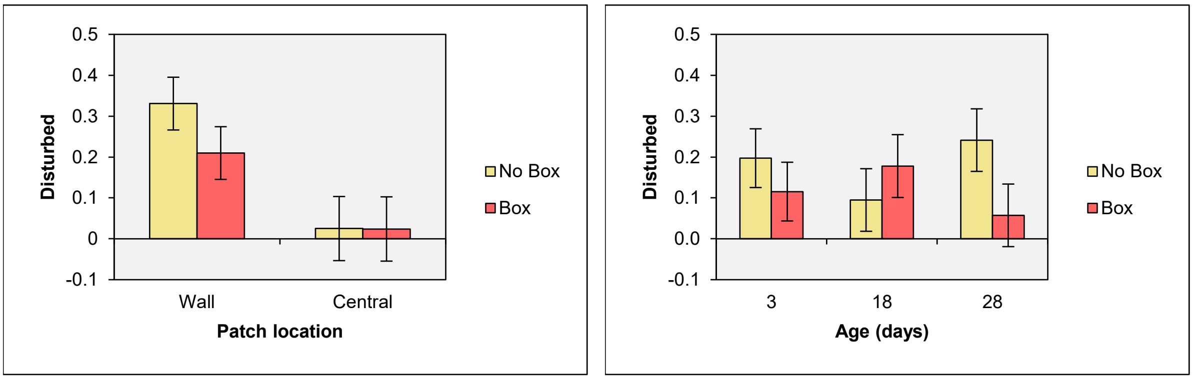


Figure 8: Effect of presence (Box) or absence (No box) of boxes in observation patches within box enriched pens on least squares mean score (0-3, ± SE) of birds disturbed in (a) wall versus central patches (Patch location, P<0.05) and, (b) at different ages.

3.5 Effect of treatment (Control or Experimental pen) on activity and resting (Prediction 5)

There were effects of treatment (*P*=0.033) and patch location (*P*=0.025) on numbers of active chickens, with more chickens active in experimental than control pens and in wall patches compared to central observation patches. No interaction between patch location and treatment was found on activity level (*P*=0.103, Table A9) although pairwise comparisons showed that the treatment effect was stronger in central patches than in wall patches (Figure 9a). There was a tendency for differences between the treatments in the number of chickens resting (*P*=0.099; higher in experimental pens than in control pens), and a significant effect of patch location (*P*=0.001), with more birds resting in wall than central patches. The interaction effect on resting was not significant (*P*=0.127; Table A9) but pairwise comparisons suggested a tendency for more resting in central patches in experimental pens than in control pens (Figure 9b).

a) b)

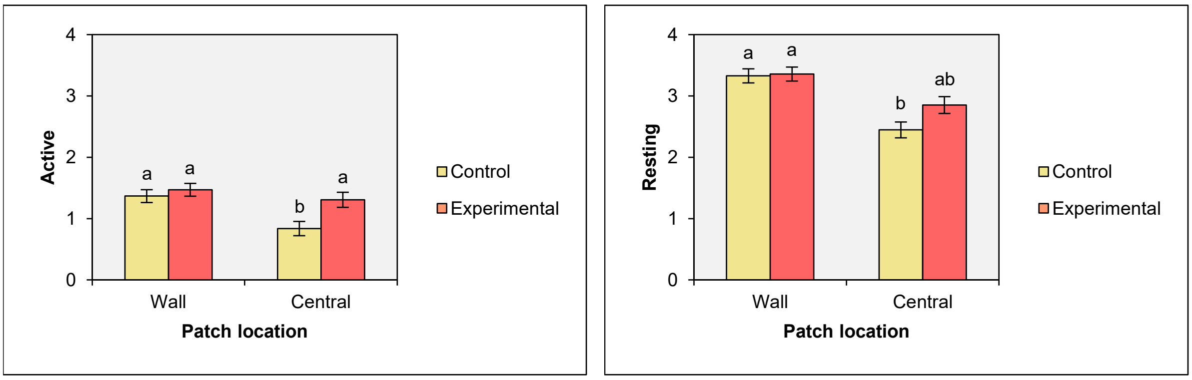


Figure 9: Effect of treatment (Control or Experimental pens) and patch location (Wall or Central) on least squares mean number (log n ± SE) of birds (a) active (Treatment, P<0.05, Patch location, P<0.05) and (b) resting (Patch location, P<0.01) in the observation patches. Different letters indicate significant differences in pairwise means for the interaction effect (Treatment x Patch location, Tukey, P<0.05).

3.6 *Effect of treatment on ground peck, ground scratch and vertical wing shaking (Prediction 6)*

There was no effect of treatment (*P*=0.930) on ground pecking but there was an age effect (*P*=0.008), with more birds ground pecking at 3 days of age than when older, whereas the interaction effect was not significant (*P*=0.656; Figure 10a, Table A8). No effect was found of treatment (*P*=0.422) on ground scratching, but there was an effect across ages (*P*=0.043), with more birds ground scratching at three days of age than when older. The interaction effect was not significant (*P*=0.328; Figure 10b, Table A8). There were no differences between the frequency of vertical wing shaking in control pens versus experimental pens (*P*=0.347), or across ages (*P*=0.275). The interaction effect on vertical wing shaking was also not significant (*P*=0.229; Figure 10c, Table A8).

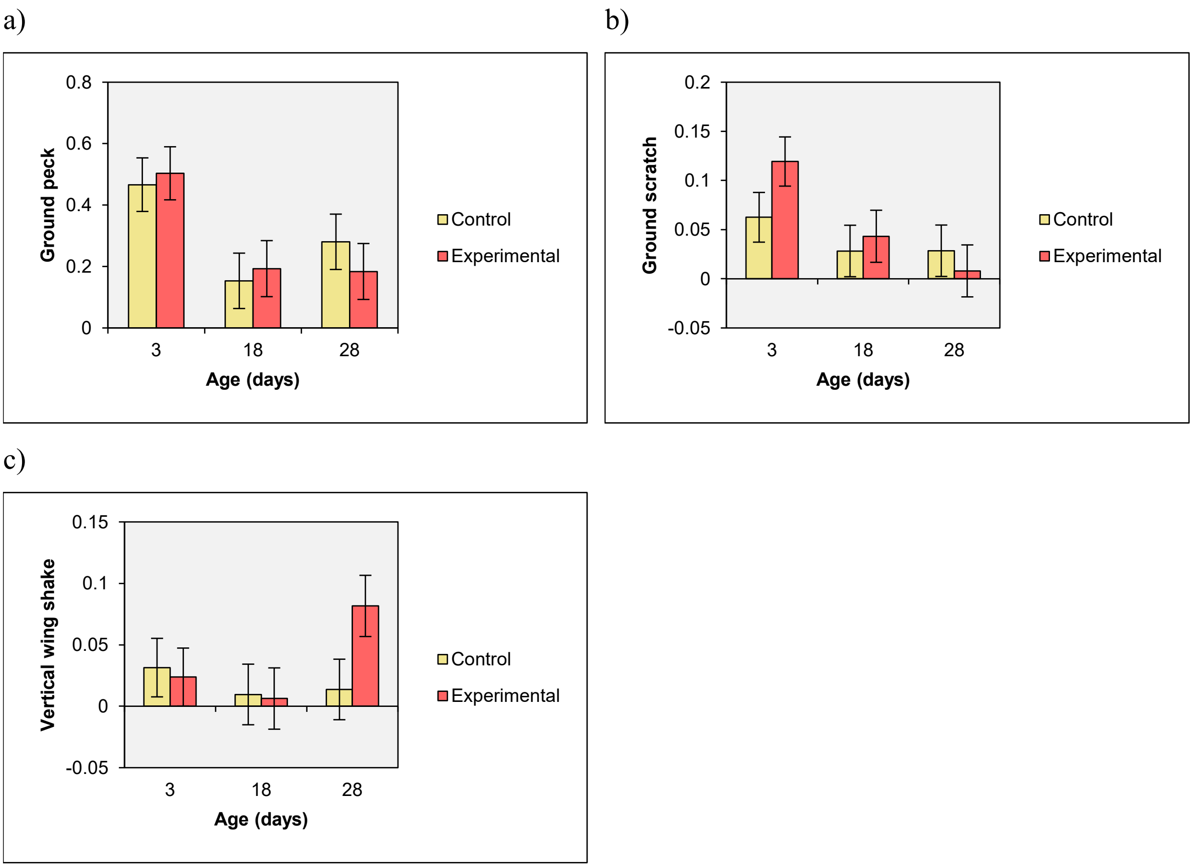


Figure 10: Effect of treatment (Control or Experimental pens) and age (days) on least squares mean frequency score (0-3) of birds (a) ground pecking (Age, P<0.01), (b) ground scratching (Age, P<0.05) and (c) vertical wing shaking (score ± SE).

3.7 *Effect of treatment on various playful appearing behaviours (Prediction 7)*

There was no effect of treatment (*P*=0.141), age (*P*=0.22) or their interaction (*P*=0.27; Figure 11a, Table A8) on the frequency of birds worm running. No treatment effect was found (*P*=0.304) on birds running, but there was effect of age (*P*=0.015), and an effect of the interaction (*P*=0.004; Figure 11b, Table A8), with birds running most at day 3 in experimental pens. There were no differences in the frequency of birds play fighting between pens (*P*=0.331), and no difference across ages (*P*=0.478) or the interaction between them (*P*=0.828; Figure 11c, Table A8). No differences were found between treatments in the frequency of wing flapping (*P*=0.850), while there was an age effect (*P*=0.042), with birds wing flapping most at 18 days of age. No interaction between treatment and age was found on wing flapping (*P*=0.909; Figure 11d, Table A8). More birds were jumping in experimental pens (*P*=0.015), and there was a tendency for differences across ages (*P*=0.091). There was an effect of the interaction between treatment and age, with most jumping at 18 days in experimental pens (*P*=0.003; Figure 11e, Table A8).

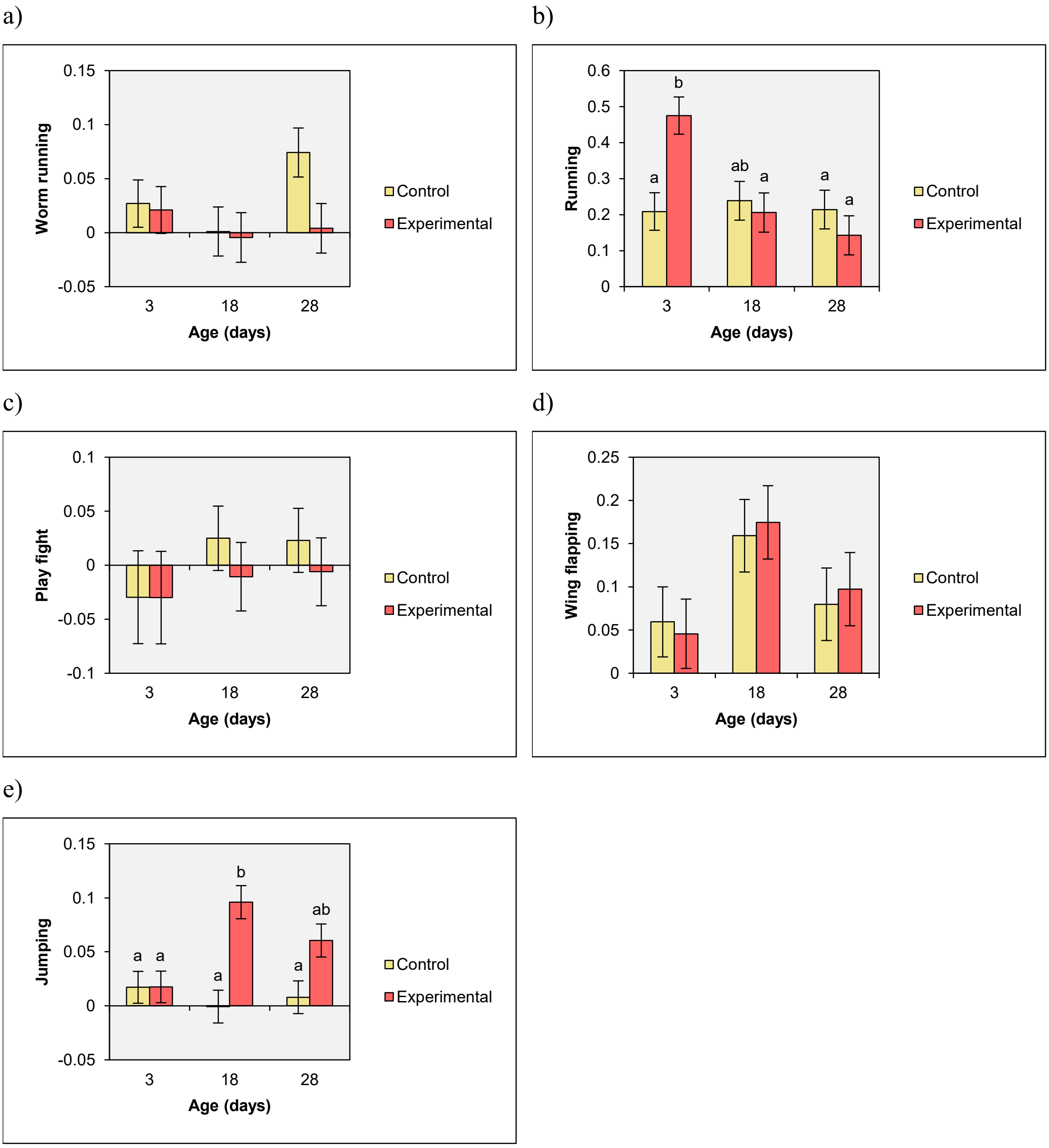


Figure 11: Effect of treatment (Control or Experimental pens) and age (days) on least squares mean frequency score (0-3) of birds (a) worm running (b) running (Age, P< 0.05) (c) play fighting (d) wing flapping (Age, P<0.05) (e) jumping (Treatment, P<0.05) (score ± SE). Different letters indicate significant differences in pairwise means for the interaction effect (Treatment x Age, Tukey, P<05).

3.8 *Effect of treatment on response to a novel object (Prediction 8)*

There was no effect of treatment (*P*=0.816), but an effect of age (*P*=0.003) on the latency to enter the outer zone of the novel object arena (longest at 18 days). No effect was found on the interaction between age and treatment (*P*=0.491; Figure 12a, Table A10). On the latency to enter inner zone there was no effect of treatment (*P*=0.671), while there was an age effect (*P*=0.007), with birds entering most quickly at 28 days of age. No interaction effect was found (*P*=0.848; Figure 12b, Table A10). No effect of treatment (*P*=0.830), age (*P*=0.060) or their interaction (*P*=0.727; Figure 12c, Table A10) were found on counts of birds in the outer zone. There was no difference in the count of birds entering inner zone between pens (*P*=0.877). An effect of age was found, with numbers entering the inner zone increasing with age (*P*=0.042), while there was no effect of the treatment and age interaction (*P*=0.691; Figure 12d, Table A10).

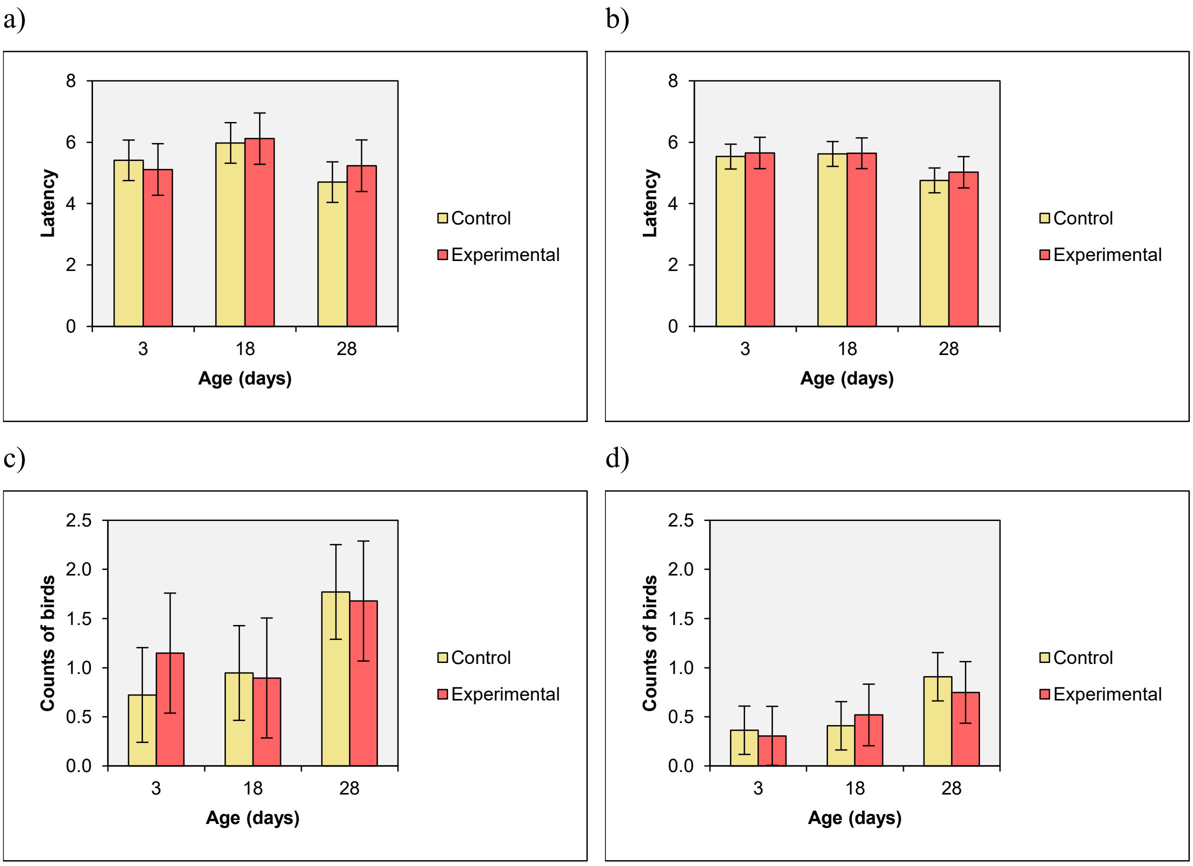
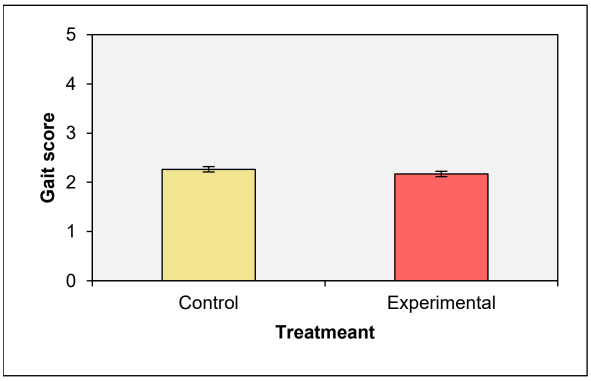


Figure 12: Effect of treatment (Control or Experimental pens) and age (days) on least squares mean latency (s) to enter (a) outer zone (Age, P< 0.01) (b) inner zone (Age, P<0.01), and counts of birds (log n ± SE) in outer zone (c) and (d) inner zone (Age, P<0.05)

3.9 *Effect of treatment on gait score (Prediction 9)*

There was no difference between control and experimental treatment on bird gait scores (*P*=0.280; Figure 13a, Table A11). Birds in Flock 3 (slow growing breed) had lower mean gait scores than birds in Flocks 1 and 2 (fast growing breed) (Figure 13b, Table A12).

a) b)



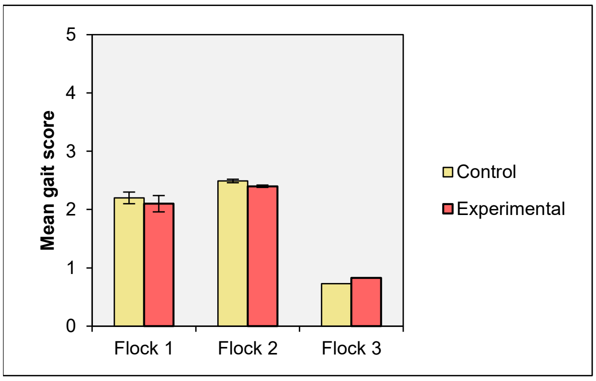


Figure 13: Effect of (a) treatment (Control or Experimental pens) on least squares mean gait score at 28 days, and (b) mean gait score per flock based on raw data, at 28 days.

3.10 *Effect of age on box use in experimental pens (Prediction 10)*

Chickens were more frequently using the box for sitting or standing on top of at days 18 and 28 than at day 3 (age: *P*=<0.000 Figure 14, Table A13). At 3 days of age, chickens were spending significantly more time under the box than when older (*P*= <0.000; Figure 14, Table A13). At day 18 and 28, birds were too big to get under the box, and therefore no registrations of "Under" were made.

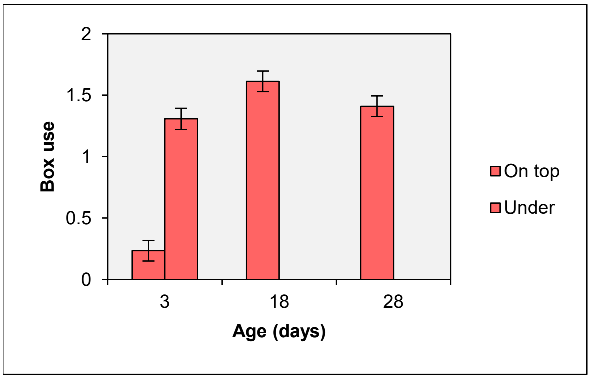


Figure 14: The effect of age (days) on least squares mean count of birds on top and under boxes (n=8/pen) in experimental pens (log n ± SE).

4 Discussion

4.1 ***Overview***

The aim of this study was to investigate the effect of cardboard boxes as an environmental enrichment on the behaviour and welfare of commercial kept broiler chickens. Providing birds with cardboard boxes resulted in birds showing more jumping at 18 days, more running at 3 days, and they also rested more compared to birds without access to boxes, indicating that presence of boxes influenced broiler behaviour. Presence of a box did not affect the birds' walking ability or their approach and avoidance tendencies towards a novel object.

4.2 ***Effect of boxes when comparing box versus no box patches within experimental pens***

Contrary to my prediction, patches with boxes did not stimulate more activity compared to patches without boxes, and the levels of general activity were low. This result is not surprising since broiler chickens are considered to be an inactive type of bird (Weeks et al., 2000; Bizeray et al., 2000), due to the strong genetic selection for improved feed conversion efficiency and energy conservation (Weeks et al., 2000). Previous studies have shown that broiler chickens spend between 70 and 80 % of their time lying down (Weeks et al., 2000; Bizeray et al., 2000) and it might be difficult to stimulate the broilers to increase their activity, especially at the end of cycle. However, Newberry (1999) found that when providing chickens with limited access to peripheral areas containing different resources, the chickens actively ran into these areas when accessible, even when 6 weeks of age. In the current study, not all patches containing a box seemed to be of interest for the chickens especially when birds were 3 and 18 days, and therefore a patch could end up with few or no active birds in it. The location of the patch seemed to be more important than whether or not it contained a box. Patches along the wall (both with box and without) were more frequently used by birds than compared to central patches.

As predicted, birds rested more in patches containing a box than in those without, and time spent resting increased as they got older. Similar studies have also found that resting increases with age (Weeks et al., 1994; Weeks et al., 2000; Thomas et al., 2011). Since the box offered birds additional places to rest against, it was not surprising that they chose to rest more in box patches. Previous studies have also found that chickens prefer to rest against vertical cover as opposed to open space (Newberry and Shackleton, 1997; Cornetto and Estevez, 2001). Regardless of the presence of a box, chickens spent more time resting than being active at all ages.

Chickens in box patches did not engage in more wing flapping, but they tended to do it more frequently as they got older. This was contrary to my prediction and might be because birds were not only observed wing flapping when trying to access the top of the box but also when they were engaging in different behaviours on the ground such as walking and running. Additionally, when chickens were younger they spent more time under the box than on top.

As predicted, chickens jumped more in box patches, and most at 18 days. The box was not very high (12 cm); if it had been higher one could maybe expect more birds to be jumping when they were older. At day 28 most chickens could take one step to get on top of the box and did not need to jump. Generally, jumping was not a behaviour that was often performed by the birds, resulting in low values (Figure 2b).

Contrary to my prediction, there was no difference between patches with a box than patches without a box on number of birds lying on the floor. It might be that the box did not provide "enough" vertical cover for the birds to feel safe and that only one box in each patch was not enough to obtain an optimal feeling of "cover". Maybe if the birds could partially see through the sides of the box, they would get a better overview of the pen and choose to lie more next to the box. Newberry and Shackleton (1997) found that birds showed increased use of cover when the cover continuity increased from 0 to 67 %, but not when it was 100% solid and there was not visibility through the wall. However, generally chickens were more attracted to lie against the wall than in a central patch regardless of presence of box. Preference for resting against the walls has also been found in previous studies (Newberry and Hall, 1990; Buijs et al., 2010). One explanation for this could be that they felt safer lying next to the walls than in central patches, and maybe because this allowed them to rest next to more birds which also contributed to a feeling of safety. In addition, when lying next to the wall they were further away from the observer, who could be perceived as a danger, than when lying in central patches. Possibly, when they moved away from the observer towards the wall, the wall blocked them from moving further so the numbers increased in that location.

Contrary to my prediction, chickens in box patches did not experience fewer disturbances by active birds than chickens in no box patches. This may be because when birds were disturbed, this often happened in highly populated areas, such as close by the wall. Even though the box provided additional locations for resting, the density of birds around the box was never as high as near the wall, thus more birds were disturbed in wall patches. Maybe the presence of boxes would have an effect on disturbance levels if the design would allow more birds to rest close to it. The level of disturbance was not affected by age. This might be because when bird density increases with age the activity level decreases, resulting in reduction in disturbance.

4.3 Effect of boxes in experimental versus control pens

The distribution of active birds was higher in experimental pens, and more birds were active in wall patches versus central. Chickens tended to rest more in experimental pens, and they rested more in wall patches than central patches. Generally, more birds were participating in both activity and resting in wall patches, and one could see most effect of boxes in central patches. The observation that the boxes seemed to distribute birds away from the wall could mean less clustering of birds near walls and better use of the available space.

Even though the distribution of birds tended to be more even in experimental pens than in control pens, which should result in better litter quality, the levels of dust bathing and exploratory behaviour were not higher in these pens. This may be because the litter mainly consisted of wood shavings, which are not the most appropriate for foraging or dust bathing behaviour. Substrates with fine particles such as peat or sand that are more effective for cleaning the feathers are highly preferred when engaging in dust bathing (van Liere et al., 1990; Shields et al., 2004; de Jong et al., 2005). Substrate preference for foraging is not so obvious, and a wider range of materials are accepted. However Sanotra et al. (1995) found a preference for foraging in straw and sand over wood shavings and feathers. Both ground scratching and ground pecking were most frequently performed at three days of age. One reason for this may be because the litter quality deteriorates over time, and this could lead to caked litter, which is not optimal for foraging behaviour. However, since both farms had underfloor heating the litter quality was quite good throughout the whole production cycle and caked litter was only observed in a few places and especially under the boxes when moving them. Another explanation may be that since the manure content of the litter increases over time the interest for exploring it goes down. Generally, the levels of birds dust bathing were low, but when they engaged in it often more birds dust bathed together and it seemed like they excavated the litter and used these special spots for this behaviour. A few birds were observed dust bathing on top of the box. In Farm 2, slower growing birds were given peat and some fresh litter, and those places were very attractive for both dust bathing and foraging.

Birds in pens with access to boxes did not show higher levels of playful behaviours apart from running and jumping. Worm running and play fighting were behaviours that rarely were observed, and the presence of boxes did not affect the prevalence of these behaviours. Play fighting was not observed until the chickens were 18 days old and it seemed like play fights were typically conducted in open spaces where the density of birds was low, which could explain why the boxes did not contribute to higher levels of this behaviour. When chickens were worm running they would move over longer distances and it appeared random if they would run through a patch with box. It did not seem like the box contributed to worm running.

As previously discussed, wing flapping did not differ between box versus no box patches, and neither did it differ between control pens and experimental pens. This might be for the same reason, that wing flapping happened when birds were standing on the ground and not so often when they were using the box to get on or off. More running occurred in pens with boxes than without, and they were running most often at 3 days of age. This might be because at 3 days chickens were sometimes using the box as a shelter to stay underneath. Maybe this made them feel more secure and so they engaged in more running.

Chickens spent the most time on top of and beside the boxes on days 18 and 28. On top, chickens had the opportunity to lie while getting a better overview of the surroundings which may have contributed to a feeling of safety similar to that hypothesized to occur when poultry sit on perches. Newberry et al. (2001) observed that layer pullets preferred to perch on the highest perches, where they were hypothesized to feel safest, but this preference declined when the groups were larger, giving them more safety in numbers on the floor. Birds were also observed running from the feeder to drinker lines. It might be that they would also use the boxes to run from box to box, as both boxes and feeder and drinker lines give some cover whereas in between they were more exposed. As previously mention chickens are motivated to seek areas with cover structure (Newberry and Shackleton, 1997), which could be interpreted as anti-predator behaviour. There was more jumping in box pens, not surprisingly, since there was nothing to jump on and off in control pens, and they did not jump often for other reasons except rarely when playing. The observation that most jumping occurred at 18 days somewhat naturally correlates to the highest frequency of sitting and standing on top of the boxes.

*4.4 **Effect of boxes on novel object test***

It has been found that access to enrichments reduces fear (Jones et al., 1991; Jones and Waddington, 1992), however this was not the case in the present study as birds in pens with access to boxes did not approach a novel object faster than birds without access. Generally, birds seemed more curious than afraid of the novel object, but the curiosity did not maintain after they had explored the object. They were then most interested in the paper, which the object was attached to, and would peck at it, scratch it with their feet and lie on it. When they were older, they destroyed the whole arena with scratching, making it somewhat difficult to count birds in the different zones. The reason for choosing this type of paper was to not confound fear of the test arena with fear of the object. However, a material different from feeding paper would have been more optimal to use, and maybe a material that was not so interesting for the chickens to peck and scratch at. It might be that corrugated, cardboard which was used to prevent chicks from getting their head stuck in the netting fence, would be more appropriate. At least they would not be able to destroy it, but most likely they would still peck and scratch at it.

Generally, birds would spend more time lying in the outer zone, but counts of chickens entering the inner zone increased with age. This may be due to the fact that when birds were older they would take up more space in the arena, resulting in higher counts of birds in the inner zone. Perrè et al. (2002) compared behaviour of brooded and non-brooded pullets and found that brooded pullets, when presented with a novel object in a familiar environment, appeared less fearful as they went nearer the object. This indicated that early mothering experiences influenced their behaviour. The box was thought to provide chicks with shelter and to derive some aspects of being brooded by a mother hen. It did not seem like the box functioned as an adequate substitute for this purpose. Maybe if the box more closely resembled a mother hen and made sound to attract chicks, the feeling of comfort when staying under it would increase and perhaps influence their behaviour in a positive way, making them feel more secure.

*4.5 **Effect of boxes on gait score***

Contrary to my prediction, birds with access to boxes did not have a lower gait score at the end of the production cycle than birds with such access. Presence of boxes did not lead to birds engaging in more vigorous activities beside jumping and running. One could assume that more exercise through jumping and running would contribute to better leg health, and previous studies have shown that increased activity has been correlated with improved walking ability (Reiter and Bessei, 1995; Bailie et al., 2013). However, the box might have been too low for birds to achieve health benefits from jumping on and off it. Also, the general prevalence of jumping was low. There was a difference between the fast and slow growing breeds' mean gait score. Whether this could partially be explained by differences in activity levels is uncertain, but most likely various aspects of the birds living conditions and genetics have an influence. Slower growing birds have in several studies been shown to have better leg health (Kestin et al., 1992; Bessei, 2006) because the selection for rapid growth has not been so intense in these breeds, (Bokkers and Koene, 2003). They are also given a diet consisting of less protein, and their rearing conditions often differ from fast growing chickens, with different light regimes and lower animal density, amongst others. All of these factors may contribute to better the leg health of these birds. Both slow and fast growing birds were observed performing ground pecking, ground scratching and eating in a sitting posture so they were not counted as performing these behaviours according to my ethogram. Similar findings have been reported in other studies (Weeks et al., 1998; Bokkers and Koene, 2003). Birds would also sometimes not get up fully when walking, and only take a few steps before lying back down on the ground. This indicates that walking represents a cost for the chickens and may even be painful. Danbury et al. (2000) found that lame broilers self select food containing the analgesic drug carpofen, and that the amount of carpofen consumed increased with the severity of lameness. These finding suggest that chronic lameness is painful and that birds suffer more pain with increased disability. Many birds were also observed lying with one leg extended at right angle to their body for many minutes at a time. This appeared to be an abnormal behaviour and it is assumed to relate with lameness. Weeks et al. (2000) also observed this long duration of leg extension in lame broilers and suggested that this posture was adopted to relieve pain or discomfort in their legs. Lameness has been a prevalent problem in the commercial broiler industry (Su et al., 1999; Sorensen et al., 1999). Selection for high live weights and rapid growth, which leads to high loads being placed at relatively immature bone structure and joints, is proposed to be the main cause for this (Kestin et al., 1999; Kestin et al., 2001; Bailie et al., 2013).

4.6 ***Box use***

As predicted, chickens spent more time under the box when they were younger. The design of the box did not allow chickens to stay under the box as they got larger. It may be that if they could get inside, they would continue this use when older. The density around the box was generally high. When birds were young, it seemed like some boxes were more popular than others and their location was typically closer to the wall than in central rows of the pen. When they got older and more of the available space was used, they were using all boxes in each row. They would rest on top of the box, and they were also observed trying to perch while sitting on the edge of the box. When doing this, they would grasp their feet at the end of the box trying to get a good grip, but this was difficult as the box design was not appropriate for perching and also the top was slippery.

4.7 ***Areas for future research***

One could speculate if the boxes would have had a greater effect with some changes to the design. Some farmers are currently using plastic boxes and these boxes are also very popular amongst the birds. These types of boxes allow the birds to get a better grip when getting on and off and when sitting on top of them because of the small gaps on top and around the box. They are also higher than the cardboard boxes used in this study, so birds can still get underneath them at an older age. Air and light also flows through the gaps, which can contribute to making them more attractive, and also the accumulation of manure on top may not be as high as on the cardboard boxes. Maybe the box in combination with other enrichment items such as Pecka Blocks (cereal-based environmental enrichment device) or straw bales which have been correlated with improved walking ability (Bailie et al., 2013) and contribute to higher levels of dust bathing and ground scratching (Guy and Wright, 2003) would have a greater effect on the birds' behavior. Perhaps adding more boxes to the pen could also contribute to gain higher benefits from the box as enrichment because this would allow more birds to engage in jumping, and provide more vertical cover.

4.8 ***Practical considerations***

Advantages of the cardboard boxes are that they are cheap to use and easy to implement. A disadvantage is that they cannot be recycled whereas plastic boxes can be re-used for multiple flocks. The disadvantage with the plastic boxes is that they are very difficult to clean, making this a rather time consuming task that most farmers don't appreciate. The cardboard boxes break down and can be thrown out with the litter before washing the barn. The farmers participating in this study either put them in the garbage or delivered them to cardboard and paper recycle. One tried to burn them but did not proceed, as the smell was very intense due to the manure that was stuck on the box. Some farmers are concerned about putting in boxes from day 1 in the chicken houses. They fear that the presence of box could lead to birds eating less, piling up underneath boxes and potentially suffocating. Many believe that after one week, when the chicks have adapted to the environment and can easily locate water and feed, would be a better time. The results of this study indicate that putting boxes in from day one was without complications, as the boxes did not have any noticeable effect on growth, culling or mortality in the first week or over the whole flock cycle.

4.9 ***Conclusions***

In conclusion, the presence of boxes increased activity levels and resulted in more jumping and running at 18 and 3 days of age, respectively. Responses to novel objects or gait scores were not influenced by boxes. Even though provision of boxes did not have an effect on many of the variables measured, there was no doubt that the birds did use them in various ways. The results of this study support the continued use of boxes as a source of environmental enrichment for broiler chickens.

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Appendix

Table A1. Model 1: Analysis based on the comparison of behaviour within experimental pens in different patch types (No box versus Box).

	DF	F	P
Active (standing on floor + standing on box in patch)			
Location	1,4	0.60	0.483
Patch type	1,4	0.04	0.852
Location*Patch type	1,4	0.16	0.706
Age	2,8	0.61	0.568
Location*Age	2,8	0.27	0.771
Patch type*Age	2,8	1.08	0.383
Flock	2,2	2.71	0.269
Number expected	1,45	2.19	0.145
Resting (number of birds lying on floor +on box in patch)			
Location	1,4	8.88	0.040
Patch type	1,4	8.45	0.043
Location*Patch type	1,4	0.71	0.445
Age	2,8	3.45	0.083
Location*Age	2,8	0.6	0.573
Patch type*Age	2,8	1.13	0.37
Flock	2,2	1.29	0.435
Number expected	1,45	8.95	0.004
Wing flapping			
Location	1,4	3.05	0.155
Patch type	1,4	0.16	0.713
Location*Patch type	1,4	0.66	0.461
Age	2,8	3.83	0.068
Location*Age	2,8	3.74	0.071
Patch type*Age	2,8	0.19	0.830
Flock	2,2	2.01	0.332
Number expected	1,45	1.36	0.249
Jumping			
Location	1,4	2.25	0.208
Patch type	1,4	19.28	0.011
Location*Patch type	1,4	0.04	0.856
Age	2,8	2.98	0.107
Location*Age	2,8	0.32	0.732
Patch type*Age	2,8	6.54	0.020
Flock	2,2	4.78	0.172
Number expected	1,45	6.20	0.016
Lying on floor			
Location	1,4	8.89	0.040
Patch type	1,4	3.94	0.118
Location*Patch type	1,4	0.26	0.637
Age	2,8	2.86	0.115
Location*Age	2,8	0.81	0.478
Patch type*Age	2,8	1.85	0.218
Flock	2,2	0.94	0.514
Number expected	1,45	10.44	0.002
Disturbed			
Location	1,4	8.16	0.046
Patch type	1,4	1.02	0.370
Location*Patch type	1,4	0.95	0.386
Age	2,8	0.04	0.960
Location*Age	2,8	0.03	0.973
Patch type*Age	2,8	2.09	0.186
Flock	2,2	2.42	0.292
Number expected	1,45	0.28	0.602

Table A2. Model 2: Analysis based on the comparison of behavior in experimental pens (with boxes) versus control pens (without boxes).

	DF	F	P
Active (standing on floor, and on box if box patch)			
Treatment	1,6	7.57	0.033
Location	1,8	7.52	0.025
Treatment*Location	1,8	3.39	0.103
Age	2,16	5.47	0.015
Treatment*Age	2,16	0.64	0.538
Location*Age	2,18	0.03	0.972
Flock	2,6	9.66	0.013
Number expected	1,41	11.1	0.001
Resting (lying on floor, and on box if box patch)			
Treatment	1,6	3.79	0.099
Location	1,8	23.5	0.001
Treatment*Location	1,8	2.90	0.127
Age	2,16	2.39	0.123
Treatment*Age	2,16	0.08	0.923
Location*Age	2,18	0.79	0.468
Flock	2,6	0.99	0.426
Number expected	1,41	9.39	0.003
Ground pecking	,		
Treatment	1,6	0.01	0.930
Location	1,8	0.01	0.908
Treatment*Location	1,8	0.33	0.584
Age	2,16	6.48	0.008
Treatment*Age	2,16	0.43	0.656
Location*Age	2,18	0.36	0.702
Flock	2,6	3.12	0.118
Number expected	1,41	1.65	0.206
Ground scratching			
Treatment	1,6	0.74	0.422
Location	1,8	2.36	0.162
Treatment*Location	1,80	0.39	0.550
Age	2,16	3.84	0.043
Treatment*Age	2,16	1.20	0.328
Location*Age	2,18	0.72	0.502
Flock	2,60	1.01	0.418
Number expected	1,41	3.08	0.086
Vertical wing shaking			
Treatment	1,60	1.04	0.347
Location	1,8	1.04	0.336
Treatment*Location	1,8	1.39	0.272
Age	2,16	1.40	0.275
Treatment*Age	2,16	1.62	0.229
Location*Age	2,18	0.54	0.591
Flock	2,6	1.64	0.270
Number expected	1,41	1.71	0.197
Worm running			
Treatment	1,6	2.87	0.141
Location	1,8	1.42	0.266
Treatment*Location	1,8	0.46	0.518
Age	2,16	1.64	0.224
Treatment*Age	2,16	1.40	0.274
Location*Age	2,18	0.41	0.667
Flock	2,6	0.19	0.829
Number expected	1,41	3.03	0.089
Running			
Treatment	1,6	1.26	0.304
Location	1,8	9.37	0.015

Treatment*Location	1,8	0.08	0.778
Age	2,16	5.19	0.018
Treatment*Age	2,16	7.56	0.004
Location*Age	2,18	0.81	0.462
Flock	2,6	18.53	0.002
Number expected	1,41	23.05	<.000
Play fighting			
Treatment	1,6	1.11	0.331
Location	1,8	0.35	0.571
Treatment*Location	1,8	0.09	0.774
Age	2,10	0.79	0.478
Treatment*Age	2,10	0.19	0.828
Location*Age	2,12	0.23	0.797
Flock	2,6	0.55	0.601
Number expected	1,29	0.64	0.429
Wing flapping			
Treatment	1,6	0.04	0.850
Location	1,8	3.31	0.106
Treatment*Location	1,8	0.08	0.778
Age	2,16	3.87	0.042
Treatment*Age	2,16	0.10	0.909
Location*Age	2,18	4.06	0.035
Flock	2,6	3.51	0.097
Number expected	1,41	0.32	0.576
Jumping			
Treatment	1,6	11.23	0.015
Location	1,8	1.05	0.334
Treatment*Location	1,8	0.06	0.807
Age	2,16	2.78	0.091
Treatment*Age	2,16	8.34	0.003
Location*Age	2,18	0.41	0.672
Flock	2,6	3.13	0.117
Number expected	1,41	5.61	0.022

Table A3. Model: 3 Analysis based on novel object test.

	DF	F	P
Latency (seconds) to enter outer zone			
Treatment	1,5	0.06	0.816
Age	2,15	8.28	0.003
Treatment*Age	2,15	0.74	0.491
Flock	2,5	3.02	0.138
Density	1,5	2.52	0.173
Latency (seconds) to enter inner zone			
Treatment	1,5	0.20	0.671
Age	2,15	6.89	0.007
Treatment*Age	2,15	0.17	0.848
Flock	2,5	4.32	0.081
Density	1,5	0.45	0.532
Number of birds in outer zone			
Treatment	1,6	0.05	0.830
Age	2,15	3.41	0.060
Treatment*Age	2,15	0.32	0.727
Flock	2,6	3.34	0.106
Density	1,15	0.08	0.780
Number of birds in inner zone			
Treatment	1,6	0.03	0.877
Age	2,15	3.92	0.042
Treatment*Age	2,15	0.38	0.691
Flock	2,6	4.14	0.074
Density	1,15	0.46	0.506

Table A4. Model 4: Analysis based on gait score.

	DF	F	P
Gait score			
Treatment	1,5	1.46	0.281
Flock	2,5	45.53	0.000
Age	1,1	2601	0.012
Density	1,5	1.46	0.280

Table A5. Model 5: Analysis based on scans of 8 boxes in experimental pens.

	DF	F	P
Numbers of birds on top of box			
Age	2,8	74.45	<0.000
Density	1,3	9.51	0.054
Numbers of birds under box			
Age	2,8	74.75	<0.000
Density	1,3	3.53	0.157

Table A6. Effect of the interaction of patch type (presence or absence of a box) and age (days) on least squares mean (± SE) behaviour counts (log n) and frequency scores (0-3) in experimental pens.

Box present	No			Yes			Interaction Patch type*Age		
Age (days)	3	18	28	3	18	28	DF	F	P
Active (log n)	1.05±0.19	1.39±0.20	1.43±0.20	1.37±0.19	1.18±0.20	1.41±0.20	2,8	1.08	0.383
Resting (log n)	2.71±0.13	2.87±0.14	3.27±0.14	3.15±0.13	3.24±0.14	3.35±0.14	2,8	1.13	0.371
Wing flapping (score)	0.04±0.04	0.16±0.05	0.09±0.05	0.03±0.04	0.20±0.05	0.11±0.05	2,8	0.19	0.830
Jumping (score)	0.01±0.02	-0.00±0.02	-0.00±0.02	0.02±0.02	0.18±0.02	0.11±0.02	2,8	6,54	0.020
Lying on the floor (log n)	2.70±0.13	2.87±0.14	3.27±0.14	3.13±0.13	3.09±0.14	3.22±0.14	2,8	1.85	0.218
Disturbed (score)	0.19±0.07	0.09±0.07	0.24±0.07	0.11±0.07	0.17±0.07	0.05±0.07	2,8	2.09	0.186

Table A7. Effect of patch location (wall or central) and patch type (presence or absence of a box) on least squares mean (± SE) count (log n) of birds lying on the floor and frequency score (0-3) of resting birds disturbed by active birds in experimental pens.

Location	Wall		Central		Interaction Location*Patch type		
Box present	No	Yes	No	Yes	DF	F	P
Lying on floor (log n)	3.20±0.10	3.35±0.10	2.69±0.13	2.94±0.13	1,4	0.26	0.637
Disturbed (score)	0.33±0.06	0.20±0.06	0.02±0.07	0.02±0.07	1,4	0.95	0.386

Table A8. Effect of the interaction of treatment (experimental or control pens) and age (days) on least squares mean (± SE) behavioural frequency scores (0-3).

Treatment	Control			Experimental			Interaction Treatment*Age		
Age (days)	3	18	28	3	18	28	DF	F	P
Ground peck	0.46±0.08	0.15±0.09	0.28±0.09	0.50±0.08	0.19±0.09	0.18±0.09	2,1	0.43	0.656
Ground scratch	0.06±0.02	0.02±0.02	0.02±0.02	0.11±0.02	0.04±0.02	0.00±0.02	2,1	1.20	0.328
Vertical wing shake	0.03±0.02	0.00±0.02	0.01±0.02	0.02±0.02	0.00±0.02	0.08±0.02	2,1	1.62	0.229
Worm running	0.02±0.02	0.00±0.02	0.07±0.02	0.02±0.02	-0.0±0.02	0.00±0.02	2,1	1.40	0.274
Running	0.20±0.05	0.23±0.05	0.21±0.05	0.47±0.05	0.20±0.05	0.14±0.05	2,1	7.56	0.004
Play fight	-0.02±0.04	0.02±0.02	0.02±0.02	-0.02±0.04	-0.01±0.03	-0.00±0.03	2,1	0.19	0.828
Wing flapping	0.05±0.04	0.15±0.04	0.07±0.04	0.04±0.04	0.17±0.04	0.09±0.04	2,1	0.10	0.909
Jumping	0.01±0.01	-0.00±0.01	0.00±0.01	0.01±0.01	0.09±0.01	0.06±0.01	2,1	8.34	0.003

Table A9. Effect of treatment (experimental or control pens) and patch location (wall or central) on least squares mean bird count (log n ± SE).

Treatment	Control		Experimental		Interaction Treatment*Location		
Location	Wall	Central	Wall	Central	DF	F	P
Active (log n)	1.36±0.10	0.83±0.11	1.46±0.10	1.30±0.12	1,8	3,39	0.103
Resting (log n)	3.32±0.11	2.44±0.12	3.35±0.11	2.85±0.13	1,8	2.90	0.127

Table A10. Effect of treatment (experimental or control pens) and age (days) on least squares mean (± SE) latency to enter (s) and bird counts (n) in the outer (0.5-1.0 m from novel object) and inner (0-0.5 m from novel object) zones of the novel object arena during 5-min exposures to a novel object (cat toy).

Treatment	Control			Experimental			Interaction Treatment*Age		
Age (days)	3	18	28	3	18	28	DF	F	P
Latency (s)									
Outer zone	5.41±0.66	5.97±0.66	4.70±0.66	5.11±0.84	6.11±0.83	5.23±0.84	2,15	0.74	0.491
Inner zone	5.53±0.40	5.61±0.40	4.75±0.40	5.65±0.51	5.64±0.50	5.02±0.51	2,15	0.17	0.848
Birds (n)									
Outer zone	0.72±0.48	0.94±0.48	1.77±0.48	1.14±0.61	0.89±0.61	1.67±0.61	2,15	0.32	0.727
Inner zone	0.36±0.24	0.40±0.24	0.90±0.24	0.30±0.31	0.51±0.31	0.74±0.31	2,15	0.38	0.691

Table A11. Effect of treatment on least squares mean (± SE) gait score (0-5, where 0 is best) at 28 days of age.

Treatment	Experimental	Control	DF	F	P
Gait score	2.26±0.05	2.17±0.05	1,5	1.46	0.280

Table A12. Mean gait score (0-5, where 0 is best) per flock.

Treatment	Age (days)	Flock	Birds scored (n)	Mean Gait Score	Flock Density (n/m^2)
Control	28	1	100	2.20±0.10	16.08
	28	2	100	2.49±0.03	13.75
	28	3	30	0.73	8.15
Experimental	28	1	100	2.10±0.14	16.08
	28	2	100	2.40±0.02	13.75
	28	3	30	0.83	5.43

Table A13. Effect of age (days) on least squares mean bird counts (n ± SE) on top and under boxes in experimental pens.

	Age (days)					
Box use	3	18	28	DF	F	P
On top (n)	0.23±0.08	1.61±0.08	1.41±0.08	2,8	74.45	<0.000
Under (n)	1.30±0.08	0	0	2,8	74.75	<0.000

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