The Effects of Environmental Enrichment on the Health, Behaviour, and Welfare of Fast-Growing Broilers

by

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ABSTRACT

THE EFFECTS OF ENVIRONMENTAL ENRICHMENT ON THE HEALTH, BEHAVIOUR, AND WELFARE OF FAST-GROWING BROILERS

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The objectives of this research were to test 1) the effects of environmental enrichments (platform, swinging scale, peckstone, suet feeder) on the health and behaviour of broilers; 2) the effects of body weight on the mobility and activity of broilers; 3) if a commercially available accelerometer, Actical®, can be used to automatically measure the activity levels of broilers. The enrichments used in this study had no effect on the body weight, mobility, or activity level of broilers according to the time spent standing during a latency-to-lie test, the number of crossings during an obstacle test, and their overall time budget. However, birds from non-enriched pens performed more play behaviour than birds from enriched pens during standardized tests, likely due to boredom which could lead to animals being more easily stimulated. Light birds had better mobility and were more active than heavy birds. Actical® is also likely a useful device to measure the activity levels of individual broilers.
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I cannot wait to start the next journey in my life by making a positive change in an animal’s life.
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List of Abbreviations

E: enriched
H: heavy
L: light
LTL: latency-to-lie test
NE: non-enriched
Chapter 1
Literature Review

1.1 The Broiler Chicken (Past and Present)

More than 8000 years ago, chickens (*Gallus gallus domesticus*) were domesticated from the red jungle fowl (*Gallus gallus*) in Southeast Asia for cockfighting and spiritual rituals (Griffin & Goddard, 1994). In the Roman Empire (27 BC-476 AD), they were primarily favoured as food and breeding animals (Jackson & Diamond, 1996).

By the late 1800s, some well-characterized chicken breeds were established, these being mainly used in small-scale egg production (Stevens, 2005). Starting in the 1900s, commercial production systems were implemented. However, improving egg production was the main focus of commercial breeding at the time, and chicken meat was mostly a by-product of the egg industry. At that time, sexing chicks was difficult. By the time people were able to tell males and females apart, it was more profitable to rear males and sell them for meat than euthanize them (Griffin & Goddard, 1994; Stevens, 2005). The selection of meat-type chickens (broilers) began in the early 1930s after sexing skills were imported from Japan. White Rock and Cornish dual-purpose breeds were used, due to their relatively broad breasts and fast growth rates. These two breeds were the foundation of modern broiler breeds (Griffin & Goddard, 1994).

In the 1960s, broilers were mostly sold as whole birds. However, demand has been growing for birds that have higher breast muscle yield (Griffin & Goddard, 1994; Schmidt et al., 2009). For example, in 1962, 83% of broilers were sold whole, whereas in 2005 the percentage dropped to 11% in North America (Zuidhof et al., 2014). Driven by demand, the major aim of broiler selection over the past few decades has been to increase growth rate, meat yield, breast muscle mass, and efficiency of converting food into body tissues (Havenstein et al., 2003;
Schmidt et al., 2009; Zuidhof et al., 2014; Karcher & Mench, 2018). Schmidt et al. (2009) compared a modern broiler line with a heritage line that had remained unselected since the 1950s. They found that the breast muscle mass accounted for 9% of a bird’s total body weight in the heritage line at week 5, whereas the breast muscle weight constituted 18% of the total weight of a modern broiler at the same age. Moreover, they also found a decrease in the growth of heart muscle after day 14 in the modern broiler line, indicating that genetic selection has shifted the energy used to develop the heart muscle into growing breast muscle. The growth rate of broilers increased by 400% from 1957 to 2005 according to another comparison carried out by Zuidhof et al. (2014).

Modern broilers have also become better at efficiently converting feed into muscle. For example, Zuidhof et al. (2014) found that a 2005 broiler requires 1.2 less grams of feed to produce 1 gram of chicken meat than a 1957 broiler. The time a broiler takes to reach a marketable weight has also been greatly reduced. In 1950, it took broilers 70 days to reach 1.4 kg; however, due to decades of commercial genetic selection, in 2010, it only took a broiler about 34 days to reach 2.2 kg (Butterworth, 2010). The more rapid growth rate and higher feed conversion efficiency have shortened each rearing cycle, which significantly lowers costs associated with the production such as feed, labour, heating, lighting, water, and housing, thus reducing the product price (Griffin & Goddard, 1994).

As broiler meat production became more efficient, consumer demands for broiler products also increased. In 2007, the broiler industry produced around 36 billion pounds of chicken meat in the US alone (Schmidt et al., 2009). In Canada, boneless chicken meat has exceeded boneless beef in terms of per capita disappearance of animal protein sources since 2009.
The increasing demand for chicken products is a driving force for even more intense genetic selection with the aim of further increasing growth rate, muscle yield, and feed conversion efficiency in broilers.

1.2 Defining Animal Welfare

It is now widely agreed that the welfare of an animal depends on its physical and physiological states, affective states (how the animal feels), and the interactions among the three (Dawkins, 2006). In the past, animal welfare was mostly assessed through physical indicators such as growth rate, health, and productivity (Hewson, 2003). However, even though physical health is crucial to achieve good animal welfare, there are limitations of measuring animal welfare only in terms of the body (Hewson, 2003; Dawkins, 2006). For example, wild animals in captivity may show stereotypic behaviour, and have poor welfare, despite being physically healthy (Hewson, 2003).

Physiological indicators such as measures of stress response are one way to assess an animal’s welfare that may not be detectable through physical examination. Indicators usually include levels of stress hormones such as corticosteroids and levels of autonomic responses such as increased blood pressure and heart rate (Hewson, 2003; Dawkins, 2006). However, interpreting physiological parameters can be difficult. For example, increased heart rate can indicate both positive (a dog seeing its owner) and negative (escaping from a predator) experiences. Moreover, an elevated level of corticosteroids can be the consequence of restraining the animal when collecting samples rather than an indicator of poor welfare (Dawkins, 2006).
When carried out objectively, behavioural tests provide a non-invasive and often more direct measure of animal welfare (Dawkins, 2006). Even though it is uncertain if animals experience feelings in the same way as we do, based on our similar brain regions and gene codes (Panksepp, 1998), it is presumed that animals are sentient and perform specific behaviour patterns that may indicate their affective states (Panksepp, 1998; Hewson, 2003). Therefore, behavioural measures can be good indicators of an animal’s emotional states. For example, inactivity in a generally active animal may be an expression of pain or discomfort (Gigliuto et al., 2014).

1.3 Welfare Concerns for Broilers

After decades of genetic selection, broilers now have fast growth rate, high breast muscle yield, and high feed conversion efficiency (Bessei, 2006). However, broilers’ high productivity comes at a cost as their welfare is compromised as a result of their fast growth rate and breast muscle yield (Bessei, 2006; Meluzzi & Sirri, 2009). Among all welfare concerns for broilers, heart failure, leg disorders, footpad dermatitis, and inactivity are the most common.

1.3.1 Heart Failure

Conventional (fast-growing) broilers are susceptible to heart failure especially during catching and transportation due to stress (Bessei, 2006; Olkowski, 2007; de Jong et al., 2012). Heart failure can be further categorized into chronic heart failure such as ascites (accumulation of fluids in the abdominal cavity) and acute heart failure such as sudden death syndrome (SDS) (Bessei, 2006). Ascites is considered to be a result of broilers’ fast growth rate which may be related to the shift in muscle development from heart muscle to peripheral/breast muscle. It was found that when growth is slowed down by feed restriction from day 7 to 14, the incidence of
ascites may be reduced (de Jong et al., 2012). Chronic heart failure such as ascites results from heart pump and blood flow inefficiency (Bessei, 2006). Due to the high carbon dioxide tension in blood caused by broilers’ high metabolic rate, the oxygen requirement may exceed the maximum pulmonary and cardiac capacity. However, due to broilers’ high prevalence of heart abnormality (left ventricular failure), the situation may be worsened, leading to a critically low oxygen concentration in blood and thus a higher incidence of cardiac arrhythmia (Korte et al., 1999; Olkowski, 2007). Broilers that die from SDS usually have long-term cardiac rhythm disturbances. SDS is considered to be associated with broilers’ high growth rate because the incidence of arrhythmia may be lowered in birds under feed restriction (Olkowski, 2007).

1.3.2 Leg Disorders and Footpad Dermatitis

Leg disorders, which can be infectious, developmental or degenerative (Bradshaw et al., 2002), are the result of multiple factors such as genetics, fast growth rate, diseases, and rearing conditions (Kestin et al., 1994; Bradshaw et al., 2002; Knowles et al., 2008). Although leg disorders are less prevalent nowadays than a decade ago due to better management, genetic selection, veterinary care, and nutrition, they are still one of the main welfare issues affecting modern broilers (Karcher & Mench, 2018). Leg disorders vary in degree of severity, and they may affect a bird’s walking ability, behaviour, and affective state (Bradshaw et al., 2002; Bassler et al., 2013). Affected birds tend to be less active and usually sit on litter for a prolonged period of time. In worst cases, birds may walk with great difficulty, use their wings to balance, and have trouble accessing food and water (Vestergaard & Sanotra, 1999; Bradshaw et al., 2002). As a result, affected birds may suffer from distress, frustration, and hunger. Another well-documented welfare problem is that broilers with leg disorders experience pain as a result of associated inflammation.
of the skin and joints. Affected birds seek feeds containing analgesic drugs when given the opportunity to choose (McGeown et al., 1999; Danbury et al., 2000).

The most common disorders are bacterial chondronecrosis with osteomyelitis (BCO) and contact dermatitis (e.g. footpad lesions) (Bradshaw et al., 2002). There is a sex difference regarding the incidence of leg disorders. Male broilers, which have faster growth rates than females, are reported to have double the prevalence of leg disorders at older age (Bradshaw et al., 2002; Knowles et al., 2008).

Bacterial chondronecrosis with osteomyelitis (BCO) is one of the major infectious causes of leg weakness and lameness in fast-growing broilers (Bradshaw et al., 2002). Unlike other leg disorders that mostly affect birds at later life stages, BCO occurs at a high rate regardless of the birds’ age. BCO mainly affects femur and tibiotarsus, and it results from the spread of bacterial pathogens such as E. coli (Wijesurendra et al., 2017). If left to progress, BCO may lead to pathological bone fracture which is a painful situation, leading to the inability to walk and access food and water (Bradshaw et al., 2002; Wijesurendra et al., 2017).

Developmental leg disorders such as tibial dyschondroplasia (TD) also result in poor broiler welfare (Lilburn, 1994; Rath et al., 2000). After decades of being genetically and nutritionally manipulated, broilers’ high growth rate is accompanied by fast bone growth during the first few weeks of age, resulting in accumulation of uncalcified chondrocytes and poor vascularization and mineralization, leading to TD (Rath et al., 2000; Bradshaw et al., 2002). In severe cases, broilers’ walking ability can be greatly impaired, and they usually have higher lameness score (Kestin et al., 1994; Bradshaw et al., 2002). However, TD has become less common recently as a result of genetic selection and proper nutrition (de Jong et al., 2012).
Contact dermatitis is the most common degenerative disorder of the skin in broilers (Bradshaw et al., 2002). The lesions are also called “skin burns” due to the similarity of tissue damage. Erosions begin at the superficial surface of skin (epidermis) and then develop quickly into ulcers, resulting in painful inflammation (Kjaer et al., 2006; Bassler et al., 2013). Lesions usually occur on the sites of the body that are in contact with wet and dirty litter for a long period of time because high litter moisture traps heat, increases litter temperature, and creates a suitable environment for ammonia synthesis. High concentration of ammonia causes “ammonia burns” on the skin which leads the skin to be more susceptible to cutaneous bacterial and fungal infections, leading to inflammation and necrotic lesions on footpads (Kjaer et al., 2006; Bassler et al., 2013). High stocking density with poor ventilation is partially responsible for litter quality deterioration (Ekstrand et al., 1997; Bradshaw et al., 2002; Dawkins et al., 2004). Water spillage and some dietary contents (such as soybean meal and high fat that increase water intake which in return results in sticky feces) are also responsible (Bradshaw et al., 2002). Sticky feces tend to adhere to birds’ bodies and feet, which increases the prevalence of contact dermatitis. Breast lesions and hock lesions develop more slowly than footpad lesions which is why footpad lesions are more frequently observed (Bradshaw et al., 2002; Haslam et al., 2006). Birds with footpad lesions are reported to be less willing to move, and in severe cases, their growth rate may be impaired (Ekstrand et al., 1998). Footpad lesions are a particularly painful condition that may lead to difficulty in walking and accessing food and water (Bradshaw et al., 2002).

1.3.3 Inactivity

Even after years of domestication, domestic chickens and junglefowl are still the same species and inbreeding between the two is still possible (Dawkins, 1989; Garnham & Løvlie,
Despite the selection during domestication for growth and fertility, domestic chickens and red junglefowl still show similar behaviour patterns but in different rates (Garnham & Løvlie, 2018). Therefore, behaviour performed by red junglefowl could be considered “natural” behaviour in domestic chickens even though the frequencies may differ (Dawkins, 1989; Garnham & Løvlie, 2018). Moreover, based on a study conducted by Bokkers and Koene (2003) where the behaviour of fast and slow growing broilers was compared until 12 weeks of age, both fast and slow-growing broilers showed the same behaviour elements such as eating, drinking, preening, foraging, stretching, walking, and dustbathing. However, the time budget of each behaviour was different between the two strains with fast-growing broilers spending more time being inactive. This indicates that conventional (fast-growing) broilers are still motivated (although generally less motivated due to specific genetic selection) to perform all types of “natural” behaviour (Bokkers & Koene, 2003; Marino, 2017). However, they became much more inactive as they grew heavier and when compared to broilers selected to grow at a slower rate (slower-growing broilers) despite still being juveniles and facing similar external limitations such as crowding, indicating their ability to perform those behaviour became restricted most likely due to their fast growth rate and heavy body weight rather than their age (Bokkers & Koene, 2003). Alternately, fast-growing broilers could have been selected for low-activity rates which contribute to weight gain and good feed conversion rates. Some comparative and descriptive studies have shown that conventional broilers spend less time walking, running, frolicking, and performing other active behaviour as they grow heavier and heavier; instead, they spend significantly more time sitting, and being inactive (Weeks et al., 2000; Tickle et al., 2018). It may be argued that this behaviour change could be normal developmental change as an animal becomes mature (Spear, 2004). However,
broilers are typically slaughtered at 5-6 weeks of age when they are still juveniles and sexually immature (Yamada, 1999; Weeks et al., 2000; Spear, 2004). Decades of selection for heavy muscling and fast growth rate, which results in skeletal disorders and cardiovascular challenge, may be the reason for these behaviour changes (Vestergaard & Sanotra, 1999; Weeks et al., 2000). The inability of performing natural behaviour such as foraging, frolicking, and in severe cases, even eating and drinking, may lead to frustration and distress in broilers (Bassler et al., 2013).

1.3.4 Housing Conditions

Besides welfare problems of broilers that directly result from selection for performance traits, management also plays an important role in their welfare (Haslam et al., 2006; Buijs et al., 2009).

Stocking density plays an integral role in broiler welfare. NFACC (2017) set the normal stocking density to 31kg/m² for broiler chickens. The maximum stocking density allowed is 38 kg/m² if certain environmental and monitoring system requirements are met. Some studies have shown that feed intake and growth rate may be reduced when stocking density is higher than 30 kg/m² (Hall, 2001; Buijs et al., 2009). Deep litter, which traps moisture and heat, worsens the condition by creating heat stress (such as deep panting) in broilers (Ekstrand et al., 1998; Haslam et al., 2006). According to Hall (2001), the incidence of leg weakness, lameness, and footpad lesions increased at a higher stocking density (34kg/m² vs 40 kg/m²). Grashorn & Kutritz (1991), Buijs et al. (2009), and Skrbic et al. (2009) also found similar results as Hall. BenSassi et al. (2019) also found that densities above 32.1 kg/m² were associated with more birds with walking difficulties, more birds with welfare problems overall, more birds rejected at slaughter, and lower growth rates and productivity when compared to densities below 32.1 kg/m².
However, even though high stocking density remains a major concern, housing conditions such as water supply systems, litter condition, temperature, ventilation, and lighting also have a profound impact on broiler welfare (Homidan et al., 2003; Carey et al., 2004; Dawkins et al., 2004). The negative impacts of high stocking density can be attenuated by implementing systems that better regulate litter humidity, ammonia level, and temperature (Dawkins et al., 2004). For example, under-floor ventilation helps control litter conditions, nipple drinkers minimize water spillage and are thus better than bell drinkers, and more highly absorbent bedding materials such as wood shavings are better than straw (Dawkins et al., 2004). However, this does not mean that litter should be kept as dry as possible. Low litter moisture leads to the production of dust which irritates respiratory systems and transports odour (Carey et al., 2004). Ideally, depending on specific housing conditions, litter moisture is optimal between 25 to 35% (Carey et al., 2004). However, regardless of better management systems, high stocking density can reduce broiler welfare due to crowding which restricts space for performing desired behaviour as well as increasing the incidence of injuries (Hall, 2001).

1.4 Assessing Broiler Welfare

1.4.1 Leg Disorders and Contact Dermatitis

In order to reduce incidences of leg weakness, it is necessary to assess it. The conventional gait scoring system was developed by Kestin et al. (1992). Birds are scored from 0 (normal) to 5 (most severe lameness) based on their walking ability. The walking ability of birds with a score of 3 or higher is greatly reduced, and the welfare of those birds is considered to be compromised. Webster et al. (2008) adapted the 5-point scale by Kestin et al. (1992) into a 3-point scale with 0 being “no impairment”, 1 being “obvious impairment”, and 2 being “severe
impairment”. Due to high efficiency, the gait scoring systems are widely used on large numbers of birds to detect leg disorders regardless of the pathology of the leg conditions (Sørensen, et al., 2000; Sanotra et al., 2001). However, assessing leg weakness based on gait scores is relatively subjective as different observers may score the same bird differently or the same observer may give birds with the same level of lameness different scores. Flooring types and fearfulness may also influence gait scores. Therefore, comparing results from different studies is difficult.

The Latency-to-lie (LTL) test was developed to assess the severity of lameness in broilers (Weeks et al., 2002; Berg & Sanotra, 2003). This test has shown to be consistent with conventional gait scoring system and thus is a better alternative to measure leg weakness because it is more subjective than gait scoring (Caplen et al., 2014). LTL test takes place in warm-water filled tanks (around 30 °C in temperature and 3cm-4cm in depth). Birds are usually tested between 28 to 43 days of age, in individual compartments with or without visual contact (Berg & Sanotra, 2003; Caplen et al., 2014) or in groups with both physical and visual contact (Weeks et al., 2002). The time each bird spends remaining standing in water is recorded and compared. The total test time is usually 10 or 15 min; however, a bird is promptly removed from water if it fails to stand. The results from LTL tests and gait scores are highly correlated. Caplen et al (2014) found that birds with lower gait scores (sound walking ability) remained standing for longer periods of time during LTL tests. Therefore, LTL test is likely a reliable test to measure the mobility and lameness of a bird. (Weeks et al., 2002; Berg & Sanotra, 2003; Caplen et al., 2014).

Caplen et al. (2014) developed a test to assess mobility in broiler chickens. Lameness is a huge issue if birds cannot access critical resources such as food and water. Before the test, feeders are removed for a short period to increase feeding motivation. During the test, an obstacle is
placed between feeders and drinkers, and birds have to cross the obstacle to feed and drink. The frequency of crossing the obstacle is recorded over a 5-hour period. Broilers are highly motivated to eat and drink, but lame birds tend to be less active and have fewer feeding/drinking bouts. Therefore, with proper habituation to the object before the test, the results should reflect the inability or unwillingness of lame birds to cross the obstacle to access food and water. The results from obstacle test also correspond well with gait scores (lameness level) (Caplen et al., 2014).

Various scoring systems are used to assess footpad lesions. Martenchar et al. (2002) scored lesions from 0 to 3 based on the percentage of footpads affected. Feet with no lesions are 0; lesions on less than 25% of footpads are 1; lesions on more than 25% but less than 50% of footpads are 2; and lesions on more than 50% of feet are 3. Other methods include scoring the severity (Ekstrand et al., 1998) and absolute size (Dawkins et al., 2004) of the lesions.

1.4.2 Behaviour and Activity
1.4.2.1 Play Behaviour

Play behaviour has been recorded in many animal species including mammals and some avian species such as parrots, corvids, hornbills, and Eurasian babblers (Špinka et al., 2001; Diamond & Bond, 2003). However, defining “play” is difficult as play comes in different forms, and varies greatly among and within species. Although the absolute functions of play are still being debated, based on common characteristics of play among species, most ethologists agree that play is most common during infancy and juvenile periods. It is also agreed that the function of play is not seen at the time of performing the behaviour. Play may contain some but not all elements of the serious form of such behaviour and does not result in injurious consequences (e.g. play fighting vs. real fighting). It also may be performed repeatedly but not in a stereotypical way,
and typically happens when stress is absent (Held & Špinka, 2011). Specific types of play include locomotion play such as running, jumping, and hopping, object play such as interacting with an inanimate object, and social play which involves more than one animal (Špinka et al., 2001; Diamond & Bond, 2003; Held & Špinka, 2011).

Avian play occurs in the same three forms as mammalian play (locomotion, object play, and social play) (Špinka et al., 2001; Diamond & Bond, 2003). Different forms of play may happen individually or in combination. For example, play fighting in parrots involves both locomotion and social play (Bekoff, 1984; Diamond & Bond, 2003). It consists of a limited portion of patterns displayed in the adult form of aggressive fighting and does not result in injurious consequences. Some predatory bird toy with their prey before consuming it, which has been described as a form of object play (Diamond & Bond, 2003).

In domestic chickens, worm-running occurs when a bird picks up a worm-like object (rope or rod) and runs with it while other birds chase after the worm-running bird (Kruijt, 1964; Cloutier et al., 2004). It was first described as feeding behaviour (i.e. food running) and was proposed as a way to attract other birds to tear up a larger food item into smaller pieces for consumption (Kruijt, 1964). However, Cloutier et al (2004) suggested that worm-running with inedible objects fits the criteria for play behaviour which involves all three forms (locomotion, object play, and social play) because (1) it has no immediate biological effect as it occurs when food is abundant (fed ad libitum); and (2) it happens even when the object material is non-nutritive.

Sparring (play fighting) is also observed in domestic chickens (Bekoff, 1984; Baxter et al., 2019). Birds performing play fighting display a portion of the elements of adult fighting such
as jumping, and physical contact without aggressive pecking and injuries (Diamond & Bond, 2003; Baxter et al., 2019). Moreover, play fighting usually occurs in birds that are only a few weeks of age when they are still juveniles. Therefore, play fighting fits the criteria of play.

Frolicking, which consist of rapid running, wing flapping, wing-assisted running and jumping, usually disappears when broilers reach 10 weeks old (Baxter et al., 2019). Frolicking may resemble escape behaviour in prey animals; however, it occurs without the presence of predatory stimuli. It is also socially contagious in groups, and happens in non-stereotypical repeated fashion (Baxter et al., 2019). Therefore, frolicking is also considered a form of play.

Play behaviour is usually considered an indication of a lack of negative physical and affective states because its frequency is often reduced when animals are under biological challenge (Held & Špinka, 2011; Ahloy-Dallaire et al., 2018). For example, castration of domestic lambs leads to depressed play for at least 3 days (Thornton & Waterman-Pearson, 2002). Similar results have also been recorded in humans as play is reduced in individuals with physical or mental illness (McGrath et al., 1990; Coplan et al., 2004). Because of findings as listed above, it is generally agreed that animals that are free from diseases, injuries, and negative affective states would play more, indicating better welfare.

However, it is worth mentioning that there are also some counter examples when play is increased when animals are experiencing negative affective states (Held & Špinka, 2011; Ahloy-Dallaire et al., 2018). For example, kittens separated from their mothers perform more play behaviour than controls, and rats engage in more rough-and-tumble play when a medium dose of a stress hormone (ACTH) is injected (see review Ahloy-Dallaire et al., 2018).
Therefore, even though the occurrence of play behaviour is a good indicator of physical health (Held & Špinka, 2011; Ahloy-Dallaire et al., 2018), and is also likely to be a reliable sign of affective states in juvenile animals, it is still unclear if play reflects absolute positive affective states. However, it has also been argued that the performance of play behaviour may be exciting and pleasurable, and thus enhance welfare (Špinka et al., 2001).

Some tests have been used to stimulate play-like behaviour in domestic fowl. One method is to displace broilers by walking through their home pen to create an open space; birds are then seen performing different types of play behaviour while re-entering the open space (Baxter et al., 2019). Another method is to open up more space outside broilers’ home pen. Pettit-Riley et al (2002) reported that birds will run towards the new empty pen after getting through a period of fear. The worm-running test stimulates object play behaviour in chickens (Cloutier et al., 2004). During the test, a worm-like object (e.g. ropes) is thrown into the home pen. Chickens will peck at the worm, run with the worm, or chase after the worm-running bird. Since worm-running is a possible form of play, and also because young animals that play more are likely to have better welfare, both the worm-running test and tests that stimulate other play behaviour may be a good way to measure affective states and animal welfare.

1.4.2.2 Activity Level

Many studies on both humans and non-human animals have used wearable automatic recording devices to monitor activity level. For example, automatic data loggers have been used in sheep to differentiate between grazing, ruminating, and resting behaviour (Giovanetti et al., 2017) as well as predict lameness (Barwick et al., 2018), to determine the cessation of activity in
broilers (Dawson et al., 2007), to detect jumping and landing force in laying hens (Banerjee et al., 2014) and walking activity of turkeys (Stevenson et al., 2019). Researchers are interested in using automatic recording devices because if proven accurate, they can potentially reduce the need of conducting labour-intensive behavioural observations.

Inactivity is common in broiler chickens which may be due to their rapid growth rate and heavy body weight (Weeks et al., 2000; Tickle et al., 2018). Leg disorders and cardiovascular issues are among the most common welfare concerns in broiler chickens (Bessei, 2006; Meluzzi & Sirri, 2009), which may contribute to reduced activity, as animals suffering from pain and discomfort are less likely to be active (Weary et al., 2009). Visual inspection either through live observation or video recordings is a common method used to measure activity and behaviour in research studies. However, it can be labour-intensive. Moreover, visual inspection is not sensitive enough to detect sub-clinical conditions that may cause slight deviations in behaviour (Weary et al., 2009). Therefore, automated measures of broiler activity levels may be helpful for monitoring animal health and behaviour, as well as providing early detection of welfare concerns such as lameness.

The Actical® (Philips Respironics, Bend, Oregon, USA) is a commercially available non-invasive and omni-directional accelerometer that detects a subject’s movement in all directions (Casey-Trott & Widowski, 2018). As a way to replace labour-intensive behaviour observation and quantify activity levels, Actical® has been validated in many species such as non-human primates (Mann et al., 2005), cats (Andrews et al., 2015), and laying hens (Casey-Trott & Widowski, 2018). However, Actical® has not been validated in broilers and it remains unclear if
the device is able to accurately record broilers’ activity levels, and to what extent the activity levels differs between different behaviour and life stages.

1.5 Strategies to Improve Broiler Welfare

Various strategies that can be used to improve broiler welfare include nutrition, genetic selection and housing. For example, certain dietary compositions have shown to improve broiler welfare by reducing heat stress through lowering heat production during digestion, or by improving litter quality, which helps reduce the incidence of contact dermatitis (Bradshaw et al., 2002). Incidence of some leg disorders such as valgus and varus deformation (VVD) has been reduced through genetic selection (Bradshaw et al., 2002), and ascites may be reduced by feed restriction to slow down growth rate between days 7 and 14 (de Jong et al., 2012).

Improving housing conditions by implementing proper lighting, ventilation and drinking systems in combination with lowering stocking density is also beneficial (Ekstrand et al., 1998; Martrenchar et al., 2002; Pettit-Riley et al., 2002; Dawkins et al., 2004). Environmental enrichment has also been suggested as a strategy to improve broiler welfare (Bailie et al., 2018; Riber et al., 2018).

1.5.1 Environmental Enrichment

Environmental enrichment may be defined as modifications to the environment which captive animals live in with the aim of improving their biological functioning and affective states (Newberry, 1995). Although domestication has altered broilers’ behaviour quantitatively and qualitatively, they still perform a wide range of “natural behaviour” such as preening, dustbathing, foraging, and perching, which have also been observed in their ancestor, the red junglefowl (Dawkins, 1989). However, broilers are usually reared in barren environment with
only feeders and drinkers on a litter floor (Norring et al., 2016). The unstimulating rearing environment and the inability to perform motivated behaviour may reduce welfare in captive animals by causing negative emotional states such as boredom and frustration, and by decreasing activity leading to decreased physical health (Newberry, 1995). Increasing environmental complexity to promote activity and natural behaviour has been shown to improve health and affective states in many domestic animals. For example, pigs (Douglas et al., 2012) and rats (Brydges et al., 2011) housed in enriched pens are more likely to judge an ambiguous situation positively (optimistic cognitive biases). Environmental enrichment also triggers wound repair and increases the lifespan in cancer-bearing mice (Bice et al., 2017).

Some studies have shown that exercising may enhance broilers’ leg strength by improving bone and muscle development (Bizeray et al., 2002; Reiter & Bessei, 2009). However, broilers’ use of traditional perches, which need be grasped by birds’ feet, is low, likely due to their conformation which causes difficulty in reaching and balancing on the perch (Bailie et al., 2018). As a result, attempts have been made to create an alternative resource that encourages perching behaviour and increases physical activity level, with the aim of improving leg condition (Ventura et al., 2010; Ventura et al., 2012; Bailie & O’Connell, 2015; Vasdal et al., 2018). Broilers favour horizontal platforms that are easily accessible over traditional perches (Bailie et al., 2018; Riber et al., 2018). Angled platforms were less preferred but were still used (LeVan et al., 2000; Bailie et al., 2018). Moreover, since perching allows broilers to temporarily escape dirty litter and allows for better air flow under their body, platforms may help with heat dissipation and reduce the incidence of contact dermatitis (Riber et al., 2018). Platforms also increase utilization of vertical space, which could lower the effect of high stocking density (Riber et al., 2018). However, the
results among different studies on effects of perches on leg health are inconsistent. Moving platforms, such as those used for automatic weighing in poultry houses, may also benefit broiler chickens because they stimulate musculo-skeletal activity as birds jump or walk onto them, and require some degree of balancing as the platforms swing. However, there are no reports in the literature as to any effects of swinging platforms on broiler health and welfare.

Provision of oral enrichment may offer a means for stimulating pecking, foraging, or exploratory behaviour, and is a requirement in some enhanced welfare labeling schemes (GAP, 2017; RSPCA, 2017). Bales of straw are suggested to stimulate activity and reduce leg problems (Riber 2018). Bailie and O’Connell (2015) found a small improvement in gait score of broilers provided with strings to peck, although this result was not supported by a latency-to-lying test.

Some more complex enrichment such as access to outdoors has been studied (Riber et al., 2018). However, depending on the farms, regions, climates, and countries, results vary among studies in terms of the effects of outdoor access on foot health. Outdoor access also exposes broilers to predators, parasites, and other disease-bearing animals (Riber et al., 2018). Therefore, the provision of outdoor access for fast-growing broilers does not seem to be practical.

1.6 Conclusion

Broilers suffer from welfare issues due to decades of genetic selection for fast growth rate, high feed efficiency, and heavy breast muscle yield. This includes health problems such as leg weakness, lameness, and contact dermatitis, as well as negative affective states such as frustration resulting from living in a barren environment that leads to the inability to perform motivated behaviour such as foraging, and other antipredator behaviour such as hiding. Some studies have been conducted to test the effects of different perches and platforms on broilers’ physical health
and activity level. However, the results among different studies are inconsistent. There is also limited literature on the provision of oral enrichment for broiler chickens. Moreover, different enrichment resources have often been studied individually, and some other enrichment ideas such as the provision of swinging automatic scales (swinging perches) that may better exercise broilers’ muscles, hanging suet feeders filled with compressed wood shavings, and commercially-available peck stones have not been previously tested. In addition, play behaviour may be an indicator of positive affective states (better welfare), However, it has not been used to investigate the effects of environmental enrichment.

Given the potential benefits of providing broilers with environmental enrichment, an experimental study that investigated the effects of multiple environmental and oral enrichment on broilers’ welfare (i.e. physical and affective states) was needed.

1.7 Research Objectives and Hypotheses

1. To determine the effects of environmental enrichment on the activity levels, mobility, and footpads of fast-growing broilers. I hypothesized that broilers reared in an environment enriched with a platform, swinging scale, peck stone, and suet feeder filled with compressed wood shavings would be more active, have better mobility, and healthier footpads than birds reared in non-enriched environments.

2. To quantify how often broilers make use of the various enrichments and any changes in use as the birds grow. I hypothesized that the use of enrichments requiring climbing would decrease with age.
3. To determine the relationships between an individual’s body weight and its activity levels and mobility. I hypothesized that heavy birds would be less active and less mobile than light birds.

4. To determine the effects of environmental enrichment on spontaneous play in natural states, as well as play behaviour through worm-running and free-space tests. I hypothesized that birds reared in enriched pens would perform more play behaviour in the spontaneous play context, worm-running, and free-space tests.

5. To validate the Actical® accelerometer in order to determine if the device is able to accurately measure activity levels in broiler chickens.

The study was divided into three Chapters. Chapter 2 addresses objectives 1 to 3 by testing the effects of environmental enrichment and body weight on mobility and activity. Chapter 3 addresses objective 4 by testing the effects of environmental enrichment on play behaviour in broilers. Chapter 4 addresses objective 5 by discussing the use of the Actical® activity monitor for automated recording of broiler activity levels.
2 Chapter 2
Effects of Environmental Enrichment and Body Weight on Mobility and Activity

2.1 Abstract

The objectives of this study were to determine if providing broilers with environmental enrichment (E) (raised platform, ramp, swing scale, peck stone, and suet feeders filled with wood shavings) affected their body weight (BW), mobility, and time budget compared to birds in non-enriched pens (NE); and to test if birds lighter (L) in BW have better mobility and are more active than heavy birds (H). The BW measures were taken on days 0, 15, 28, and 42. Two Light and two Heavy birds within in each pen were identified as focal animals. Latency-to-lie (LTL) test was conducted on a sample of focal birds on day 41 or 42, and an obstacle test was performed on day 34 to measure broilers’ mobility. Instantaneous scan samples were collected from continuous video recordings at 20-minute intervals between 1100h and 1500h on days 23, 27, and 30 to determine any effects of enrichment and body weight on time budget and to determine resource use in the enriched pens. Generalized linear mixed models were used to test the effects of enrichment, age, and sex on BW, as well as the effects of enrichment, age, and BW on mobility, time budget, and resource use (only in E treatment). No difference was found in BW (P=0.511) or mobility (Latency-to-lie: P=0.664; Obstacle Test: P=0.462) between NE and E birds; however, L birds spent longer time standing during LTL (P<0.0001) and performed more crossings in the obstacle test (P=0.003) than H birds. No treatment effect was found in time spent performing low-energy activities (sitting, sidelying) (P=0.063) and high-energy activities (walking, running, wing-assisted running, foraging) (P=0.791). H birds engaged in significantly
less high-energy activities (P=0.035). E birds spent more time on the obstacles before stepping off than NE birds (P=0.047); and L birds spent more time on the obstacles than H birds (P=0.0003). The results indicate that the provision of the environmental enrichments used in this study did not have an impact on the broilers’ BW, mobility, or time budget. Instead, BW played a more important role in broiler’s mobility and activity levels.

2.2 Introduction

Global consumption of poultry meat has increased considerably in the past 40 years. While broiler chickens were once primarily marketed as whole birds, demand has grown for birds that have higher breast muscle yield for the cut-up market (Griffin & Goddard, 1994; Schmidt et al., 2009). Driven by this demand, the major aim of broiler selection over the past decades has been to increase growth rate, meat yield, breast muscle mass, and feed efficiency (Griffin & Goddard, 1994; Jackson & Diamond, 1996; Konarzewski et al., 2000; Havenstein et al., 2003; Schmidt et al., 2009; Zuidhof et al., 2014). After decades of intense genetic selection, modern broilers now grow at a considerably faster rate than those of previous decades. For example, in 2010, it only took a broiler about 34 days to reach 2.2 kg compared to 70 days for a 1950 broiler to reach 1.5 kg (Butterworth, 2010). The broiler chicken’s high productivity comes at a cost as their welfare is compromised as a result of selection for production traits (Bessei, 2006; Meluzzi & Sirri, 2009).

Among welfare concerns for broilers, leg disorders, footpad dermatitis, and inactivity are most common (Bradshaw et al., 2002; Knowles et al., 2008; Tickle et al., 2018). Leg disorders are the result of multiple factors such as genetics, fast growth rate at a young age, and rearing in
relatively barren conditions at high stocking densities (Kestin et al., 1994; Bradshaw et al., 2002; Knowles et al., 2008). Affected birds tend to be less active and usually sit on litter for a longer period of time. In the worst cases, birds may walk with great difficulty, use their wings to balance, and may have trouble accessing food and water (Vestergaard & Sanotra, 1999; Bradshaw et al., 2002). Constant contact with litter can also lead to contact dermatitis, the most common disorder of the skin in broilers (Bradshaw et al., 2002). Lesions usually occur on the sites of the body that are in contact with wet and dirty litter for a long period of time such as footpads, toes, hocks, and breasts because high litter moisture traps heat, increases litter temperature, and creates a suitable environment for ammonia synthesis, leading to ammonia burns and subsequent bacterial and fungal infections (Kjaer et al., 2006; Bassler et al., 2013).

Studies have shown that conventional, fast-growing broilers spend less time walking, running, frolicking, and performing other active behaviour as they grow heavier. Instead, they spend more time sitting, and being inactive (Weeks et al., 2000; Tickle et al., 2018). It may be argued that this behaviour change could be normal developmental change as an animal matures (Arain et al., 2013). However, broilers are typically slaughtered at 5-6 weeks of age when they are still juveniles (Provenza & Malechek, 1986; Yamada, 1999; Weeks et al., 2000; Arain et al., 2013).

One approach suggested to alleviate some of their welfare problems is to increase broilers’ activity level, by stimulating more active behaviour through enriching the rearing environment (Reiter & Bessei, 2009; Pichova et al., 2016; Riber et al 2018). Although domestication has altered behaviour quantitatively, broilers still perform a wide range of “natural behaviours” such as preening, dustbathing, foraging, and perching, which have also been
observed in their ancestor, the red jungle fowl (Dawkins, 1989). However, broilers are usually reared in a relatively barren environment with only feeders, drinkers and a litter substrate (Norring et al., 2016). The unstimulating rearing environment and the inability to perform motivated behaviour may reduce broiler welfare because of negative emotional states such as boredom and frustration, as well as decreased physical health (Newberry, 1995).

Perching is a common behaviour in layer chickens when they have access to a perch. However, the use of traditional perches is low in broilers, likely due to their conformation which causes difficulty in reaching and balancing on the perch (Bailie et al., 2018). As a result, attempts have been made to create an alternative resource that encourages perching behaviour and increases physical activity level, with the aim of improving leg condition (Ventura et al., 2010; Ventura et al., 2012; Bailie & O’Connell, 2015; Vasdal et al., 2018). Studies have shown that broilers favour horizontal platforms that are easily accessible over traditional perches (Bailie et al., 2018; Riber et al., 2018). Angled platforms were less preferred but were still used (LeVan et al., 2000; Bailie et al., 2018). Moreover, since perching allows broilers to temporarily escape dirty litter and allows for better air flow under their body, platforms may also help with heat dissipation and reduce the incidence of contact dermatitis (Riber et al., 2018). Raised platforms also increase utilization of vertical space, which could lower the effect of high stocking density (Riber et al., 2018). However, the results among different studies on effects of perches on leg health are inconsistent. Moving platforms, such as those used for automatic weighing in poultry houses, may also benefit broiler chickens because they stimulate musculo-skeletal activity as birds jump or walk onto them, and require some degree of balancing as the platforms swing.
However, there are no reports in the literature as to any effects of swinging platforms on broiler health and welfare.

Other enrichment types may also improve broiler welfare. Provision of oral enrichment may offer a means for stimulating pecking, foraging or exploratory behaviour and is a requirement in some enhanced welfare labeling schemes (GAP, 2017; RSPCA, 2017). In terms of physical health, Bailie and O’Connell (2015) found a small improvement of gait score in broilers provided with strings to peck, although this result was not supported by a tests for leg strength.

Although the results among different studies are inconsistent, different enrichment resources have often only been studied individually. Some other enrichment options such as the provision of swinging automatic scales (swinging platforms) that may better exercise broilers’ muscles, hanging suet feeders filled with wood shavings, and commercially available peck stones have not been previously tested in broilers. The objective of the study reported in this chapter was to determine if conventional broilers reared in an environment enriched with a platform, swinging scale, peck stone, and suet feeder filled with compressed wood shavings are more active, have better mobility, and healthier footpads than birds reared in non-enriched environments. We also wanted to quantify how often broilers made use of the various enrichments and any changes in use as the birds grew. We hypothesized that the use of enrichments requiring climbing such as platforms and ramps would decrease with age due to broilers being less mobile as they grow. As it has been suggested that broilers’ compromised welfare is a result of their heavy body weight (Knowles et al., 2008; Bassler et al., 2013; Tickle et al., 2018), another objective of this study was to determine the relationships between an
individual’s body weight and its activity levels and mobility. We hypothesized that heavy birds would be less active and less mobile than light birds due to their heavy weight.

2.3 Methods

All procedures were approved by the Animal Care Committee at the University of Guelph (Animal Utilization Protocol #3746).

2.3.1 Animals, Housing, and Management

A total of 456 Ross 708 broiler chickens were obtained from a commercial hatchery at 1 day of age and housed in 12 floor pens (160 × 238 cm; width × length; 38 birds/pen, stocking density=29kg/m², 19 females and 19 males) with one round feeder (diameter: 33.75cm), a line of nipple drinkers (5 nipples per pen) and new pinewood shavings (approximately 6 cm in depth). The floor pens were divided by solid white plastic walls. Birds were sexed after hatch and a small amount of animal-safe pink paint (KONK Livestock Markers, Canada) was applied on the heads of female birds. Birds were kept until 43 days of age and had ad libitum access to water and antibiotic-free, all-vegetable feed that was milled on-site. All birds were fed with a starter diet from day 1 to day 14, a grower diet from day 15 to day 28, and a finisher diet after day 28. All of the birds received Bronchitis vaccines (mass type, live virus; Merial, Québec, Canada; method: spray), Newcastle-Bronchitis vaccines (B1 type, B1 strain, Mass & Conn types; Merial, Québec, Canada; method: spray), Marek’s disease vaccines (Ceva, France; method: subcutaneous injection), and Immucox® 5 vaccines (Ceva, France; method: oral). The experimental room was climate-controlled at approximately 32 °C on day 1, 31 °C on day 5, 29 °C in week 2, 27 °C in week 3, 24 °C in week 4, and 21 °C in week 5. Light:dark schedule was
maintained at 23:1 h from day 1 to day 4, and 16:8 h after day 4. After day 4, the lights were turned on at 0615h and turned off at 2215h with a dawn/dusk period. The light intensity was kept at 56 Lux from day 1 to day 4, 20 Lux from day 5 to day 28, and was reduced to 7 Lux from day 29 until the end of the trial. The weekly mortality rate was 0.19% for the first, second, and third week, and increased to 1.74% in the fourth week. Mortalities were evenly found in most pens (both NE and E). Necrotic enteritis was diagnosed when the birds were 28 days of age, and the condition was controlled by treating broilers with antibiotics via drinking water (Pot-Pen, Vetoquinol, Lavaltrie, QC) from day 29 to 34. Mortality rate decreased to 0.39% in week 5, and there was no more mortality from day 33 until the end of the trial. Birds were checked twice daily for health status and no moribund birds were found. All chickens were processed on day 43.

2.3.2 Experimental Design

Half of the pens had enrichment (E) while the other half were barren (NE). Enrichment included a raised platform (Red Rooster Mark 2 Black Slats, Clark Ag Systems, Caledonia, ON, Canada, 58×39 cm, length×width) with a 25° ramp using the same material as the platform (79×39 cm, length×width) placed at the back of each pen, a swinging scale (Ohaus, Dundas, Ontario, Canada, diameter: 51cm) in the middle of the pen, a mineral peck stone (PECKstone, Protekta, ON, Canada, 12×19×11 cm, length×width×height) against the left pen wall, and a suet feeder (Scotts, Hagersville, Ontario, Canada, 13×5×13 cm) filled with wood shavings suspended above the peck stone (Figure 2.1). The pinewood shavings in suet feeders were refilled every other day from the pen’s litter.
The pens were divided into 6 blocks based on the temperature gradient in the room (Figure 2.2) with treatment pens systematically distributed across blocks. Within the experimental room, there was also one extra enriched pen and one extra non-enriched pen with 38 birds/pen. When a mortality occurred, another bird of the same sex, similar body weight, and reared under the same treatment condition (E or NE) was added in order to maintain the stocking density in the experimental pens. A total of 8 E birds and 6 NE birds were replaced over the course of the experiment.

2.3.3 Body Weight and Selection of Focal Animals

Birds were individually weighed on day 0, 15, 28, and 42. On day 22, we selected one of the lightest and one of the heaviest males from each pen as focal animals for automated measures of activity. Body weight variation was considered when making the selection. Within a block, one light bird from E and one light bird from NE was selected and matched by body weight. Similarly, one heavy bird from each treatment was selected and matched by body weight. Body weight difference of selected Light or Heavy focal birds, respectively, from pens within the same block was less than 100g. Actical ® activity monitors (Philips Respironics, Bend, Oregon, USA; 29 mm x 37 mm x 11 mm; 22g) were put on these birds’ backs until final processing (see Chapter 4 for more details). On day 34, one additional heavy and one additional light bird from each pen were selected as focal animals using the same method described above. These focal birds did not carry the Actical ® devices and served as controls to determine the effect of wearing the device on broiler behaviour. The four focal birds within each pen (Light-Actical ®, Light-No Actical ®, Heavy-Actical ® and Heavy-No Actical ®) (N=12 for each body weight by Actical®
group) were marked using different colours of livestock paint for individual identification. The body weights of these focal birds were individually measured on days 15, 22, 28, 34, 40, and 42.

2.3.4 Latency-to-Lie (LTL) Test

The latency-to-lie test (LTL) is a quantitative method used to measure the mobility of broilers (Weeks et al., 2002; Berg & Sanotra, 2003), and research trials have shown that LTL is a reliable and objective alternative to relatively more subjective gait scoring (Caplen et al., 2014). All focal birds (4 birds per pen) were tested on day 41 or day 42.

The LTL test was conducted in a clear plexiglass tank (98×48×103 cm; length×width×height) with a plastic mesh divider in the middle to separate and accommodate two birds at the same time while allowing for visual and vocal communication (Figure 2.3). The glass tank had a wood cover (measurement included in reported height) with a door on top to provide easy access for researchers while preventing birds from escaping. The water was kept at 4cm in depth, and a heating pad was placed under the glass tank to keep the water temperature at approximately 28 °C.

During the test, two researchers placed two focal birds from the same pen in the test tank at the same time, and the moment when both birds were placed standing in the water was the start point of the test. The end point of the test was defined as follows: 1. When a bird sat in the water and remained sitting (breast touching water, could be leaning forward, backward, or sitting flat) for at least 3 seconds, the last second during the 3-second sitting period was the end-point. 2. When a bird dipped into water at least 3 times within 10s, the time at which the third dip was made was the end time of the test (definition of dipping: breast touched water but bird
immediately stood up). 3. When a bird remained standing for 10 mins after being placed into water. Video recordings were made to calculate the total standing duration of each bird. The test did not end until both birds reached the end point. After the test, birds were dried and returned to their home pens.

2.3.5 Obstacle Test

An obstacle test was performed in each pen on day 34 based on the procedure described by Caplen et al. (2014). In this test, a wooden barrier (160×9×9 cm; length×width×height) was placed in the middle of each pen, creating an obstacle between the feeder and drinkers (Figure 2.4). During the tests, the birds had to cross (step on and off) the obstacle to gain access to food and water. Barriers were placed in the pens 24 hours before the test to allow birds to habituate to the presence of obstacles. Barriers were positioned to allow access to the feeder and drinkers during the habituation period. One hour before the test, all feeders were removed from home pens in order to increase the birds’ motivation to cross the obstacle at the beginning of the test. Access to water was allowed during that one hour. Subsequently, birds were gently directed to the drinker line (the back of the pen) using a board, the obstacle was placed, and the feeder was returned. Researchers then left the room.

A digital video camera (Sony Digital High Definition Video Camera; HDR-CX405 and DCR-SR68 models; Sony, Japan) was positioned above the front of each pen on a monopod (Digiant MP-3606 Professional Video Monopod 70”, Zhejiang, China), angled towards the obstacle to record all 12 pens at the same time for 5 hours. Continuous video recordings were observed for 5-hour period between 1100h and 1600h using the Observer XT software (Version 12.0, Noldus Information Technology, Wageningen, NL) to count the number of times each bird
stepped on and off the obstacle. Each combination of a step-on and step-off, regardless of
direction was considered a “crossing”. On the whole pen level, the sum of crossings was
recorded by adding up the number of crossings by all birds. For focal birds, the total number of
crossings per bird and the duration of time (minutes per 5 hour) that a bird spent on the barrier
before stepping off were also recorded.

2.3.6 Litter Moisture

Five samples of litter were collected from each pen on day 15, 28, and 42 to represent the
litter condition. All samples were collected using a square metal box (10 ×10×10 cm) that
spanned the full depth of litter and sealed in plastic bags before being processed on the same day.
The five samples from a pen were mixed thoroughly in a clean container, placed in an individual
aluminum foil container and weighed (to the nearest 0.1g) before being kept in a drying oven at
65 ° Celsius for 24 hours. The combined sample of each pen was divided into 2 so that each pen
had two replicates of litter sample. After 24 hours, the litter samples were again weighed, and the
moisture content was calculated by subtracting dry weight from wet weight. The moisture
content of each pen was the average of both replicates.

2.3.7 Time Budget and Resource Use

Video recordings of each pen were made on day 23, 30, and 37. Video recordings started
at 1100h and stopped at 1500h. Starting from minute 10 of the recording, instantaneous sampling
was conducted at 20-min intervals using the Observer XT software (Version 12.0, Noldus
Information Technology, Wageningen, NL) so that twelve samples were collected within the 4-
hour observation period. At each scan, the total number of birds in view, the number of birds
performing each state behaviour, as well as the number of birds at each location (on litter, on
platform, on ramp, under platform/ramp, and on scale) were recorded. Definitions of the recorded behaviours and locations are given in Table 2.1. Two trained observers followed the definitions to record behaviour and location using the Observer XT software (Version 12.0, Noldus Information Technology, Wageningen, NL). The reliability of observers was tested using Observer software. They had to achieve a Kappa coefficient greater than 0.6 (McHugh, 2012) against the main researcher of this study. The Kappa coefficients of the observers were 0.67 and 0.85. The percentage of time birds spent performing different behaviour and at different locations was calculated by dividing the total number of birds performing each behaviour or being at different locations by the total number of birds in view at that scan. The behaviour and location of the focal birds were also individually recorded at each scan.

2.3.8 Footpad Lesions

Footpads of all birds were scored on day 42. Lesions on both feet of each bird were scored from 0 to 3 based on the percentage of footpads affected (Martrenchar et al., 2002). Feet with no lesions were 0; lesions on less than 25% of foot pads were scored 1; lesions on more than 25% but less than 50% of foot pads were scored 2; and lesions on more than 50% of feet were scored 3.

2.3.9 Statistical Analyses

All statistical analyses were computed in SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). Generalized linear mixed models were used for all tests. To account for different pen locations in the room, block was included as a random effect in all analyses. Treatment and sex as well as the interaction were fixed effects for footpad lesions. For litter moisture, treatment and age were fixed effects. For body weight, treatment, age, sex, and their interactions were included
as fixed effects. To compare the preference of birds for different resources, age and resource (litter, platform, ramp, under platform/ramp, scale) were fixed effects. For time budget, treatment, body weight, age, and their interactions were fixed effects. For results from the LTL test and obstacle test, treatment, body weight, wearing Actical ® or not, and their interactions were fixed effects. Since repeated measurements were made on each experimental unit at different ages for body weight, litter moisture, resource use, and time budget, compound symmetry (CS), which is a covariance structure that includes within-subject correlated errors was included in those models. Data were assessed for normality using Shapiro-Wilk analyses. The body weight and time spent on obstacles were log-transformed to normalize the data. LTL test, the total number of crossings for focal birds and for the whole pen level, litter moisture, and the number of feet with any lesion scorings other than 0 followed Gaussian distribution. In terms of time budget, low-energy activities (sum of sitting and sidelying), high-energy activities (sum of walking, running, wing-assisted running, and foraging), all standing (standing with no other behaviour, feed while standing, drink while standing, and preen while standing), all sitting (sitting with no other behaviour, preen while sitting, and sidelying), feed while standing, and drink while standing followed Gaussian distribution.

In focal birds, low-energy activities and all sitting, as well as the use of each resource (on litter, on platform, on ramp, on scale, and under ramp/platform) at different ages all followed Gaussian distribution. High-energy activity time budget, all standing, feed standing, and drink standing were log-transformed to normalize the data. Data for resource use by E birds on day 23 followed Gaussian distribution. Resource use data on day 30 and 37 were log-transformed to normalize the data. The least square means (LSmeans) and standard errors of data following
Gaussian distribution, and the back-transformed LSmeans and standard errors of log-transformed data are reported in results.

Statistical significance was set to \( P < 0.05 \).

### 2.4 Results

#### 2.4.1 Body Weight

There was an age × sex interaction for body weight at all ages \( (P=0.0001 \) for all comparisons) but no treatment effect was found \( (P=0.511) \) (Figure 2.5). Males were significantly heavier than females at all ages \( (P<0.0001) \) (Figure 2.6). BW data collected on days 15, 22, 28, 34, 40, and 42, for focal birds indicated that the heavy birds remained significantly heavier than light birds from day 22 \( (P<0.001) \) for the duration of the trial. The growth trend is shown in Figure 2.7.

#### 2.4.2 Latency-to-Lie Test

There was no treatment effect \( (P=0.664) \) on time spent remaining standing during the latency to lie test. NE birds stood for 376±34.5 s, and E birds stood for 353±33.5 s (Figure 2.8). There was also no effect of wearing the Actical ® \( (P=0.997) \). Birds wearing Actical ® devices stood for 364±33.5 s, and birds without Actical ® stood for 364±34.5 s. However, there was a body weight effect \( (P<0.0001) \) (Figure 3.8). Lighter birds stood significantly longer than heavier birds \( (L: 511.6±34.46 \text{ s}; H: 217.1±33.51 \text{ s}) \). There was no treatment × weight effect \( (P=0.677) \).

#### 2.4.3 Obstacle Test

There was no treatment effect \( (P=0.462) \) on the number of crossings per hour during the obstacle test \( (\text{NE: } 2.0±0.24; \text{E: } 2.4±0.24) \) (Figure 2.9). There was a BW effect \( (P=0.003) \) in the
number of crossings per hour in focal birds. Lighter birds performed significantly more crossings than heavier birds, regardless of their treatment (L: 2.9±0.23; H: 1.7±0.23) (Figure 2.9). There was also no effect of wearing the Actical ® (P=0.892) on the number of crossings per hour in focal birds (With Actical ®: 2.3±0.23; without Actical ®: 2.3±0.23) There was a treatment effect (P=0.047) on the time spent on the obstacle by focal birds. Focal E birds spent significantly more time on the obstacle before stepping off than focal NE birds (E: 4.9±1.16 min; NE: 2.6±0.59 min) (Figure 2.10). There was also a BW effect (P=0.0003) on time spent on the obstacle in focal birds. Lighter birds spent significantly longer time on the obstacle than heavier birds (L: 6.8±1.55 min; H: 1.9±0.45 min) (Figure 2.10). However, no treatment × BW effect (P=0.873) was found. There was no Actical ® effect (P=0.729) in time spent on obstacles (with Actical ®: 1.3±0.23 min; without Actical ®: 1.2±0.23 min)

2.4.4 Litter Moisture

There was an age effect on litter moisture with all ages differing from one another. The litter had the lowest moisture on day 15 (13.0%±1.03%), the highest litter moisture on day 28 (23.0%±1.03%) with intermediate values on day 42 (19.5%±1.03%). However, there was no treatment effect (p=0.2740) (E: 19.5%±1.30%; NE: 17.5%±1.30%) or treatment×age effect (P=0.177)

2.4.5 Time Budget

Pen level observations. The average percentage of birds “in view” was 83.3% for NE, and 83.7% for E for all of the scans.
There was no treatment (P=0.063) or treatment × age effect (P=0.326) on time spent performing low-energy activities (sitting and side lying combined) (NE: 63.2%±0.77%; E: 65.3%±0.73%). However, there was an age effect (P=0.015) with birds spending more time in low-energy activities on day 23 compared to day 30 (P=0.013; Figure 2.11). Although birds tended to spend more time in low-energy activities on day 37 compared to day 23, the difference was not statistically significant (P=0.084). The percentage of time spent in low-energy activities did not differ between days 30 and 37 (P=0.642; Figure 2.11)

No treatment (P=0.791), age (P=0.695) or treatment×age effects (P=0.476) were found for high-energy activities (walking, running, wing-assisted running, foraging) (NE: 9.9%±0.77%; E: 9.6%±0.74%).

**Focal bird observations.** The average percentage of scans when a focal bird was “in view” was 81.9% for L birds, and 88.4% for H birds, regardless of their rearing environment. The percentage was 88.4% for NE, and 82.1% for E, regardless of their BW.

Although H birds spent more time doing low-energy activities (sitting and sidelying) numerically, the difference was not statistically significant (P=0.364) (L: 54.4%±3.90%; H: 60.5%±4.37%; Figure 2.12). Lighter birds spent significantly more time performing high-energy activities (walking, running, wing-assisted running, foraging) than did heavier birds (P=0.035) (L: 13.4%±1.45%; H: 9.9%±1.07%) (Figure 2.12). No age effects were found for either low- (P=0.070) (day 23: 62.7%±4.63%; day 30: 52.6%±4.47%; day 37: 63.0%±4.96%) or high-energy activities (P=0.3638) (day 23: 11.6%±1.61%; day 30: 12.9%±1.52%; day 37: 10.2%±1.27%)
The percentages of time birds spent performing standing (includes standing with no other behaviour, feed standing, drink standing, and preen standing), sitting (includes sitting with no other behaviour, preen sitting, and sidelying), feeding (feed standing), and drinking (drink standing) are reported in Table 2.3 based on their ages, treatment types (for pen level), and BW (for focal birds).

The occurrence of crawling, play fighting, and dust bathing was very low, and thus the data were not analyzed.

2.4.6 Resource Use

The percentages of time that birds in the E pens spent using different resources was not affected by age. The birds were observed on the litter floor 80.6%±1.18%, 79.7%±1.15%, and 81.2%±1.15% of the time on days 23, 30 and 27, respectively (P=0.113). The percentages of time spent on the hanging scale (day 23: 4.6%±0.56%; day 30: 5.3%±0.52%; day 37: 4.7%±0.52%; P=0.517), ramp (day 23: 1.2%±0.26%; day 30: 1.4%±0.24%; day 37: 0.7%±0.24%; (P=0.081), platform (day 23: 7.9%±0.73%; day 30:7.7%±0.67%; day 37:7.1±0.67; P=0.717), and under platform/ramp (day 23: 5.3%±0.83%; day 30: 5.7%±0.77%; day 37: 6.2%±0.77%; P=0.647) were all similar across ages.

In order to determine resource preference, the area of each location was calculated, and the percentage of birds per m² at different ages (days 23, 30, and 37) are reported in Figure 2.13. There was a location effect (P<0.0001) on days 23, 30, and 37. Overall, litter, platform, and scales were equally used, and the ramps were the least favoured resource (P<0.05). The use of
oral enrichment was included in the ethogram. However, peck stones and suet feeders were not in view in most videos, so no analysis was able to be done.

2.4.7 Footpad Lesions

There were few footpad lesions overall and no score 3 lesions were found. Therefore, scores 1 and 2 were combined to determine the percentages of footpads with lesions. The occurrence of foot lesions was not affected by treatment (E: 5.2%±2.13%; NE: 3.3%±2.13%; P=0.060), sex (F: 5.2%±2.13%; M: 3.3%±2.13%; P=0.0608), or treatment×sex interaction (P=0.385).

2.5 Discussion

According to our results, providing broilers with the described environmental enrichment had no effects on their body weight, activity levels (based on time budget), or mobility (based on LTL and obstacle test). The only treatment effect we found was that E birds spent significantly more time than NE birds on the obstacle before stepping off during the obstacle test. However, E birds did use the resources, and preferred the platforms and scales regardless of their ages.

Although there is still debate on whether the provision of perches can improve broilers’ leg health significantly, a recent systematic review indicated that six out of nine studies did not find a significant improvement (Pedersen & Forkman, 2019). New research by Malchow et al. (2019) showed an insignificant increase in activity in fast-growing broilers (P=0.073) reared in environment with elevated grids. However, no improvement in walking ability was found. The E birds in our study, housed with a combination of enrichment (raised platforms, ramps, scales, pecking stones, and suet feeders) also did not show better mobility than the NE birds. The whole
idea of providing broilers with enrichment in the hope of improving their leg health is by increasing their activity level, in part, based on a study by Reiter & Bessei in 2009, where the locomotion ability of broilers was improved by training broilers to walk on a treadmill. However, such improvement was not found in most studies that offered broilers perches as well as in our study that offered a combination of different enrichments. One possible explanation is that broilers used the platform mostly as a resting place. As a result, the provision of platforms did not increase locomotion and activity level as well as training broilers to use a treadmill did. This explanation is also supported by our time budget results where no difference was found between NE and E birds in terms of time spent in low-and high-energy activities. Our results agree with Bailie et al. (2013) who found that provision of perches did not affect activity. BenSassi et al. (2019) also did not find an effect of enrichments on walking ability.

Interestingly, even though there was a numerical increase in the performance of low-energy activities and a numerical decrease in the performance of high-energy activities as the birds got older, the difference was not statistically significant, and they spent most of the time being inactive at all ages. However, high-intensity activities such as running and wing-assisted running are usually short in duration, so it is likely that occurrences of the high-intensity activities were missed during scans at 20-min intervals. Moreover, the broilers were already around 1kg when first observed at the age of 23 days, and there could have been a difference in activity levels if data had been collected at earlier ages. It is worth mentioning that the categorization of low-energy (sitting and sidelying) and high-energy activities (walking, running, wing-assisted running, foraging) agree with results from the Actical ® activity monitors reported in Chapter 4.
Although the provision of enrichments did not influence broilers’ mobility based on the LTL and obstacle tests, E birds did spend significantly more time on the obstacle before stepping off, and this was the only treatment effect found. It is likely due to E birds being exposed to different enrichments from a young age and thus were better at, less fearful of or more comfortable with using the obstacle as a perch (Riber et al., 2007).

Our results indicated that an individual bird’s BW had a significant impact on their mobility and some measures of activity. For example, H birds spent significantly less time performing high-energy activities. H birds also numerically spent more time performing low-energy activities and sitting, and less time standing. Similar results on mobility were also reported by Kristensen et al., (2006) who found that higher body weight increased the probability of poor gait score. Lighter birds also spent more time on the obstacles than heavier birds during the obstacle test, perhaps due to them being more able to balance themselves on the “perch”.

Another reason for the lack of significant effects of environmental enrichment could be due to the relatively small sizes of the pens. A study conducted using dairy cows showed that cows reared in larger pens moved longer distance and demonstrated more movement than those kept in smaller pens even though the stocking densities were the same (Telezhenko et al., 2012). Newberry and Hall (1990) also reported that the total space used by broilers from 4-9 weeks of age was greater in large than small pens at the same stocking density. Broiler studies where an increase in active behaviour was found in E pens were also conducted in larger pens in commercial settings (Ventura et al., 2012; Vasdal et al., 2018).
The provision of straw bales and separation of resources is suggested to be effective in improving mobility (Pedersen & Forkman, 2019). However, more research should be conducted to confirm the effects of straw bales and separation of resources on leg health and activity level of broilers.

In conclusion, contrary to the hypothesis, enrichments had no effect on the growth, mobility, and activity levels in broilers. Further research should look into other enrichment types that can motivate broilers to stay active instead of resting on a provided enrichment. Managing broilers’ BW also seems to be a more effective way to improve their mobility and welfare.
Table 2.1: Behaviour and location definitions for time budget and resource use. All behaviour were mutually exclusive.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting</td>
<td>Immobile, entire breast touching the ground</td>
</tr>
<tr>
<td>Sidelying</td>
<td>Similar to sitting but is lying on their side. Usually leg is stretched</td>
</tr>
<tr>
<td>Standing</td>
<td>Immobile on two legs, body not touching the ground</td>
</tr>
<tr>
<td>Walking</td>
<td>Slow forward movement, breast above the ground, using legs</td>
</tr>
<tr>
<td>Crawling</td>
<td>Slow forward movement, breast touching the ground, using legs</td>
</tr>
<tr>
<td>Running</td>
<td>Faster forward movement, using legs; NO wings involved</td>
</tr>
<tr>
<td>Wing assisted running</td>
<td>Faster forward movement, using legs and wings</td>
</tr>
<tr>
<td>Feed standing</td>
<td>Downward pecking in feeder while standing</td>
</tr>
<tr>
<td>Feed sitting</td>
<td>Downward pecking in feeder while sitting</td>
</tr>
<tr>
<td>Drink standing</td>
<td>Pecking at nipple drinkers while standing</td>
</tr>
<tr>
<td>Drink sitting</td>
<td>Pecking at nipple drinkers while sitting</td>
</tr>
<tr>
<td>Preen standing</td>
<td>Moving the beak through the feathers while standing</td>
</tr>
<tr>
<td>Preen sidelying</td>
<td>Moving the beak through the feathers while sidelying</td>
</tr>
<tr>
<td>Preen sitting</td>
<td>Moving the beak through the feathers while sitting</td>
</tr>
<tr>
<td>Foraging</td>
<td>Ground scratching using both legs accompanied by pecking on the ground</td>
</tr>
<tr>
<td>Play fight</td>
<td>Two birds, hopping and chest bumping; while facing on another. No forceful pecking.</td>
</tr>
<tr>
<td>Dustbathing</td>
<td>Vertical wing shakes in a lying position, combined with time spent ruffling feathers and bill raking.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>On platform</td>
<td>≥ half body on the horizontal part of the platform</td>
</tr>
<tr>
<td>On ramp</td>
<td>&gt;half body on the slope of the ramp</td>
</tr>
<tr>
<td>Under platform/ramp</td>
<td>&gt;half body under platform or ramp</td>
</tr>
<tr>
<td>On scale</td>
<td>Both feet on the scale</td>
</tr>
<tr>
<td>On stone</td>
<td>Both feet on the stone</td>
</tr>
<tr>
<td>On litter</td>
<td>On the litter but not under the platform or ramp</td>
</tr>
<tr>
<td>On drinker line</td>
<td>Perching on drinker line</td>
</tr>
</tbody>
</table>
Table 2.2 Mean percentage of time (±SE) of birds performing low- and high-energy activities on days 23, 30, and 37 regardless of their treatment. LSmeans within a column sharing a superscript are not different (P≥0.05).

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Low-energy activities</th>
<th>High-energy activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% time</td>
<td>SE (%)</td>
</tr>
<tr>
<td>23</td>
<td>60.39</td>
<td>1.367</td>
</tr>
<tr>
<td>30</td>
<td>67.13</td>
<td>1.315</td>
</tr>
<tr>
<td>37</td>
<td>65.23</td>
<td>1.315</td>
</tr>
</tbody>
</table>
Table 2.3 Mean percentage of time (±SE) that birds were observed performing standing, sitting, feeding, and drinking based on their treatment (NE or E) and BW (L or H) across ages (days 23, 30, and 37). Behaviours are not mutually exclusive. LSmeans with the same letters within row for age (a,b) or treatment and body weight (c,d) are not different (P>0.05).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Day 23</th>
<th>Day 30</th>
<th>Day 37</th>
<th>NE</th>
<th>E</th>
<th>Day 23</th>
<th>Day 30</th>
<th>Day 37</th>
<th>L</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing</td>
<td>28.2±1.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.5±1.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.9±1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.8±0.79&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.2±0.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.8±3.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.1±4.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.3±3.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.5±3.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.1±2.73&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sitting</td>
<td>66.2±1.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.3±1.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.3±1.30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>68.8±1.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70.4±1.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>68.8±4.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.8±4.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.0±4.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.7±3.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>66.4±3.81&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feeding</td>
<td>15.6±0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.6±0.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.1±0.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.0±0.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.9±0.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.9±2.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.7±2.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.7±2.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.9±2.36&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.5±1.97&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Drinking</td>
<td>5.3±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.9±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.0±0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.8±0.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.1±1.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.8±1.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.8±1.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.1±1.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.5±1.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Figure 2.1 Picture showing an enriched pen with its resources (an elevated platform, an angled ramp, a swinging scale, a peck stone, and a suet feeder filled with wood shavings). The brown paper was used to train the chicks how to eat and drink, and was removed after a week.
Figure 2.2 The layout of the experiment and temperature gradient of the room. 12 pens were used (pen 9-20).
Figure 2.3 Two broilers during the Latency-to-Lie test

Figure 2.4 Broilers during the Obstacle test. The obstacle is the white wooden beam in the middle, separating the feeder and drinkers.
**Figure 2.5** The mean body weight (kg) (±SE) of all birds reared in NE and E environment at days 0, 15, 28, and 42. There was no effect of environment on BW at any age. LSmeans with the same letters are not different (P≥0.05).
Figure 2.6 The mean body weight (kg) (LSmeans ±SE) of male and female birds at days 0, 15, 28, and 42, regardless of their treatment types. Males were heavier than females at all ages. LSmeans with the same letters are not different (P≥0.05).
Figure 2.7. Mean body weights (± SE) of Heavy (H) and Light (L) focal birds from day 15 to day 42. The heavy birds were significantly heavier than light birds from day 22 to 42 (P<0.001).
Figure 2.8 Mean standing time (s) (±SE) of birds reared in non-enriched (NE) or enriched (E) pens, and Light (L) or Heavy (H) in BW during LTL test. LSmeans with the same letters within main factor (treatment or BW) are not different (P≥0.05).
**Figure 2.9** The mean total number of crossings per hour (±SE) in non-enriched (NE) and enriched (E) pens, and Light (L) and Heavy (H) focal birds during the obstacle test. LSmeans with the same letters within main factor (treatment or BW) are not different (P≥0.05).
Figure 2.10 Mean time (min) (±SE) spent on obstacles per 5 hour in non-enriched (NE) and enriched (E) pens, and Light (L) and Heavy (H) focal birds in BW during the obstacle test. LSmeans with the same letters within main factor (treatment or BW) are not different (P≥0.05).
Figure 2.11 Mean percentage of time (±SE) that birds at different ages (days 23, 30, and 37) were observed performing low-and high-energy activities. LSmeans with the same letters for each category of activity are not different (P≥0.05).
**Figure 2.12** Mean percentage of time (±SE) that light (L) or heavy (H) birds were observed performing low- and high-energy activities. LSmeans with the same letters for each category of activity are not different (P≥0.05).
Figure 2.13 Location preference (% birds per m²) (±SE) at day 23(a), 30(b), and 37(c). LSmeans with the same letters are not different (P≥0.05)
Chapter 3
Effects of Environmental Enrichment on Play Behaviour in Broilers

3.1 Abstract

The objective of this study was to determine the effects of environmental enrichment on play behaviour of broiler chickens. Using the same birds as in Chapter 2, 3 worm running tests (on days 10, 24, and 38), and 3 free-space tests (on days 8, 21, and 35) were performed to stimulate play behaviour such as worm exchange, worm pecking, worm chasing, worm running, frolicking (running, wing-assisted running, and wing flapping), and sparring. Video recordings were also made on days 23 and 30 to observe spontaneous play. Generalized linear mixed models were used to test the effects of enrichment and age on the occurrence of different forms of play behaviour per bird per 5 min. Non-enriched (NE) birds performed more worm exchange, worm running, and worm exchange than enriched (E) birds at all ages during worm-running tests. In free-space tests, the occurrence of all play behaviour (sum of running, wing-assisted running, wing flapping, and sparring; P<0.0001), running (P<0.0001), and wing-assisted running (P=0.016) were higher in NE birds than that in E birds on day 8. The magnitude of the treatment difference decreased on day 21 (all play behaviour: P=0.062; running: P=0.048; wing-assisted running: P=0.67), and disappeared on day 35 (all play behaviour: P=1.000; running: P=0.997; wing-assisted running: P=0.997). The occurrence of worm chasing declined in both NE and E birds over time. The occurrence of running, wing-assisted running and all play behaviour combined decreased in NE birds as they aged, but remained unchanged in E birds. The occurrence of stationary play behaviour such as worm pecking (P=0.004) and wing flapping (P=0.0004) increased as birds aged. There was no treatment effect (P=0.32) on spontaneous play.
(sum of running, wing-assisted running, wing flapping, and sparring) likely due to relatively short observation time (5min). Although the age difference was not statistically significant, birds performed less play on day 30 than day 23 (P=0.084). NE birds did not differ from E birds in terms of spontaneous play but were more responsive than E birds during tests intended to stimulate play behaviour in broilers. This was possibly because of the larger contrast between their barren environment and test conditions, and / or because they were bored as a result of the unstimulating environment which made them more easily aroused. The findings also show that worm-running and free-space tests are effective in stimulating play behaviour.

3.2 Introduction

Play behaviour is common in young animals (Špinka et al., 2001; Diamond & Bond, 2003). Although the function of play is still being debated, based on common characteristics of play among species, most ethologists agree that play is most common during infancy and juvenile periods, is not critical to present survival, may occur repeatedly but not stereotypically, and typically occurs when stress is absent (reference). There are different categories of play behaviour that include locomotor play such as running, jumping, and hopping, object play which involves interacting with inanimate objects, and social play which involves more than one animal (Špinka et al., 2001; Diamond & Bond, 2003; Held & Špinka, 2011), for example, rough-and-tumble play (as reviewed in Ahloy-Dallaire et al 2018).

In domestic chickens, worm-running occurs when a bird picks up a food item or object and runs with it while other birds chase after it (Kruijt, 1964; Cloutier et al., 2004). It was first described as feeding behaviour (i.e. ‘food running’) and was proposed to function as a way to attract other birds to tear up large food items into smaller pieces for consumption (Kruijt, 1964).
However, Cloutier et al (2004) suggested that worm-running with inedible objects fits the criteria for play behaviour since it involves all three forms (locomotion, object play, and social play) and because it has no immediate biological effect as it occurs when food is abundant (fed ad libitum), and when the object material is non-nutritive.

Sparring, which is also known as play fighting, is also observed in domestic chickens (Bekoff, 1984; Baxter et al., 2019). Birds performing play fighting display a portion of the elements of adult fighting such as jumping, and physical contact but without aggressive pecking and injuries (Diamond & Bond, 2003; Baxter et al., 2019). Moreover, play fighting occurs in birds when they are still juveniles. Therefore, play fighting fits the criteria of play. Frolicking, which consists of rapid running, wing flapping, wing-assisted running, and jumping, usually disappears when broilers reach 10 weeks old (Baxter et al., 2019). Frolicking resembles escape behaviour in prey animals; however, it occurs without the presence of predatory stimuli. It is also socially contagious in groups, and happens in non-stereotypical repeated fashion (Baxter et al., 2019). Therefore, frolicking may also be considered a form of play.

Some conditions have been reported to stimulate play-like behaviour in domestic fowl. One involves displacing broilers by walking through their home pens. Birds were reported to re-enter the empty area by performing different types of play behaviour (Baxter et al., 2019). Another involves creating more accessible space outside broilers’ home pen. Newberry (1999) reported that opening a gate to give daily temporary access to an open pen next to the home pen led to broilers running into the open space, often with wings flapping, and she suggested that this could have been related to boredom in the home pen. A ‘worm-running test’ stimulates object play behaviour in chickens (Cloutier et al., 2004). During the test, a worm-like object (e.g. pipe
cleaner or twisted paper) is thrown into the home pen. Chickens will peck at the “worm”, run with the “worm”, or chase after the worm-running bird.

Play behaviour has been considered an indication of a lack of negative physical and affective states as its frequency is often reduced when an animal is under biological challenge (Held & Špinka, 2011; Ahloy-Dallaire et al., 2018). For example, castration of domestic lambs leads to depressed play for at least 3 days (Thornton & Waterman-Pearson, 2002). Similarly, in humans play is reduced in individuals with physical or mental illness (McGrath et al., 1990; Coplan et al., 2004). Because of findings such as above, it is generally agreed that animals free from diseases, injuries, and negative affective states play more, indicating better welfare. However, there are also some counter examples when play is increased when the animal is experiencing negative affective states (Ahloy-Dallaire et al., 2018). For example, kittens separated from their mothers performed more object play behaviour than controls, and rats engaged in more rough-and-tumble play when a moderate dose of a stress hormone (ACTH) was injected (Arelis, 2006). Possible explanations for these counter-examples are 1) play may be exciting and pleasurable, and thus may be performed to alleviate some forms of stress (Špinka et al., 2001) or 2) play may enable animals to better prepare for challenging situations (e.g. in the kittens’ case) (Ahloy-Dallaire et al., 2018).

Generally, play is considered as a good indicator of the absence of poor welfare, and a lower frequency of play behaviour in a generally playful species and at an age when play would be expected may indicate poorer welfare. Based on this logic, if environmental enrichment is indeed able to improve broiler welfare, either by reducing lameness or by increasing activity level, birds reared in an enriched environment should play more.
The objective of the study reported in this chapter was to determine the effects of environmental enrichment on spontaneous play in natural states, as well as play behaviour through worm-running and free-space tests. We hypothesized that birds reared in an enriched environment would play more as a result of increased mobility and welfare.

3.3 Methods

All procedures were approved by the Animal Care Committee at the University of Guelph (Animal Utilization Protocol #3746). Details on housing, management and experimental design are given in Chapter 2.

3.3.1 Worm Running Test

A total of 3 worm-running tests were performed on all pens on days 10, 24, and 38 between approximately 1000h-1100h (lights on: 0615-2215h). The procedure was adapted from Cloutier et al., 2004. “Worms” were made from white twisted tissue paper and were 5cm long at week 2, 6cm long at week 4, and 7cm long at week 6. This colour was chosen after tests with non-experimental birds showed fear responses to red, purple and blue tissue paper. The diameter of the paper worms was 3 mm. Before conducting the test, a researcher stood in front of the pen for approximately one minute to allow the birds to habituate to the researcher’s presence. Once the birds returned to their undisturbed behaviour, a “worm” was gently thrown into the pen. The entire test was observed carefully, and a new “worm” was supplied as soon as the old “worm” was destroyed, eaten, or lost. Two digital video cameras (Sony Digital High Definition Video Camera; HDR-CX405 and DCR-SR68 models; Sony, Japan) mounted on monopods (Digiant MP-3606 Professional Video Monopod 70”, Zhejiang, China) were used for each pen to record the tests for 10 minutes, ensuring there was at least 5 minutes of video recording when at least
one “worm” was present. Pens within the same block were tested concurrently, and all “worms” were removed after the tests.

Scan sampling of the video recordings was used, starting from the moment the “worm” landed on the pen floor. Every scan lasted 10s, and 30 worm-present scans were made for each pen to account for a total of 5 minutes of observation time per pen. During each scan, the following recordings were made: (1) the presence of at least one “worm” (yes or no) during at least part of the scan period, (2) the number of birds performing worm pecking, worm running, and worm chasing. Scans during which a worm was not present were skipped to conduct the next scan. Only scans during which a worm was present were used to account for the 5-min observation time. Occasionally, a missing worm reappeared during the observation period, and when this occurred, the replacement worm was followed.

Continuous behaviour recording was also performed for the whole 5-min observation period to record how many times a worm exchange occurred. The videos were played at slow speed (at least 1/2 of normal speed) so that the behaviour of individual birds could be tracked. The observer for both samplings (Z.L.) was not blinded to the hypothesis. The Kappa coefficient for intra-observer reliability was 0.92. The ethogram used for both methods of data collection is provided in Table 3.1.

3.3.2 Free Space Test

A total of 3 free space tests were performed on all pens on days 8, 21 and 35 between approximately 1000h to 1100h. The procedure and ethogram were adapted from Newberry et al. (2018) and Baxter et al. (2019). During the test, a researcher walked into the pen with a divider
and gently pushed all birds back to the drinker line. In the meantime, a second researcher removed the feeder in order to create the same amount of ‘new’ open space in all pens (E pens still had all of the enrichments present so total amount of open space differed between treatments). After approximately 10 seconds, the divider was removed and both researchers quietly moved to the next non-adjacent pen and repeated the whole procedure in the same way. Four non-adjacent pens were recorded simultaneously for 5 min. Two digital video cameras (Sony Digital High Definition Video Camera; HDR-CX405 and DCR-SR68 models; Sony, Japan) were positioned on monopods ((Digiant MP-3606 Professional Video Monopod 70”, Zhejiang, China) above the front of each pen to record the test for 5 minutes. Feeders were returned to the pens after the tests. Afterwards, another round of four non-adjacent pens were tested and recorded. A total of three rounds were made because there were 12 pens in total. The testing order was systematically determined beforehand to avoid any disturbance to a recording pen, but two NE and two E pens were included in each round.

Continuous all-occurrence sampling was used for the whole observation period (5 min) to record every occurrence of running, frolicking (wing-assisted running and wing flapping), sparring, dustbathing, and aggression (Table 3.2) in order to determine the frequencies of each behaviour. The observer (Z.L.) was not blinded to the hypothesis. The intra-observer reliability was 0.91 (Kappa Coefficient). Jumping, which is part of frolicking behaviour, was excluded from ethogram because birds housed in enriched pens had structures from which they could jump, which may have artificially increased the frequency.
3.3.3 Spontaneous Play

Video recordings were made on all pens concurrently on days 23 and 30 between 1100h to 1500h to observe spontaneous play behaviour in a non-test context. One digital video camera (Sony Digital High Definition Video Camera; HDR-CX405 or DCR-SR68 model; Sony, Japan) was placed on a monopod (Digiant MP-3606 Professional Video Monopod 70”, Zhejiang, China) in front of each pen for video recording. Continuous all-occurrence sampling was used for the whole observation period (5 min for each day) to record every occurrence of running, frolicking (wing-assisted running and wing flapping), sparring, dustbathing, and aggression in order to determine the frequency of occurrence of each behaviour. The ethogram used was the same as for free-space tests (Table 3.2).

3.3.4 Statistical Analyses

For worm-running tests, the occurrence of defined behaviours from each 10-second scan was summed to account for the total occurrence of each behaviour for a total of 5 minutes of observation time. Both worm-running and free-space tests as well as spontaneous play were corrected for the number of birds per pen, and the behaviour unit reported in this chapter is occurrence per bird per 5 min.

All statistical analyses were computed in SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). Generalized linear mixed models were used for worm-running, free-space tests, and spontaneous play. To account for different pen locations in the room, block was included as a random effect. Treatment, age, and their interactions were included as fixed effects. Since repeated measurements were made on each experimental unit at different ages, compound symmetry (CS), which is a covariance structure that includes within-subject correlated errors was
included in the model. Data were assessed for normality using Shapiro-Wilk analyses. Worm exchange, chasing, and running data from worm-running tests, and running, wing-flapping, and all play behaviour (sum of running, wing-assisted running, wing-flapping, and sparring) from free-space tests, and sum of all play behaviour from spontaneous play observations followed Gaussian distribution; their LSmeans and standard errors are reported in results. Worm pecking, and wing-assisted running were log-transformed to normalize the data, and the log-transformed LSmeans and standard errors were back transformed and reported in results as well. The occurrence of sparring was low, and thus it was not analyzed by itself. Instead, sparring was grouped with other play behaviour into “all play behaviour”. Statistical significance was set to P<0.05.

3.4 Results

3.4.1 Worm Running Test

There were overall treatment effects on worm exchange (P=0.034), worm chasing (P<0.0001), and worm running (P=0.035), with NE birds performing more of those behaviours than E birds (Figure 3.1). The treatment difference in worm pecking was not statistically significant (P=0.231).

There was an overall decrease in the occurrence of worm chasing per bird per 5 min as the birds aged (P<0.0001), and an increase in the occurrence of worm pecking on day 38 (P=0.004; Figure 3.2). The difference in terms of worm exchange (P=0.007) and worm running (P=0.002) varied inconsistently across days of observation (Figure 3.2). There was no treatment × age interaction in worm exchange (P=0.507), pecking (P=0.222), chasing (P=0.130), or running (P=0.651).
3.4.2 Free Space Test

There were effects of treatment, age, and treatment × age on all play behaviour (P<0.0001 for all effects), running (P<0.0001 for all effects), and wing-assisted running (P=0.016, P=0.0004, P=0.003 respectively; Figure 3.3). NE birds performed more running than E birds on days 8 and 21, but not on day 35. The occurrence of running decreased at each age tested for NE birds but not for E birds, which had a lower running frequency at all three ages observed. NE birds performed significantly more wing-assisted running than E birds only on day 8, as the frequency of this behaviour declined considerably over time in NE birds. The frequency of wing-assisted running was stably low in E birds at all ages. The occurrence of all play behaviour was significantly higher in NE than E birds on day 8; the difference decreased on day 21 and completely disappeared on day 35. The occurrence of all types of play declined at each age tested for NE birds but not for E birds.

There was no treatment effect (P=0.251) or treatment × age interaction (P=0.107) on frequency of wing-flapping. Wing flapping occurred less often on day 8 (0.05±0.02 occurrences per bird per 5 min) than on day 21 or 35 (0.19±0.02 and 0.12±0.02 occurrences per bird, respectively; P=0.0004).

3.4.3 Spontaneous Play

Since the occurrence of each individual play behaviour was low, only total play (sum of running, wing-assisted running, wing flapping, and sparring) was statistically analyzed. There was no treatment effect (P=0.319) (E: 0.13±0.0192; NE: 0.10±0.0185 occurrences per bird per 5 min) (Figure 3.4) and no treatment by age interaction (P=0.478). There was also no difference in
total play on days 23 and 30 (Day 23: 0.14±0.017; day 30: 0.10±0.017 occurrences per bird; P=0.084).

3.5 Discussion

Contrary to our hypothesis, the results show that NE birds played more than E birds in test contexts. We hypothesized that E would play more because animals from enriched environments are generally thought to have fewer negative, and perhaps, more positive affective states. Several studies have indicated that animals such as mink (Vinke et al., 2005) and rats (Morley-Fletcher et al., 2003) living in an enriched environment play more than the controls. Another reason for our hypothesis of more play in E birds was that we expected E birds to have better mobility and thus would be more able to play; however, the results from Chapter 2 indicated that this was not the case.

NE birds performed significantly more play behaviour than E birds on day 8 (worm-running test) and day 10 (free-space test); the differences declined on day 21 (worm-running test) and day 24 (free-space test), and completely disappeared on day 35 (worm-running test) and day 38 (free-space test). A decrease in play behaviour over time was also in line with a slight increase in inactivity we found in Chapter 2. Other studies (Weeks et al., 2000; Tickle et al., 2018) on broilers have shown similar results, where the broilers spent less time walking, running, and performing other active behaviour as they got older. Vasdal et al. (2019) also found a decrease in play (running, worm-running, play fighting) from day 16 to day 30 in broilers. It may be argued that this behaviour change could be a normal developmental change as an animal becomes mature (Spear, 2004). However, a decrease in play behaviour with age was not found in layer pullets at even older ages (Cloutier et al., 2003). Moreover, broilers are typically
slaughtered at 5-6 weeks of age when they are still juveniles and sexually immature, and thus play could be expected to persist throughout the productive life of a broiler (Yamada, 1999; Weeks et al., 2000; Spear, 2004). The increase of stationary play behaviour (e.g. worm pecking) and decrease of locomotor play behaviour (e.g. worm chasing) are more likely due to the broilers’ fast growth rate and heavy body weight, rather than their age (Bokkers & Koene, 2003). Although the birds in the current experiment were diagnosed with necrotic enteritis at 29 d of age, the condition was rapidly controlled by antibiotics. Only one behavioural test (worm-running) was conducted after this point, and since the general trend of decreasing activity continued to week 6, the condition likely did not affect the final result. However, the light intensity was also reduced to 7 lux from day 29 until the end of the experiment. A few studies have found that birds rested more and preened less when exposed to lower light intensity (Newberry et al., 1988; Deep et al., 2012); however, Deep et al. (2012) found no increase in standing and walking with increasing light intensity (1, 10, 20, and 40 lux) under a 17-h light phase. Moreover, Newberry et al. (1986) found that activity decreased with age regardless of light intensity. Therefore, even though it is possible that the lower light intensity from day 29 until the end of the experiment may have contributed to a larger decrease in activity in broilers, the general trend of decreasing activity with age should remain unaffected, and thus, our interpretation that the increase of inactive play behaviour and decrease of active play behaviour are more likely due to the birds’ fast growth rate and heavy body weight still stands.

There may be several explanations for NE birds being more engaged than E birds during worm-running and free-space tests. To begin with, there are counter examples where animals were observed to play more after being put under more negative affective states. For example, a
rebound effect has been described, where calves subject to spatial restriction performed more play than calves housed in more spacious pens when calves from both environments were tested in a novel environment (Rushen & de Passillé, 2014). The larger contrast between the NE environment before and during the tests (i.e. opening up extra space and offering “worms”) compared to that in the E pens may have led the NE birds to be more easily stimulated to play during the tests. According to this interpretation, the higher play behaviour in the NE birds reflected transiently higher responsiveness in the test context rather than reflecting an underlying state of greater positive welfare when kept in a NE environment. Another explanation is that NE birds could have been experiencing boredom and thus were more responsive during the tests. Boredom and its effect on captive animals are reported in literature (Burn, 2017), and it has been suggested that animals, such as mink (Meagher & Mason, 2012) and pigs (Stolba & Wood-Gush, 1980) living in unstimulating barren environment suffer from boredom, are more easily aroused, and show more interest when stimuli are offered. Meagher and Mason (2012) found that NE mink showed increased interest in all types of stimuli (aversive, ambiguous, and rewarding). Stolba and Wood-Gush (1980) also found that the more barren the environment, the stronger the interest that pigs exhibited towards a stimulus. The results from worm-running and free-space tests could also be suggesting boredom in NE birds. Therefore, it is possible that NE birds were experiencing negative welfare and play was an indicator of that. A different interpretation could be NE birds had better welfare than E birds and thus played more. Since we did not find any other “positive” effects of enrichment, this explanation might be the case. The significant difference in play between NE and E disappeared at day 35 and 38 which could be due to reduced space in pens as the birds got bigger, or due to possible physical restrictions. However,
as birds got older and heavier, even though NE birds may have still wanted to play, they may have become less able to play as a result of decreasing mobility (Weeks et al., 2000; Tickle et al., 2018), and thus the difference between NE and E birds disappeared. There was evidence of this interpretation from the worm-running tests as broilers spent more time performing stationary play behaviour such as pecking than worm chasing as they aged.

Play was recorded during 5-min observations in the free-space tests and spontaneous play context using the same methods. From these data, it is evident that the level of all play behaviour was 5-20 times (in NE pens) and 5-10 times (in E pens) higher (depending on test dates) in the tests than during spontaneous conditions. The level of worm chasing in the worm-running tests was of a similar magnitude to all play behaviour in the free-space tests. These results indicate that the two tests were effective in stimulating play as predicted, as well as in detecting treatment differences. Therefore, these tests can be valuable for use in future research on responses in these contexts. Because the level of spontaneous play was very low, caution is needed in interpreting the results because any possible treatment differences may have occurred below the level of detection. Nevertheless, it is interesting that none of the results revealed higher levels of play in the E than the NE treatment, contrary to the prediction that the enriched environment would stimulate more play. As discussed earlier, studies indicated suppression of play under adverse conditions, as the adverse conditions could have been perceived as life-threatening. This was not the case in the current study, as chickens grew at similar rates in both treatments and in accordance with expected growth according to the breeder manual (Aviagen, 2019). In addition, mortality rates were comparable and within rates typical for the breed. The findings show the
importance of considering the context under which play is observed and show that the timing of
observations relative to stimulation of activity is critical to the interpretation of play data.

In conclusion, NE birds performed more play behaviour under worm-running and free-
space test conditions. The occurrence of play also generally declined as the birds got older. NE
birds might be more responsive as a result of a higher contrast between their barren environment
and test stimuli (the presence of worms and extra space) and/or boredom resulting from an
unstimulating environment. Birds performed less active play behaviour and became more
sedentary as they aged, likely due to their heavy body weight and physical inability.
Table 3.1 The ethogram of worm-running tests as adapted from Cloutier et al. (2004).

All behaviour patterns were mutually exclusive, with each bird being assigned to only one behaviour per scan.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worm pecking</td>
<td>Bird pecks at “worm”. The worm is usually seen to move when impacted by the beak, and may be lifted off the ground, but not carried or pecked at while being carried.</td>
</tr>
<tr>
<td>Worm running</td>
<td>While walking or running, bird carries a “worm” projecting from its beak. The bird makes rapid changes of direction which typically attracts other birds to follow. If the bird also performs worm pecking or worm chasing in the same scan, it was only record as worm running.</td>
</tr>
<tr>
<td>Worm chasing</td>
<td>While walking or running, a bird follows or moves along with the bird carrying the worm and may attempt to grab the worm from its beak. If this bird also performs worm pecking in the same scan, it was recorded as worm chasing.</td>
</tr>
<tr>
<td>Worm exchange</td>
<td>A worm is transferred from one bird’s beak to another when grabbed from a worm running bird by a worm chasing bird, or from a stationary bird holding a worm with at least one end off the ground.</td>
</tr>
</tbody>
</table>
**Table 3.2** The ethogram of free-space tests and spontaneous play as adapted from Baxter et al. (2018). All behaviour patterns were mutually exclusive.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Running</td>
<td>Forward movement at 2 to 3 times or more of the normal walking speed.</td>
</tr>
<tr>
<td>Wing-assisted running</td>
<td>Forward movement at 2 to 3 times or more of the normal walking speed with wing assistance, including sudden direction change.</td>
</tr>
<tr>
<td>(part of frolicking)</td>
<td></td>
</tr>
<tr>
<td>Wing-flapping</td>
<td>Rapid up and down movements of wings. No running involved.</td>
</tr>
<tr>
<td>(part of frolicking)</td>
<td></td>
</tr>
<tr>
<td>Sparring (play fight)</td>
<td>Two birds, hopping and chest bumping while facing one another, no forceful pecking, short and non-aggressive.</td>
</tr>
<tr>
<td>Aggression</td>
<td>Forceful and rapid pecking or kicking.</td>
</tr>
<tr>
<td>Dustbathing</td>
<td>Vertical wing shakes in a lying position. Only the number of dustbathing initiations was recorded.</td>
</tr>
</tbody>
</table>
Figure 3.1 The mean occurrence (±SE) of worm exchange, worm pecking, worm chasing, and worm running per bird per 5 minute during worm-running tests for non-enriched (NE) and enriched (E) birds on days 10, 24, and 38. LSmeans with the same letters within each behaviour are not different (P≥0.05).
Figure 3.2 The mean occurrence (±SE) of worm exchange, worm pecking, worm chasing, and worm running per bird per 5 minute during worm-running tests on days 10, 24, and 38 regardless of their treatment types (non-enriched or enriched). LSmeans with the same letters within each behaviour are not different (P≥0.05).
Figure 3.3 Mean occurrence (±SE) of all play behaviour (sum of running, wing-assisted running, wing flapping, and sparring), running, and wing-assisted running of non-enriched (NE) and enriched (E) birds during free-space tests on days 8, 21, and 35. LSmeans with the same letters within each behaviour and age are not different (P≥0.05).
Figure 3.4 Mean occurrence (±SE) of all play behaviour (sum of running, wing-assisted running, wing flapping, and sparring) for non-enriched (NE) and enriched (E) birds during spontaneous play observations on days 23 and 30. LSmeans with the same letters within each behaviour are not different (P≥0.05).
4 Chapter 4
Use of the Actical® Activity Monitor for Automated Recording of Broiler Activity Levels

4.1 Abstract
Remote sensing equipment can be useful for automatically quantifying activity. The objectives of this study were to validate a commercially available Actical® accelerometer device for quantifying general activity levels in fast-growing broilers by 1) matching and comparing the Actical® counts with corresponding behavioural data, and 2) by analyzing the Actical® counts for different times of day and different ages of birds. Additionally, activity counts were compared between birds in treatment groups (Enriched/E vs. Non-enriched/NE) and body weights (BW) (Heavy/H vs. Light/L). Actical® data were compared and matched with behaviour observation data to determine which acceleration values (Actical® counts) (counts per second) corresponded with specific behaviour patterns. The Generalized linear mixed models were used to test if the Actical® counts differed for different behaviours, time of day, age, treatment and BW groups. The Actical® counts generally corresponded with level of activity of specific behaviour patterns, with running, wing-assisted running and foraging generating the highest counts and sitting and side-lying generating the lowest (P<0.0001). The Actical® counts did not differ between NE and E birds. H and L birds also had similar counts in E pens; however, H birds had significantly higher counts than L birds in NE pens. We suspect that loose straps and some “inactive” behaviour such as pecking at straps and body shaking, as well as the gait may have registered high counts in some birds. Overall, Actical® was able to generally reflect the intensity of specific behaviour patterns and is likely a reliable way to monitor the behaviour and activity levels in broilers.
4.2 Introduction

Different kinds of automatic recording devices have been used to detect location, behaviour changes and health conditions in a variety of animals. For example, automatic data loggers have been used in sheep to differentiate between grazing, ruminating, and resting behaviour (Giovanetti et al., 2017) as well as predict lameness (Barwick et al., 2018), to determine the cessation of activity in broilers (Dawson et al., 2007), to detect jumping and landing force in laying hens (Banerjee et al., 2014) and walking activity of turkeys (Stevenson et al., 2019). Researchers are interested in using automatic recording devices because they can potentially reduce the need for conducting labour-intensive behavioural observations.

Reduced activity is common in broiler chickens which may be due to the interaction between their rapid growth rate and heavy body weight (Weeks et al., 2000; Tickle et al., 2018). Leg disorders and cardiovascular issues are among the most common welfare concerns in broiler chickens (Bessei, 2006; Meluzzi & Sirri, 2009), which may contribute to reduced activity, as animals suffering from pain and discomfort are less likely to stay active (Weary et al., 2009). Visual inspection either through live observation or video recordings is a common method used to measure activity and behaviour in research studies. However, it can be labour-intensive. Moreover, rapid visual inspection of a flock may not able to detect an issue until the animals are already ill (Weary et al., 2009). Therefore, automated measures of broiler activity levels may be helpful for monitoring animal health and behaviour, as well as providing early detection of welfare concerns such as lameness.

The Actical® (Philips Respironics, Bend, Oregon, USA) is a commercially available non-invasive and omni-directional accelerometer that detects a subject’s movement in all
directions. As a way to replace more labour-intensive behaviour observation and quantify activity levels, the Actical® has been validated in non-human primates (Mann et al., 2005), dogs (Hansen et al., 2006), cats (Andrews et al., 2015), and laying hens (Casey-Trott & Widowski, 2018) to accurately monitor activity change in response to illness or veterinary treatments. However, Actical® has not been validated in broilers and it remains unclear if the device is able to accurately record broilers’ activity levels.

The objective of the study reported in this chapter was to determine if Actical® was able to accurately measure activity levels in broiler chickens. The validation procedure was conducted in two ways: 1) matching and comparing the Actical® counts with corresponding behavioural data, and 2) by analyzing the Actical® counts for different times of a day and different ages of birds. Broilers are more active during the day than night (Deep et al., 2012), and more active at a younger age (Bokkers & Koene, 2003); therefore, if Actical® can accurately register counts in accordance with the intensity of an activity, the counts are expected to be higher during the day and at earlier ages. Additionally, activity counts were compared between different birds in treatment groups (E vs NE) and body weights (BW) (H vs L). We hypothesized that E and L birds would register higher Actical® counts as a result of increased mobility.

4.3 Methods

All procedures were approved by the Animal Care Committee at the University of Guelph. (AUP#3746).
4.3.1  Actical® Validation

The dimension of the device is 28 x 27 x 10mm, and it weighs 22g. In order to securely hold the device while minimizing its disturbance to the bird, two light-weight beige coloured elastic cotton straps were used to wrap the device under a bird’s wings so that it was worn like a backpack (Figure 4.1). The straps were checked at least every other day to ensure that two fingers would fit comfortably between a bird’s wings and the straps to minimize discomfort.

One heavy male and one light male per pen were fitted with the Actical® on day 22. The birds were given at least 24 hours to adjust to wearing the device on their backs before starting data collection. On day 23, one digital video camera (Sony Digital High Definition Video Camera; HDR-CX405 or DCR-SR68 models; Sony, Japan) was placed on a monopod (Digiant MP-3606 Professional Video Monopod 70”, Zhejiang, China) in front of each pen for video recording. All recordings were made between 1100h to 1500h. For validation purposes, 6 out of the 12 pens were used, resulting in a sample from 12 birds (6 L and 6 H birds). All selected birds wore the Actical® devices from day 22 to day 42. After the trial was completed, the Actical® backpacks were removed, and all data were uploaded to the Actical® software for summarization and analysis.

4.3.2  Actical® Validation Data Collection

In order to achieve maximum sensitivity, all Actical® devices were programmed based on subject size and weight. As determined by a study on laying hens (Casey-Trott & Widowski, 2018), the following settings were made: 1 sec epochs, height 10.0cm, weight 0.5kg, gender female, and age 2 (as per software reference values for humans).
After video recordings were made, two trained observers followed a detailed ethogram (Table 4.1) to record behaviour using the Observer XT software (Version 12.0, Noldus Information Technology, Wageningen, NL). Beginning at minute 10 and ending at minute 250, twelve 10-minute periods were continuously observed at 20-min intervals, generating 2 hours of behavioural data from each bird for day 23. The reliability of observers was tested using Observer software, and the Kappa coefficients were 0.95 and 0.96. Observers were also balanced for the videos they observed (NE vs E)

Actical® data were uploaded, compared, and matched with Observer data to determine which acceleration values (Actical® counts) (counts per second) corresponded with specific behaviour patterns. The Actical® data collected during a behaviour transition (e.g. from sitting to standing, from standing to walking, etc.) and when a bird was performing both state behaviour and point behaviour at the same time were excluded in order to accurately reflect the Actical® counts of each state behaviour. The unit of comparison between different behaviour patterns was counts per second.

Prior to matching the recorded Observer and Actical® data, a data pre-processing multi-step approach was performed. First, the date fields were made uniform across all the files by adopting a “dd-mm-yyyy” format in the Actical® data files. The same format was already present in the Observer files. Second, the “point” behaviour lines and “stop” states from the Actical® data files were filtered out. Third, all spaces were replaced with underscores (“_”) in the header labels and the behaviour state labels to ensure an error-free matching process between columns with corresponding information. Finally, the first letter for all behaviour state labels was capitalized to ensure uniformity across the data. The two data files were then combined by
matching the 2nd column values from the recorded Observer files with the 4th column values from the Actical® files. Both columns contained time values in the generic format “hh:mm:ss”.

Finally, the total number of seconds were calculated for each behaviour state corresponding to the information present in the Actical® data files (Figure 4.2). All data processing was performed using bash scripts and Perl version 5.26.1 on a Linux Mint HP Z840 system. The average Actical® counts per second were then calculated for each occurrence of a behaviour pattern per second.

Actical® counts (average count per 15s) from all birds wearing Actical® (N=24: 12 H, 12 L; 12 NE, 12 E) during the daytime and nighttime on days 25, 32, 36, and 38 were analyzed and compared to results from Chapter 3. Lights were turned on at 0615h and turned off at 2215h. Daytime period was 16-h long and nighttime period was 8-h long (Figure 4.3).

4.3.3 Statistical Analyses

All statistical analyses were computed in SAS 9.4 (SAS Inst. Inc., Cary, NC, USA). Generalized linear mixed models were used on all data. To account for different pen locations in the room, block was included as a random effect.

To test if BW group, enrichment, and their interactions had an effect on the birds used to compare the Actical® counts of various behaviour patterns, BW, treatment, and their interactions were included as fixed effects. One value (the average counts/second for all observations of that behaviour for the individual bird) was calculated, and each individual bird was included in the model to test if the Actical® counts of various behaviour patterns differed from one another. BW, treatment, age, time of day (day or night), and their interactions were included as fixed
effects for Actical® counts to statistically compare different times of day, age, and BW. Since
repeated measurements were made on each experimental unit at different ages for Actical®
counts comparison, compound symmetry (CS), which is a covariance structure that includes
within-subject correlated errors, was included in the model. Data were assessed for normality
using Shapiro-Wilk analyses. The average counts per second of each behaviour, and the average
Actical® counts per 15 seconds of all Actical® wearing birds were log-transformed to normalize
the data, and the log-transformed LSmeans and standard errors were back transformed and then
reported in results as well. Statistical significance was set to P<0.05.

4.4 Results

4.4.1 Comparison of Activity Counts for Specific Behaviour Patterns

There was an effect of behaviour on Actical® count (P<.0001) (Figure 4.4). There was
also large variation between individual birds in the number of counts per second for the same
behaviour patterns. For example, the average count per second ranged from 4.37 to 26.99 for
running (Table 4.2).

4.4.2 Comparison of Activity Counts for Age, Time of Day, Body Weight, and Treatment

There was no enrichment effect (P=0.856) on the number of Actical® counts per 15 s in
all Actical® wearing birds on days 25, 32, 36, 39. However, an age effect (P=0.009), time of day
effect (P<0.0001), BW effect (P<0.0001), treatment × BW effect (P<0.0001), and age × BW
effect (P=0.030) were found. As expected, the Actical® counts decreased as the birds got older,
and the counts were higher during the day, and lower at night, regardless of age or BW (Figure
4.5). However, even though there was no significant BW difference between H and L birds in E
pens (P=0.348), H had significantly higher counts than L in NE pens (P<0.0001; Figure 4.6). The
Actical® counts per 15 s did not differ between L and H birds on day 25; however, the difference increased with age and H birds had significantly higher counts on days 36 and 39. The Actical® counts per 15 s for L birds decreased with age; however, such decline was not found in H birds (Figure 4.7).

4.5 Discussion

In general, Actical® data reflected differences between specific behaviour patterns that were assumed to vary according to amount of movement (acceleration in the x,y, and z directions combined) associated with each behaviour. For example, low-energy activities such as sitting and sidelying generated the lowest Actical® counts, and high-energy activities such as running, wing-assisted running, and walking registered the highest counts. Our results mostly match the observations of slow-, medium-, and high-energy activities in laying hens reported by Kozak et al. (2016) using custom built accelerometers to measure 3-dimensional accelerations associated with particular behaviour patterns. For example, Kozak et al. (2016) categorized resting, sleeping, small postural head/shoulder/neck movements as low-intensity activities, foraging as moderate-intensity activity, and walking, running, and wing-flapping as high-intensity activities. The counts of dustbathing in our study were not as high because both resting phase and active wing shaking phase were included in our behavioural observation, whereas Kozak et al. (2016) reported the accelerations for resting phase and wing shaking phase separately.

No enrichment effect was found regardless of birds’ age (N=12 per body weight by treatment interaction) which corresponds to the findings from Chapter 2. The activity counts also decreased over time which agrees with the results in Chapter 2, where birds performed more low-energy activities and less high-energy activities as they got older. However, the Actical®
counts of L and H birds contradict our findings from Chapter 2. In Chapter 2, L birds were more active and had better mobility than H birds, as indicated by both latency-to-lie and obstacle tests. However, the Actical® counts of H birds were higher than those of L birds in NE pens, and no BW effect was found in E pens. It is possible that the H birds in NE pens were less active (sitting, sidelying) which led to other birds pecking at the devices. NE birds may have pecked at the straps more than the E birds due to boredom (explained in Chapter 3), resulting in higher counts in H birds in NE pens but not E pens. However, such pecking difference was not observed in video recordings or at other times. Future studies of similar nature should consider putting on a mock backpack on all other birds to minimize this effect. There was also a considerable amount of variation between individual birds. There are several possibilities for this finding. For example, wearing the backpack may have affected some broilers’ behaviour more than the others. Stadig et al. (2018) found that slow-growing broilers wearing loggers walked less and pecked at the data loggers more often than control birds for the first week after being fitted with a backpack. However, the effect disappeared after one week. Although wearing Actical® had no effect on the birds’ performance in LTL test and obstacle test in Chapter 2, it is possible that wearing the Acticals® affected other behaviour in the home pen. Buijs et al. (2018) only found a mild effect after equipping laying hens with backpacks and the effect became almost absent after 2 days. Moreover, the weight of an Actical® equaled 1.5% of birds’ BW on day 25 which was below the maximum of 3% for birds recommended by Phillips et al. (2003).

Moreover, certain “inactive” behaviour such as pecking at straps used to attach Actical® and body shaking may have contributed to high Actical® counts. Similar findings were reported by Lascelles et al. (2008). They found grooming and scratching in cats led to high activity counts.
even though the cat was generally inactive. Since we took out the behavioural transition counts and point behaviour counts when conducting behaviour-specific Actical® comparison, such high counts generated by “inactive” behaviour were minimized for the first part of the validation. However, it is possible that these behavioural transitions and point behaviours affected the continuous count comparisons.

The looseness of the Actical® straps may have also had an effect. Actical® attached to loose straps may register higher counts in the event of “inactive” behaviour such as pecking at straps and body shaking, as well as general activity such as walking and eating. Similar findings were reported in dogs with loose collars (Preston et al., 2012). Banerjee et al. (2014) also suggested that the accelerometer results from hens jumping off perches were influenced by the movement of the accelerometer relative to the hen’s body. The gait of the H and L birds may also have affected the activity counts. We did not measure gait scores directly; however, LTL and obstacle tests are correlated with gait scores, and the results suggest that the gait of H and L birds could have been different. Some behaviour counts in this study had a high SE which could be a result of the fact that only a limited number of birds performed a particular behaviour during the observation. For example, sitting, preening while sitting, drinking, feeding while standing, standing, crawling, and walking were observed for all 12 focal birds whereas running was only observed in 3 birds. Olsen et al. (2016) also found large Actical® inter-device variability. They suggested that the age of the device may have contributed to variable data and re-calibration of the device may be required. However, we did not check this in our study.

It may be more accurate to use Actical® to measure epochs registered as inactivity (0 counts) rather than using activity counts. Hansen et al. (2006) found that even though the
proportional increase in Actical® counts differed by dog with movement, all measurements were close to 0 when the animals were resting. Following this logic, a high Actical® count does not necessarily indicate a high-intensity active behaviour; however, a zero count is most likely an accurate reflection of inactivity. Casey-Trott and Widowski (2018) found a strong correlation between stationary inactivity through live observation and zero Actical® counts and used this measure to compare the behaviour of laying hens with and without keel fractures.

The use of automated accelerometers was also proposed to reduce the need of conducting labour-intensive visual inspection such as live observation. However, the data collected by Actical® cannot be used in real-time; data were not able to be analyzed until the device was removed from the focal birds, which could be time-consuming. In the context of effective broiler management, it may be more practical to allow accelerometers to transmit data wirelessly at regular intervals.

Overall, Actical® was able to generally reflect the intensity of specific behaviour patterns and the results are mostly in accordance with the findings from Chapter 2. Confirming our hypothesis, higher counts were registered during the day and at a younger age when broilers were more active. Actical® is likely to be a useful device to measure the activity levels and behaviour change in broilers. However, the tightness of the Actical® straps should be adjusted in a way to maximize the accurate registration of activity counts while assuring the comfort of the bird. In addition, miniaturization of the device might increase registration accuracy by allowing multiple devices to be attached to different body parts of a bird. Moreover, given the variation between individual birds, future research needs to find out if it may be more practical to compare the inactivity of broilers based on zero counts.
**Table 4.1 Actical® validation ethogram**

<table>
<thead>
<tr>
<th>Behaviour (S-state; P-point)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting (S)</td>
<td>Immobile, entire breast touching the ground</td>
</tr>
<tr>
<td>Sidelying (S)</td>
<td>Similar to sitting but is lying on their side. Usually leg is stretched</td>
</tr>
<tr>
<td>Standing (S)</td>
<td>Immobile on two legs, body not touching the ground</td>
</tr>
<tr>
<td>Walking (S)</td>
<td>Slow forward movement, breasts above the ground, using legs</td>
</tr>
<tr>
<td>Crawling (S)</td>
<td>Slow forward movement, breasts touching the ground, using legs</td>
</tr>
<tr>
<td>Running (S)</td>
<td>Faster forward movement, using legs; no wings involved</td>
</tr>
<tr>
<td>Wing assisted running (S)</td>
<td>Faster forward movement, using legs and wings</td>
</tr>
<tr>
<td>Feed standing (S)</td>
<td>Downward pecking in feeder while standing</td>
</tr>
<tr>
<td>Feed sitting (S)</td>
<td>Downward pecking in feeder while sitting</td>
</tr>
<tr>
<td>Drink standing (S)</td>
<td>Pecking at nipple drinkers while standing</td>
</tr>
<tr>
<td>Drink sitting (S)</td>
<td>Pecking at nipple drinkers while sitting</td>
</tr>
<tr>
<td>Preen standing (S)</td>
<td>Moving the beak through the feathers while standing</td>
</tr>
<tr>
<td>Preen sidelying (S)</td>
<td>Moving the beak through the feathers while sidelying</td>
</tr>
<tr>
<td>Preen sitting (S)</td>
<td>Moving the beak through the feathers while sitting</td>
</tr>
<tr>
<td>Foraging (S)</td>
<td>Ground scratching using both legs accompanied by pecking on the ground,</td>
</tr>
<tr>
<td>Play fight (S)</td>
<td>Two birds, hopping and chest bumping; while facing on another. No forceful pecking.</td>
</tr>
<tr>
<td>Dustbathing (S)</td>
<td>Vertical wing shakes in a lying position.</td>
</tr>
<tr>
<td>Wing flapping (P)</td>
<td>Wings flap while standing</td>
</tr>
<tr>
<td>Jump (P)</td>
<td>Push off a surface and into the air by using the muscles in legs</td>
</tr>
<tr>
<td>Wing assisted jumping (P)</td>
<td>Push off a surface and into the air by using the muscles in legs while using wings</td>
</tr>
<tr>
<td>Peck at stone (P)</td>
<td>Peck at any part of the peck stone</td>
</tr>
<tr>
<td>Peck at suet feeder (P)</td>
<td>Peck at any part of the suet feeder</td>
</tr>
<tr>
<td>Peck at backpack (P)</td>
<td>Peck at any part of the Actical® device or bra straps</td>
</tr>
<tr>
<td>Peck at scale (P)</td>
<td>Peck at the floor of the scale</td>
</tr>
<tr>
<td>Peck at platform/ramp (P)</td>
<td>Peck at the floor of the platform or the ramp</td>
</tr>
<tr>
<td>Feather pecking (P)</td>
<td>The focal bird is pecking another bird’s feathers</td>
</tr>
<tr>
<td>Peck at litter (P)</td>
<td>No ground scratching involved</td>
</tr>
<tr>
<td>Feather ruffle (P)</td>
<td>Feather erection and body shaking</td>
</tr>
<tr>
<td>Leg stretching (P)</td>
<td>Stretching one of the legs while standing</td>
</tr>
<tr>
<td>Head scratching (P)</td>
<td>Scratch its head using one foot</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>On platform</td>
<td>≥ half body on the horizontal part</td>
</tr>
<tr>
<td>On ramp</td>
<td>&gt;half body on the slope</td>
</tr>
<tr>
<td>Under platform/ramp</td>
<td>&gt;half body under platform or ramp</td>
</tr>
<tr>
<td>On scale</td>
<td>Both feet on the scale</td>
</tr>
<tr>
<td>On stone</td>
<td>Both feet on the stone</td>
</tr>
<tr>
<td>On litter</td>
<td>Anywhere but the locations listed above</td>
</tr>
<tr>
<td>On drinker line</td>
<td>Perching on drinker line</td>
</tr>
<tr>
<td>Out of view</td>
<td>Can’t be seen in the video</td>
</tr>
</tbody>
</table>
Table 4.2 The Actical® counts of each state behaviour ranked from the lowest to the highest mean. The number of birds observed performing each behaviour (N), the range and median of the counts are also reported. LSmeans with the same letters are not different (P≥0.05).

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>N</th>
<th>Range</th>
<th>Median</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting</td>
<td>12</td>
<td>0.18-2.23</td>
<td>0.46</td>
<td>0.42</td>
<td>0.105</td>
</tr>
<tr>
<td>Preen sitting</td>
<td>12</td>
<td>0.04-1.89</td>
<td>0.47</td>
<td>0.48</td>
<td>0.177</td>
</tr>
<tr>
<td>Sidelying</td>
<td>8</td>
<td>0-1.33</td>
<td>0.43</td>
<td>0.49</td>
<td>0.113</td>
</tr>
<tr>
<td>Drink standing</td>
<td>12</td>
<td>0-6.58</td>
<td>0.81</td>
<td>0.74</td>
<td>0.163</td>
</tr>
<tr>
<td>Feed standing</td>
<td>12</td>
<td>0.13-2.48</td>
<td>0.85</td>
<td>0.89</td>
<td>0.178</td>
</tr>
<tr>
<td>Preen standing</td>
<td>11</td>
<td>0-3.45</td>
<td>0.70</td>
<td>0.94</td>
<td>0.269</td>
</tr>
<tr>
<td>Feed sitting</td>
<td>4</td>
<td>0.47-3.60</td>
<td>2.46</td>
<td>1.39</td>
<td>0.702</td>
</tr>
<tr>
<td>Standing</td>
<td>12</td>
<td>0.39-5.28</td>
<td>2.19</td>
<td>1.75</td>
<td>0.419</td>
</tr>
<tr>
<td>Dustbathing</td>
<td>6</td>
<td>0-9.40</td>
<td>3.08</td>
<td>2.64</td>
<td>0.831</td>
</tr>
<tr>
<td>Crawling</td>
<td>12</td>
<td>0-19.12</td>
<td>3.44</td>
<td>4.01</td>
<td>1.859</td>
</tr>
<tr>
<td>Walk</td>
<td>12</td>
<td>1.36-59.76</td>
<td>5.33</td>
<td>5.75</td>
<td>1.949</td>
</tr>
<tr>
<td>Foraging</td>
<td>4</td>
<td>0-22.80</td>
<td>8.83</td>
<td>7.89</td>
<td>3.195</td>
</tr>
<tr>
<td>Wing-assisted run</td>
<td>5</td>
<td>1.95-62.54</td>
<td>15.86</td>
<td>8.56</td>
<td>5.786</td>
</tr>
</tbody>
</table>
Figure 4.1 A focal bird wearing the Actical® accelerometer
<table>
<thead>
<tr>
<th>Start_time Behaviour</th>
<th>Behaviour pattern</th>
<th>Start_time Actical</th>
<th>Duration behaviour</th>
<th>Sum Actical counts for duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>#2</td>
<td>11:31:26 Walking</td>
<td>11:31:25</td>
<td>7.12392</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>11:31:59 Standing</td>
<td>11:31:59</td>
<td>3.50138</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>11:32:03 Drink standin</td>
<td>11:32:03</td>
<td>16.1089</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>11:32:19 Standing</td>
<td>11:32:19</td>
<td>3.642</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>11:32:22 Drink standin</td>
<td>11:32:22</td>
<td>10.886</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>11:32:33 Walking</td>
<td>11:32:33</td>
<td>2.04112</td>
<td>11</td>
</tr>
<tr>
<td>9</td>
<td>11:32:35 Standing</td>
<td>11:32:35</td>
<td>8.84486</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>11:32:44 Walking</td>
<td>11:32:44</td>
<td>2.7215</td>
<td>30</td>
</tr>
</tbody>
</table>

**Figure 4.2** An example of behaviour-specific Actical® counts comparison by matching the behaviour data with Actical® counts. The duration of behaviour is in seconds.
Figure 4.3 Actogram showing the Actical® counts difference between day and night. The x and y axes are time of day and Actical® counts, respectively. Lights were turned off at 2215h and turned on at 0615h. Nighttime period was demonstrated between the two red lines.
Figure 4.4 The mean Actical® counts (±SE) of each state behaviour ranked from the lowest to the highest. LSmeans with the same letters are not different (P≥0.05).
Figure 4.5 Mean Actical® counts per 15 s (±SE) for birds of different ages (days 25, 32, 36, and 39), and different time of day (day, night). LSmeans with the same letters within each group are not different (P≥0.05). N=24
Figure 4.6 Mean Actical® counts per 15s (±SE) for light (L) and heavy (H) birds from enriched (E) and non-enriched (NE) pens, respectively (N=6 per BW by treatment combination). “x” shows the interaction between enrichment treatments and BW groups. LSmeans with the same letters within each group are not significantly different (P≥0.05).
Figure 4.7 Mean Actical® counts per 15s (±SE) for light (L) and heavy (H) birds on days 25, 32, 36, and 39 (N=12). LSmeans with the same letters within each age are not significantly different (P≥0.05).
Chapter 5
General Discussion

It is suggested that the leg health of broilers may be improved by increasing broilers’ activity level (Reiter & Bessei, 2009). However, the effects of environmental enrichment among different studies are inconsistent (reviewed by Pedersen & Forkman, 2019), and some potentially successful enrichments such as moving platforms had not previously been studied. Moreover, even though some studies have been conducted to compare the physical measurements and behaviour between fast- and slow-growing broilers, there is only limited research on the effects of heavy vs. light body weight on fast-growing broilers of the same age. The research presented here provides an in-depth analysis of the effects of a combination of environmental enrichment (elevated platforms with ramps, swinging scales, oral pecking enrichment) and body weight (heavy vs. light) on broilers’ leg health, time budgets, play behaviour, and activity level.

The provision of environmental enrichment had no effect on the mobility of broilers based on the standing time during the latency-to-lie test (LTL) and the number of crossings during the obstacle test. My results support six out of the nine studies that found no enrichment effect on the leg health of broilers (reviewed by Pedersen & Forkman, 2019). Time spent in low- and high-energy activity also did not differ between birds reared in enriched pens (E) and those reared in non-enriched pens (NE). That being said, E birds spent more time on the obstacle before stepping off during the obstacle test, possibly due to them being more used to moving in 3-dimensional space.

One possible explanation for the lack of enrichment effect is that broilers use platforms and swing scales mostly as resting places. As a result, the provision of described enrichment
does not increase locomotion and activity level as would training broilers to use a treadmill (Reiter & Bessie, 2009). The small pen sizes could also have been a limiting factor. A study conducted using dairy cows showed that cows reared in larger pens moved longer distance and demonstrated more movement than those kept in smaller pens even though the stocking densities were the same (Telezhenko et al., 2012). Broiler studies where an increase in active behaviour was found in E pens were also conducted in larger pens in commercial settings (Ventura et al., 2012; Vasdal et al., 2018).

Although not many treatment effects were found in the physical measurements (Chapter 2), NE birds performed significantly more play behaviour (Chapter 3) such as worm exchange, worm running, and worm chasing during worm-running tests, and more all play behaviour (sum of running, wing-assisted running, wing flapping, and sparring), running, and wing-assisted running during free-space tests. There are at least two possible explanations for NE birds being more engaged than E birds in worm-running tests at all ages and in free-space tests at age 11 and 25. First of all, the larger contrast between the NE environment before and after giving the test stimuli (i.e. opening up extra space and offering “worms”) compared to that in the E pens may have led the NE birds to be more easily stimulated to play during the tests. Moreover, NE birds could have been bored and thus more responsive during the tests. Boredom and its effect on captive animals is reported in literature, and it is suggested that most animals living in unstimulating barren environments suffer from boredom and are more easily aroused when stimuli are offered (Meagher & Mason, 2012; Burn, 2017). My results could also be suggesting this. However, using play to assess welfare has limitations, because even though play is usually considered as an indication of a lack of negative affective states, counter examples where more
play is performed in negative conditions exist (Ahloy-Dallaire et al., 2018). Therefore, the results should be interpreted in conjunction with other measurements reported in Chapter 2 and 4 before drawing any conclusions on welfare.

On the other hand, body weight (BW) had significant effects on broilers’ leg strength and time budgets. Light birds (L) had better mobility based on the results that they stood for significantly longer during the LTL test, and performed more crossings during the obstacle test. L birds also spent more time on the obstacle before stepping off, possibly due to them being more able to balance on it. L birds also performed significantly more high-energy activities than H birds. H birds performed numerically more low-energy activities than L birds.

Similar results for leg strength were also found by Kristensen et al. (2006) who found that higher body weight increased the probability of poor gait score. The occurrence of footpad lesions was very low in this study most likely due to low litter moisture. The maximum litter moisture in this study was 23%; however, ideally, depending on specific housing conditions, litter moisture is optimal between 25 to 35% (Carey et al., 2004). Lower litter moisture can lead to poorer air quality (dust) but this was not observed in my trial.

In general, the Actical® data reflected differences between specific behaviour patterns according to the energy level, or amount of movement associated with each behaviour. My results are similar to the observations of slow-, medium-, and high-energy activities in laying hens reported by Kozak et al. (2016). Regarding Actical® activity counts, I found no treatment effect (E or NE), and the activity counts also decreased over time which agree with the results in Chapter 2, where birds performed more low-energy activities and less high-energy activities as
they got older. However, the Actical® counts of L and H birds contradict the findings from Chapter 2. It is possible that the tightness of the straps used to secure the device had an effect on the registered counts. Actical® attached to loose straps may register higher counts in the event of “inactive” behaviour such as pecking at straps, litter and body shaking. The device may also register higher than usual counts when birds perform general activities such as walking and eating due to the movement of the device relative to a bird’s body. Similar findings were reported in dogs with loose collars (Preston et al., 2012). The gait of the H and L birds may also have affected the activity counts. We did not measure gait scores directly; however, LTL and obstacle tests are correlated with gait scores (Caplen et al., 2014), and the results suggest that the gait of H and L birds could have been different. The inter-device variability may have also contributed to the large variation between individual birds (Olsen et al., 2016). Future research needs to find out if it may be more practical to compare the inactivity of broilers based on zero counts. The use of alternative automated methods of assessing behaviour such as machine vision and GPS might also be more effective than attaching accelerometers, and allow for data to be examined in real time. In the context of effective broiler management, it may also be more practical to allow accelerometers to transmit data wirelessly at regular intervals.

Overall, the provision of described environmental enrichments had little effect on broilers’ leg strength and activity levels. However, it is likely that the enrichments alleviated some of the negative outcomes of living in a barren environment, such as boredom, and thus had positive effects on the affective states of the broilers. Further research should look into other enrichment types that can motivate broilers to stay active instead of resting on a provided enrichment. Managing broilers’ BW also seems to be a more effective way to improve their leg
strength and welfare. Overall, Actical® was able to reflect the intensity of specific behaviour patterns and the results are in accordance with the findings from Chapter 2. Actical® is likely to be a useful device to measure the activity levels and behaviour change in broilers. However, more studies need to be done to figure out if there is inter-device variability, how to control it, and how to more accurately compare activity levels by using Actical®.

The experiments presented in this thesis provided insights into the effects of different environmental enrichment and body weight on the health and behaviour of fast-growing broilers. This is also the first study to validate the use of Actical® to measure activity levels in fast-growing broilers which could be useful for future research. I would conduct the experiments in larger pens if I were to continue in this research area due to the reason discussed earlier. I would also research other environmental enrichments to encourage broilers to stay active instead of resting on resources.
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