The Chicken and the Tag

Automated individual-level activity tracking and the relationships between activity, body weight and leg health in broilers

Malou van der Sluis

Propositions

1. Group-level data are insufficient to assess broiler welfare.
   (this thesis)

2. The subjectivity of the current gold standard for broiler gait scoring is the main limiting factor in automation of gait scoring.
   (this thesis)

3. Presenting work to an audience outside of one's area of expertise should be stimulated to realize societal impact.

4. The reference situation in animal experiments should be defined according to the best available animal welfare standards instead of the most common welfare standards.

5. Personal experience is essential for people to take issues seriously and to take action.

6. Hybrid working improves human well-being.

Propositions belonging to the thesis, entitled

The Chicken and the Tag – Automated individual-level activity tracking and the relationships between activity, body weight and leg health in broilers

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Malou van der Sluis

Thesis committee

Promotor
Prof. Dr T.B. Rodenburg
Professor of Animal Welfare
Utrecht University and Wageningen University & Research

Co-promotors
Dr E.D. Ellen
Researcher Animal Breeding and Genomics
Wageningen University & Research

Dr Y. de Haas
Researcher Animal Breeding and Genomics
Wageningen University & Research

Other members
Prof. Dr F. van Langevelde, Wageningen University & Research
Prof. Dr J.M. Siegford, Michigan State University, United States of America
Prof. Dr N.E. O'Connell, Queen's University Belfast, United Kingdom
Dr I.C. de Jong, Wageningen University & Research

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Wageningen Institute of Animal Sciences (WIAS).

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Thesis

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Malou van der Sluis
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Abstract

Animal agriculture, including broiler farming, has intensified to meet the increasing demand for livestock products. The resulting large numbers of animals per farm make keeping track of individual broiler activity challenging. However, individual activity records are of great relevance for assessing broiler welfare and for implementation of activity in broiler breeding programs. In this thesis, it was studied whether, and how, detailed activity data on individual group-housed broilers could be collected in an automated manner throughout life. To this end, both an ultra-wideband (**UWB**) and a passive radio frequency identification (**RFID**) tracking system were implemented to collect data on distances moved by individual broilers. The recorded distances were compared to distances recorded on video. Both systems showed moderate to good agreement with video and with each other. However, RFID worked with smaller, more lightweight tags and could therefore be implemented earlier in life than UWB. Using the collected activity data, the relationships between activity, leg health and body weight were studied, with the ultimate goal of examining whether selecting on activity to improve health and welfare of broilers would be feasible. Using UWB data, general indications for relationships between gait classification and activity were observed, with lower activity levels for birds with a suboptimal gait, but an interaction with body weight was also observed. It remained difficult to distinguish gait classifications based solely on distance moved. Using RFID data, the relationship between activity patterns early in life and average daily gain (**ADG**) was further looked into. A negative correlation between ADG and the root mean square error of activity was observed, indicating that broilers with more deviations, in both directions, from the expected linear trend in activity had a lower ADG. RFID data were also used to estimate the heritability of activity. An estimated heritability of 0.31 was observed across the full production period. Overall, the results of this thesis improve our understanding of the relationships between activity, leg health and body weight in broilers and could in the future potentially help to improve broiler (leg) health and welfare, through selection on activity. Potential directions for future implementation of activity tracking in larger scale broiler systems include less detailed RFID tracking and a sensor-fusion approach of RFID and computer vision.

Contents

Chapter 1:
General introduction

1.1 Introduction

Animal agriculture, or the livestock sector, has a pivotal role in providing food. One-third of the protein intake of the worldwide human population is provided by livestock products, and there is a rapidly increasing demand for these products, among other things due to growing populations and incomes (Steinfeld et al., 2006). The global production of meat and milk is predicted to reach a level of 465 and 1,043 million tonnes, respectively, in 2050. At the same time, the livestock sector significantly contributes to environmental issues and climate change, through land degradation, water use, greenhouse gas emissions, and more (Steinfeld et al., 2006). One approach to the balancing act between increasing production and reducing the environmental impact, is intensification of animal agriculture, defined as increasing the amount of output generated per unit of input into the agricultural system (Place, 2018). Livestock production intensification started in Europe and North America, but now takes place throughout the world (Leenstra, 2013). Indeed, intensification in animal agriculture has resulted in higher outputs per unit of input (Place, 2018) and hereby in reduced environmental impacts per unit of production for dairy products (Capper et al., 2009), beef (Capper, 2011), eggs (Pelletier et al., 2014), and chicken meat (Renema et al., 2007). The reduced environmental impacts per unit output have been realized through, among other things, improvements in genetics, reproductive performance, and nutrition (Place, 2018). At the same time, the average size of production units has increased and the number of livestock producers has decreased in many parts of the world (Steinfeld et al., 2006). Nowadays, livestock are often kept in large numbers per farm. When looking at the overall average number of animals and dedicated farms in the Netherlands over the last years, it can be calculated that cattle farms house on average approximately 150 animals per farm, pig farms house on average approximately 3,000 animals per farm, laying hen farms house on average 52,000 animals per farm, and broiler farms, farming chickens kept for meat production, on average house approximately 76,000 animals per farm (Centraal Bureau voor de Statistiek, 2020). With these large numbers of animals per farm, keeping track of individual animals is challenging. However, individual records are of great relevance for assessing for example animal welfare (**Box 1.1**) and for implementation of traits in breeding programmes.

Box 1.1. Animal welfare

The welfare of an animal is defined by the individual's perception of its own physical and emotional state (Webster, 2013). Assessing an animal's welfare therefore requires an understanding of animal emotions and motivations (Webster, 2016). An animal's physical and emotional state can be affected by many factors and there are different approaches for assessing animal welfare. Here, some main approaches are discussed, to provide a general impression of how animal welfare can be assessed.

A well-known approach is the 'Five Freedoms' concept. Here, it is stated that the welfare of an animal should be considered in relation to five freedoms (FAWC, 1993):

1) Freedom from thirst, hunger, and malnutrition;
2) Freedom from discomfort;
3) Freedom from pain, injury or disease;
4) Freedom to express normal behaviour;
5) Freedom from fear and distress.

However, the focus on avoiding negative experiences in four of the five freedoms is often regarded as too limited, as this does not include positive welfare (e.g., Yeates and Main (2008)). A concept that includes both positive and negative experiences, is 'Quality of Life' (**QoL**). The FAWC (2009) distinguishes three categories for QoL: 1) a good life, 2) a life worth living, and 3) a life not worth living. For this approach, an animal's quality of life can be judged based on regular welfare assessments for the animal (FAWC, 2009). However, concerns have been raised regarding defining QoL simply as a sum of positive and negative experiences, as it may be difficult to quantify in how far positive experiences can offset negative experiences (e.g., Webster (2016)). The 'Five Domains Model' is another well-known approach, that in its most recent form consists of the domains 1) nutrition, 2) physical environment, 3) health, 4) behavioural interactions, and 5) mental state (Mellor et al., 2020). These domains are similar to the Five Freedoms, but there is more emphasis on mental states and the relationship between physiological mechanisms and affective experiences. Moreover, it emphasizes that just minimising negative states is not sufficient for good welfare, also positive experiences are required (Mellor et al., 2020).

In general, however, it remains difficult to objectively assess positive experiences, or 'a life worth living', as interpretations of others' feelings are subjective (e.g., Webster (2016)). Overall, animal welfare remains a complex concept, and welfare can be difficult to measure in practice. However, ensuring good welfare is pivotal and positive experiences can have consequences not only for the quality of life of the animals, but also their health (reviewed in Boissy et al. (2007)).

This thesis focusses on the validation and implementation of sensor technologies to track activity of individual broilers. There are several reasons why this focus on broilers, and their activity levels, is warranted. First, it has been predicted that over the period from 2020 to 2029, poultry meat will - just like in the past decade although at a slower rate - be the primary driver of meat production growth (OECD/FAO, 2020). Furthermore, compared to beef and pork, chicken meat has a lower environmental impact in terms of land use, energy use and climate change (de Vries and de Boer, 2010). Moreover, given that broilers have a uniform appearance and are kept in large groups, visual monitoring of individual broilers is challenging. This highlights the importance of sensor technologies for monitoring of broilers. At the same time, however, broilers are a challenging starting point for implementation of sensor technologies, given their small body size. Their small size means that small, lightweight sensors are needed, to avoid affecting the birds' behaviour (discussed for laying hens by Siegford et al. (2016)). A trait that warrants attention in broiler monitoring is locomotor activity, as locomotor activity has been shown to be related to different health, welfare and production parameters. For example, ill animals often spend more time sleeping and show a reduced feed intake (Gregory, 1998) and consequently likely show lower activity levels. Furthermore, at group-level, lame birds have been observed to walk a smaller percentage of the time (Weeks et al., 2000). Moreover, research has indicated that larger daily distances are walked by birds that received load reduction through a suspension device (Rutten et al., 2002), indicating an effect of body weight on locomotor activity. This thesis aims to show that specific sensor technologies can be successfully implemented to track activity in broilers and can provide pivotal information for assessing broiler health, welfare and performance.

In the remainder of this introduction, a short overview is provided of the trend towards larger-scale production of poultry - with emphasis on broilers - and the consequent challenges that arise in large groups, the importance of data at the level of the individual, and the potential of technological innovations to monitor individual broilers housed in groups.

1.2 Towards larger-scale production and its consequent challenges

Over time, not only the animals themselves have changed as a result of domestication and artificial selection (**Box 1.2**), but also the numbers and housing systems of poultry underwent changes. In 1961, the number of chickens worldwide was approximately 3.9 billion (FAO, 2021b). Between 1961 and 2019, this number increased substantially and nowadays almost 26 billion chickens are kept worldwide, compared to approximately 1.5 billion cattle and

Box 1.2. From wild jungle fowl to modern-day broiler

The wild jungle fowl is the ancestor of the modern-day broiler, which has been domesticated for 4,000 years at the very least (Brambell, 1965). Several traits have changed over time under artificial selection, but the behavioural pattern of the modern-day broiler is thought to still be similar to that of the wild jungle fowl, meaning that, among other things, they still have their gregarious nature and a social order within the group (Brambell, 1965). Studies in laying hens have also indicated that several behaviours are shown to a lesser extent than in jungle fowl, but are still present. For example, layers were observed to be less active and less involved in social interactions (Schütz and Jensen, 2001) and were shown to have a reduced frequency of exploratory and foraging behaviours (Schütz et al., 2001). Other traits have changed significantly. For broilers, the perhaps most obvious changes include the growth rate and feed conversion ratio. Havenstein et al. (2003) compared two broiler strains, one from 1957 and one from 2001, that were fed diets representative of those times. The average body weight and feed conversion ratio of these birds differed strongly at different ages and on different diets. For the 1957 strain, the feed conversion (**FC**) at 42 days old was 2.34 on 1957 feed and 2.14 on 2001 feed, and the average body weight at this age was 539 grams on 1957 feed and 578 grams on 2001 feed. For the 2001 strain, the FC at 42 days old was 1.92 on 1957 feed and 1.62 on 2001 feed, and the average body weight was 2126 grams on 1957 feed and 2672 grams on 2001 feed. Figure **B1.2.1** shows the body weight of the strains on their respective diets over time. It should be noted that the rapid growth has also been linked to negative side effects, such as cardiovascular and musculoskeletal problems (Julian, 1998). Nowadays, these are also taken into account in breeding programmes (see **section 1.3**).

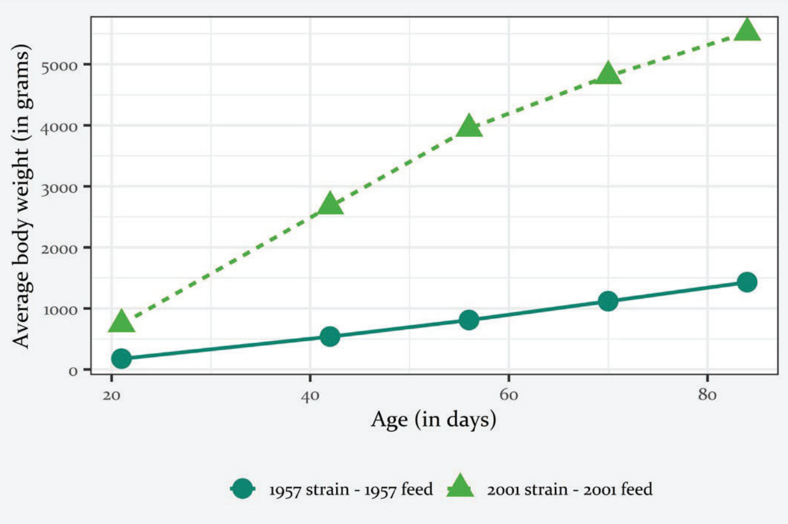

Figure B1.2.1. Body weights over time for the 1957 and 2001 strain, on feed representative of the year of origin of the strain. Based on Havenstein et al. (2003).

approximately 850 million pigs (FAO, 2021b). Over the past 60 years, the global per capita egg consumption has nearly doubled and poultry meat consumption has quintupled, and poultry now forms the main source of animal protein in the world (FAO, 2021a). It has been projected that poultry meat will account for half of all additional meat consumed over 2020-2029 and to hereby rise to a global consumption of 145 million metric tonnes (OECD/FAO, 2020).

As mentioned earlier, intensification in animal agriculture has resulted in higher outputs per unit of input (Place, 2018). One of the main characteristics of intensification involved housing animals indoors and bringing food to the animals, instead of keeping animals outside where they could forage (Brambell, 1965). Indeed, a common production system for broilers nowadays entails housing the birds in a large, open, indoor area, with bedding, feeders and automated drinkers provided (Karcher and Mench, 2018). The birds are housed in this area from the day of hatching until transport to slaughter and the farmer routinely walks through this area to monitor the birds, the equipment and the bedding (Karcher and Mench, 2018). There are restrictions to the number of broilers allowed to be kept per square meter. For example, in the EU 33 to 42 kilograms of broiler weight can be housed per square meter (European Directive, 2007), depending on, for example, farm performance regarding bird mortality and contact dermatitis, and several management aspects.

The intensification of animal production has increased the concerns about animal welfare, some of which were already raised in 1965 and persist from the past into the present (Brambell, 1965; Karcher and Mench, 2018). One of the concerns is that, within these systems, animals are kept in close confinement, which may impair their welfare by limiting their (natural) behavioural opportunities (Brambell, 1965). Hall (2001) compared the behaviour of broilers reared at two different final stocking densities, of 34 and 40 kg/m^2. The broilers kept at higher densities showed, among other things, decreased walking, i.e., a reduced number of steps taken (Hall, 2001), which is in line with other reports in literature (e.g., Lewis and Hurnik (1990)). Possibly, this indicates that, in crowded conditions, a form of physical or social restriction is exerted by conspecifics (Lewis and Hurnik, 1990). Furthermore, the broilers at the higher density showed a larger number of lying bouts and disturbances, defined as “bird stops lying and stands because other birds step on or over it”, a larger proportion of lying bouts that ended through disturbance, and a shorter duration of undisturbed rest (Hall, 2001). These findings indicate that the resting behaviour of the broilers was affected by the high density (Hall, 2001). Moreover, ground pecking was reduced in birds kept at the higher density, which was suggested to possibly be related to poorer litter quality or decreased free space for performing behaviours undisturbedly at the higher density (Hall, 2001).

Stocking density alone, however, does not necessarily explain welfare differences between flocks. Dawkins et al. (2004) studied broiler flocks housed at different densities under a range of commercial conditions and concluded that differences between producers in the provided bird environments affected welfare more than stocking density itself. One factor that was observed to affect broiler welfare was litter quality, which is also a concern with the current type of housing for broilers. Litter quality depends on, among other things, humidity levels (Weaver and Meijerhof, 1991), ventilation rates (Weaver and Meijerhof, 1991), number of drinkers per unit area (T. A. Jones et al., 2005), stocking density (Guardia et al., 2011), and water pressure of the drinkers (Carey et al., 2004). Poor litter quality can have negative consequences for birds' health, welfare and performance. For example, different types of contact dermatitis have been linked to poor litter conditions (de Jong et al., 2014; Greene et al., 1985). This includes both foot pad dermatitis (**FPD**), a dermatitis on the plantar surface of broilers' feet, and hock burn (**HB**), which is a dermatitis on the plantar surface of broilers' hocks (Greene et al., 1985). Dawkins et al. (2004) observed that higher levels of litter moisture were also related to more legs being scored as angle-out, meaning that there was an outward twist at the intertarsal joint with an angle larger than 30 degrees between the legs. Furthermore, wet litter has been reported to result in reduced body weight gain, feed intake and water intake in broilers (de Jong et al., 2014). Consequently, these birds also had lower carcass weight (de Jong et al., 2014). Furthermore, broilers on wet litter showed a higher feed conversion ratio (de Jong et al., 2014). Altogether, this shows that it is important to maintain a good litter quality.

Another concern in large groups of birds is monitoring individuals. Individual broilers are very difficult to identify, given their uniform appearance and the large group sizes in which they are kept. This poses a challenge for both manual monitoring and video-based monitoring. Birds can be visually marked to allow identification, both for live observations and for video recordings. However, this can be time-consuming, especially since markings may fade over time and birds therefore may need to be marked repeatedly. Also, it may be difficult to mark large numbers of birds in a way that is unique for each individual but still clearly recognizable from a distance. Moreover, markings may affect the behaviour of birds. For example, Zukiwsky et al. (2020) studied whether dye markings from an automated marking system affected aggressive behaviours among broiler breeder chicks, and observed that the dye markings promoted aggressive pecks at 26 days of age. As Siegford et al. (2016) note in their review on recording location and activity of individual laying hens in large groups using tracking systems, poultry studies on productivity or performance and associated risk factors often use group-level data. In this respect, Siegford et al. (2016) mention the 'ecological fallacy' limitation which has been described as the fallacy of drawing individual-level inferences based on group-level data (Diez-Roux, 1998). For example, if one would record two behaviours (A and B) at farm-level and observe a negative relationship between behaviours A and B, one would not be able to state with certainty that individual birds that show behaviour A more indeed show behaviour B less. It could be that the pattern at group-level differs from the individual-level pattern (see **Figure 1.1**, where individuals within a farm show a positive relationship between behaviour A and B). Therefore, group-level data are not always suitable for certain research questions and may result in biased conclusions.

1.3 The importance of individual data

Individual data are of great relevance in broilers for several reasons. These include, but are not limited to, the fact that welfare is experienced at the individual level and that individual data are required as input for breeding programmes.

The welfare in broiler units can be assessed in a standardized manner using for example the Welfare Quality® assessment protocol for poultry (Welfare Quality®, 2009). With this approach, scores are given for twelve welfare criteria in four main categories or principles (**Table 1.1**; adapted from Welfare Quality® (2009)). For each of these criteria, one or more measurements are made in a broiler unit. The scores for these measurements are combined into a score per criterion, and these criterion-scores are then

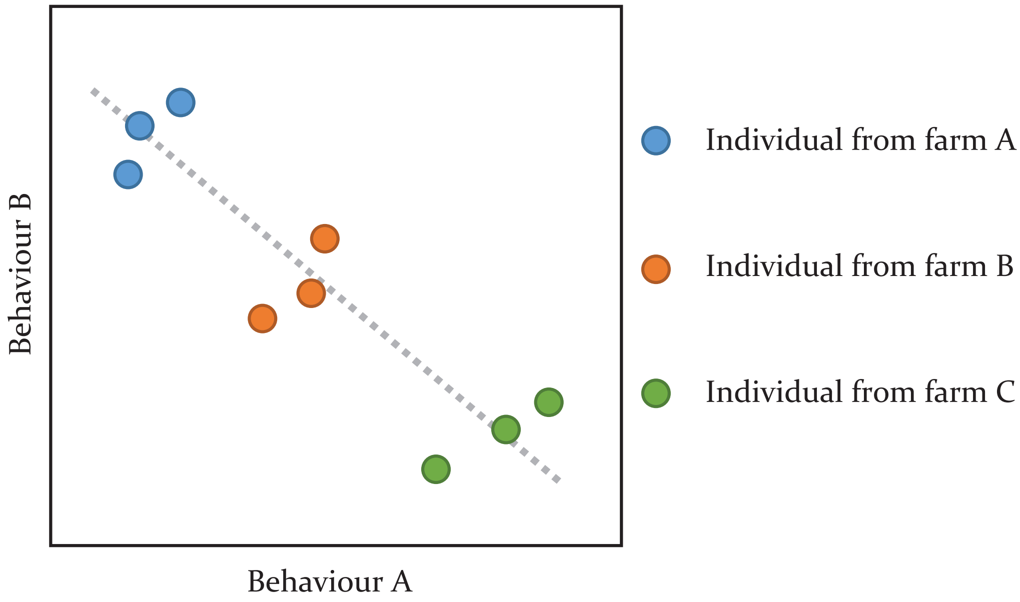

Figure 1.1. Schematic example of how group-level patterns can differ from individual-level patterns. Points represent individual recordings. The dashed line represents the negative correlation that would be observed if observations would only be made at farm-level, while within farms, the correlation appears to be positive.

combined into a score per principle. The overall welfare assessment of a broiler unit is then based on the principle-scores (Welfare Quality®, 2009). The different measurements are not always recorded for all broilers present, but are for example recorded on a subset of animals. An example of this is the welfare measure 'lameness', or gait score, which is scored on a random selection of individuals and an average flock gait score is calculated based on these results (Welfare Quality®, 2009). However, the average welfare assessment for the broiler unit might not represent all individuals in the unit equally well, as there can be broilers that deviate from the average value, be it in a positive or a negative way. By definition (**Box 1.1**), welfare is an individual experience. Therefore, to fully assess animal welfare, individual data on broilers are required. Especially for leg weakness, which is a major welfare issue in broilers (SCAHAW, 2000), individual data would be valuable. Leg weakness is a general term to describe multiple pathological states resulting in impaired walking ability in broilers (Butterworth, 1999). This impaired walking ability may affect birds' welfare through, for example, the potentially associated pain (Danbury et al., 2000) and resulting difficulty with competing for resources and performing specific behaviours (Kestin et al., 1992),

Table 1.1. Summary of measurements in poultry Welfare Quality® assessment protocol (adapted from Welfare Quality® (2009)).

	Welfare criteria	Detailed definition	Broiler specific measurements representing situation on farm
Good feeding	Absence of prolonged hunger	Animals should not suffer from prolonged hunger, i.e., they should have a suitable and appropriate diet	Emaciation
	Absence of prolonged thirst	Animals should not suffer from prolonged thirst, i.e., they should have a sufficient and accessible water supply	Drinker space
Good housing	Comfort around resting	Animals should have comfort when they are resting	Plumage cleanliness, litter quality, dust sheet test
	Thermal comfort	Animals should have thermal comfort, i.e., they should neither be too hot nor too cold	Panting, huddling
	Ease of movement	Animals should have enough space to be able to move around freely	Stocking density
Good health	Absence of injuries	Animals should be free of injuries, e.g., skin damage and locomotory disorders	Lameness, hock burn, foot pad dermatitis, breast blister
	Absence of disease	Animals should be free from disease, i.e., animal unit managers should maintain high standards of hygiene and care	E.g., on farm mortality, culls on farm, ascites, dehydration, septicaemia, hepatitis
	Absence of pain induced by management procedures	Animals should not suffer pain induced by inappropriate management, handling, slaughter, or surgical procedures (e.g., castration, dehorning)	N/A in this situation
Appropriate behaviour	Expression of social behaviours	Animals should be able to express normal, non-harmful, social behaviours (e.g., grooming)	No measure yet
	Expression of other behaviours	Animals should be able to express other normal behaviours, i.e., it should be possible to express species-specific natural behaviours such as foraging	Cover on the range, free range
	Good human-animal relationship	Animals should be handled well in all situations, i.e., handlers should promote good human-animal relationships	Avoidance distance test
	Positive emotional state	Negative emotions such as fear, distress, frustration or apathy should be avoided whereas positive emotions such as security or contentment should be promoted	Qualitative behavioural assessment

such as dustbathing or preening while standing (Vestergaard and Sanotra, 1999; Weeks et al., 2000).

Broiler breeding programmes include many traits, with numbers of 50 or more having been reported (Siegel et al., 2019). Among these traits are skeletal abnormalities, and abnormalities involving the limbs are noted to be particularly relevant (Siegel et al., 2019). **Table 1.2** shows several of the different leg health measurements that are sometimes recorded for broilers. Differences in gait score, or walking ability, have been observed between broiler crosses (Kestin et al., 1999), but impaired walking ability can have many causes, including both non-infectious origins (e.g., genetics and growth rate) and infectious origins (Bradshaw et al., 2002). For specific components of leg health, it has been observed that there is potential for selection for improved leg health. Kjaer et al. (2006) studied the heritability of foot pad dermatitis (**FPD**) in Ross 308 broilers and observed a heritability of 0.31 with a standard error of 0.12. Kapell et al. (2012) studied the development of leg health over up to 25 years of selection in three purebred commercial broiler lines, and estimated genetic parameters using data on four recent generations. They observed that the prevalence of hock burn (**HB**) decreased mainly in the first ten years of recording, with a range of -1.3 to -1.5% per year, after which it stabilized. The heritability of HB was estimated to be 0.06 to 0.09. This is similar to the estimate of 0.08 mentioned by Kjaer et al. (2006), but their estimate did not differ significantly from zero. The prevalence of tibial dyschondroplasia (**TD**) was observed to decrease by -0.4 to -1.2% per year. The heritability of TD was estimated to be 0.10 to 0.27 (Kapell et al., 2012), which is in agreement with Siegel et al. (2019) who observed heritabilities for TD of 0.13 to 0.18 in three pure lines. Siegel et al. (2019) furthermore studied the heritability of femoral head necrosis (**FHN**) in three pure lines and observed heritabilities of 0.26 to 0.30. These studies show that, when individual data on leg health are available, there is potential to reduce the prevalence of (different types of) leg health issues.

1.4 Activity as a proxy for other traits

To allow implementation of the earlier-mentioned leg health traits in breeding programs, individual records for large numbers of broilers are desired. However, it is not only time-consuming to obtain information on large numbers of individual broilers, some of the leg health problems are also

Table 1.2. Overview of several leg health measurements and the prevalence of these leg health issues.

Leg health measurement	Example scoring method	Prevalence
Gait. Walking ability (Kestin et al., 1992).	Six-point scale with 0 = normal gait with no detectable abnormalities and 5 = incapable of sustained walking on feet (Kestin et al., 1992).	E.g., Sanotra et al. (2003) observed in broiler flocks in Denmark and Sweden that 14% to 30% of the broilers had gait scores of 3 or higher.
Footpad dermatitis (FPD). Dermatitis on the plantar surface of broilers' feet (Greene et al., 1985).	Three-point scale with 0 = no lesions, only mild hyperkeratosis, no discoloration or scars and 2 = severe lesions – deep lesions, ulcers, and scabs (Ekstrand et al., 1998).	E.g., de Jong et al. (2012) observed for Dutch broiler flocks that on average 64.5% of the birds showed mild to severe FPD lesions.
Hock burn (HB). Dermatitis on the plantar surface of broilers' hocks (Greene et al., 1985).	Three-point scoring scale with 1 = not affected and 3 = severe lesions (Kjaer et al., 2006).	E.g., at four-weeks-old 0.5% of birds of a fast-growing line showed HB, at six-weeks-old this was 88% (Kjaer et al., 2006).
Tibial dyschondroplasia (TD). Lesion characterized by an avascular cartilage mass in the metaphysis of the proximal ends of the tibiotarsus and tarsometatarsus (Leach and Monsonego-Ornan, 2007).	*Post mortem*, e.g., four-point scale with 0 = normal growth plate (including slight uniform thickening of growth plate) and 3 = severe lesion with cartilage extended > 0.75 cm (Kaukonen et al., 2017).	TD is considered one of the most common problems in the poultry industry, with an incidence that may be as high as 30% in broiler flocks (Crespo, 2020).
Femoral head necrosis (FHN) – i.e., bacterial chondro-necrosis with osteomyelitis (BCO). Thought to be caused by mechanical damage to poorly mineralized columns of cartilage cells in proximal growth plates of leg bones, after which colonization by bacteria occurs via bloodstream (Wideman and Prisby, 2013).	*Post mortem*, e.g., three-point scale with 0 = normal proximal femoral head and 2 = the femoral head appeared necrotic, with a porous cartilage and brittle femoral neck (Tahamtani et al., 2018). Can also be scored using for example bacteriology, serology and histopathology (Wijesurendra et al., 2017).	In a study on Australian broiler farms BCO was detected in approximately 28% of the mortalities and culls, and 46.4% of bones with evidence of BCO were femurs (as opposed to tibias) (Wijesurendra et al., 2017).

difficult to score objectively as these depend on scores given based on visual observations by a human observer (see **Table 1.2**). Furthermore, some conditions, such as FHN, can only be accurately assessed *post mortem* (Siegel et al., 2019). For practice, this means that selection cannot be performed on the individual for which FHN is assessed, but only on its siblings, which compromises the accuracy of the breeding value (Siegel et al., 2019). Therefore, if an indicator trait that is linked to leg health could be used instead, scoring of leg health could potentially become easier and more time-efficient.

One trait that has potential as an indicator of gait, and other health, welfare or production-related traits, is locomotor activity. Relationships between activity and, for example, illness, body weight and leg health have been observed and some examples are shown in **Figure 1.2**. These examples suggest that activity is a key trait to monitor in broilers. However, manually monitoring activity of individual broilers is difficult and time-consuming. Therefore, automated ways of recording individual activity levels are desired, for example using sensor technologies.

1.5 Implementation of sensor technologies

In recent years, substantial progress has been made in sensor technologies for monitoring individual animals kept in groups. Different types of sensor technologies have potential for implementation for identification and monitoring of group-housed birds (reviewed by Ellen et al. (2019)). These sensor technologies can be divided into two main categories: remote sensor technologies, for example computer vision, and body-worn sensor technologies, where sensors are attached to the animals (Ellen et al., 2019). The remainder of this section will focus on two types of body-worn sensor technologies that have potential for use in broilers to track activity levels: radio frequency identification (**RFID**) systems and ultra-wideband (**UWB**) technology. First the methodology of these systems is explained and then examples are given of earlier implementations of these systems for poultry.

1.5.1 Radio frequency identification

The term 'radio frequency identification' refers to technology that uses radio waves to carry information (Debouzy and Perrin, 2012; Finkenzeller, 2010).

Figure 1.2. Schematic overview of the relationship between locomotor activity and illness, body weight and leg health, respectively.

Generally, RFID systems consist of three main components (Roberts, 2006):

1) *tags (or transponders)*, containing an antenna and an electronic microchip with a unique identification code, and located on the object, or animal, to be identified (Debouzy and Perrin, 2012; Finkenzeller, 2010; Ngai et al., 2008);

2) *readers (or interrogators)*, which retrieve the data from the tags through communication via antennas that work on the same radio frequency as the tags (Ngai et al., 2008), and;

3) *a host system*, which receives the information from the readers (Ngai et al., 2008).

Typically, the resulting RFID registration that is stored consists of a timestamp, the specific antenna (i.e., location) at which the tag was registered, and the ID code of the tag (Brown-Brandl et al., 2017). There are many different variants of RFID systems (Finkenzeller, 2010). The main distinctions between, and advantages and limitations of, different RFID systems are outlined in **Box 1.3**.

1.5.2 Ultra-wideband

The term 'ultra-wideband', which is also mentioned in **Box 1.3**, refers to a radio frequency signal that occupies a bandwidth greater than 500 MHz (FCC, 2002). In other words, with UWB communication, information is spread out over a wide portion of the frequency spectrum (Alarifi et al., 2016). Similar to RFID, the general means of obtaining information is communication between target (i.e., tag) and reference nodes (i.e., sensors) (Alarifi et al., 2016; Ubisense, 2011). The position of the reference nodes is known beforehand and via communication with the target node, the target's position can be determined. There are different ways to extract positional information from UWB signaling, for example by using the time of arrival of the signal or the angle of arrival of the signal (Alarifi et al., 2016). It is however important to note that active UWB tags are generally larger and heavier than passive RFID tags (**Box 1.3**), limiting the use of active UWB for small birds or early in life.

Box 1.3. Different types of RFID systems (from Ellen et al. (2019))

There are many different types of RFID systems (Finkenzeller, 2010), and an important first distinction can be made in the type of tag used. Two main types of tags that will be discussed here are passive tags and active tags (**Figure B1.3.1**, from Ellen et al. (2019)). Passive tags obtain power from the field of the reader, as they do not have their own power supply (Finkenzeller, 2010). Active tags, like UWB, on the other hand, have a battery that can be used to power the tag circuitry and to actively broadcast a signal to the reader (Angeles, 2005). Generally, active tags have longer read ranges compared to passive tags, but are also heavier and more expensive (Roberts, 2006). Another important distinction among RFID systems can be made based on the operating frequency. Often, four ranges are distinguished: low frequency (**LF**), high frequency (**HF**), ultra-high frequency (**UHF**), and microwave (Ruiz-Garcia and Lunadei, 2011). The operating frequencies differ in their read ranges and in their sensitivity to the environment. In brief, systems that operate on higher frequencies have longer read ranges and faster communication (Ruiz-Garcia and Lunadei, 2011). However, systems operating on lower frequencies are less sensitive to interference from metals and liquids than higher frequency systems (Ruiz-Garcia and Lunadei, 2011).

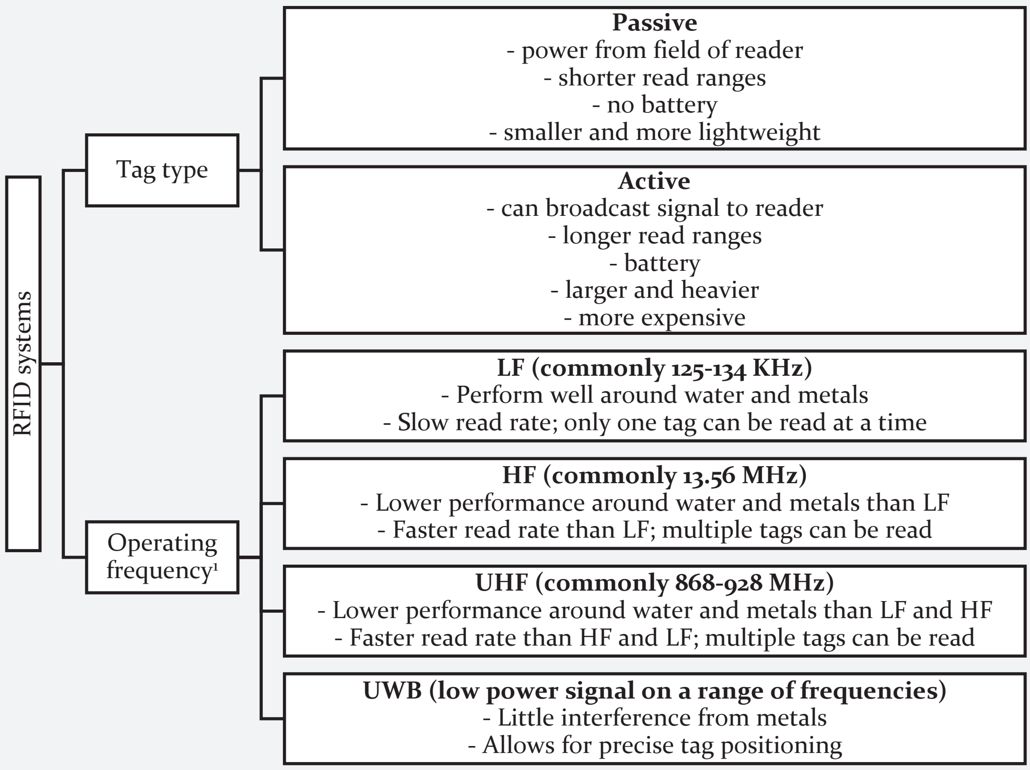

Figure B1.3.1. Overview of different radio frequency identification (RFID) systems and their main characteristics (based on Angeles (2005); Finkenzeller (2010); Nekoogar and Dowla (2011); Roberts (2006); Ruiz-Garcia and Lunadei (2011)). LF: low frequency; HF: high frequency; UHF: ultra-high frequency; UWB: ultra-wideband. [1]Microwave frequencies are excluded here, as these are not commonly used for animal tracking.

1.5.3 Examples of implementations for poultry

Both passive RFID and UWB systems have been implemented to track poultry behaviour. As indicated in **Box 1.3**, passive RFID systems can operate on different frequencies (low frequency (**LF**), high frequency (**HF**), and ultra-high frequency (**UHF**)) and this can have consequences for what applications are suitable. Passive LF RFID has been used in several studies to monitor pop holes or range use in laying hens, or nest box use (e.g., Campbell et al. (2017); Chien and Chen (2018); Gebhardt-Henrich et al. (2014b); Gebhardt-Henrich and Fröhlich (2015); Ringgenberg et al. (2015); see Siegford et al. (2016) for a review of RFID for recording range and nest box use). Furthermore, passive LF RFID has been used to monitor presence of laying hens in an environmental preference chamber (Sales et al., 2015) and as an identification support for computer vision approaches (Nakarmi et al., 2014). LF RFID has also been implemented for broilers, for example to study range visits (e.g., Taylor et al. (2017a, 2017b)), or distances ranged from the shed on a commercial farm (Taylor et al., 2020). Compared to LF systems, one of the main advantages of higher frequency systems is that these systems can have anti-collision protocols (Brown-Brandl et al., 2017), which means that multiple birds can be registered at the same antenna at the same time, instead of data collision resulting in lost information. Examples of implementations of HF RFID include monitoring of pop holes in laying hens (e.g., Hartcher et al. (2016); Thurner et al. (2009)). Examples of implementations of UHF RFID include tracking of feeding and nesting behaviour of multiple laying hens at the same time using antennas in the feed troughs and nest boxes (L. Li et al., 2017). Also for broilers, there are implementations of UHF RFID. G. Li et al. (2019) developed and tested an UHF RFID system to monitor feeding and drinking behaviour of individual broilers and concluded that the system could accurately detect and record the feeding and drinking behaviours of individual broilers in a group setting. Besides these applications of passive RFID where detecting the presence of birds at a specific location or birds passing a specific area is the main goal, there are also implementations in which the movement between antennas is used to study activity. For example, Kjaer (2017) implemented RFID to record general locomotor activity in laying hens, using a grid of antennas that covered the central part of the pen in which the birds were kept. Active UWB systems have also been implemented to study poultry behaviour. For example, Rodenburg et al. (2017) used an UWB system to track the location of individual laying hens. They equipped hens with a tag in a small backpack and compared the UWB recordings to video tracking. They observed that the results from both systems were very similar and that the UWB system could detect the bird's location with an accuracy of 85%. Furthermore, active UWB has been tested for outdoor tracking in slow-growing broilers and it was concluded that the UWB system showed great promise for the monitoring of free-range use (Stadig et al., 2018a). Approaches for, and studies on, detailed indoor tracking of individual broilers housed in groups and throughout life were still limited at the start of this project, but the discussed studies indicate that sensor technologies can aid in collecting the desired data on activity for individual broilers.

1.6 Aim and outline of thesis

The main aims of this thesis were 1) to study whether and how we can collect detailed activity data on individual broilers throughout life in an automated manner, while they are housed in a group, and 2) to study the relationship between individual activity, leg health and body weight, with the ultimate goal of examining whether it is possible to select on activity to improve health and welfare in broilers. A schematic overview of this thesis is presented in **Figure 1.3**. We first aimed to implement and validate an UWB tracking system for assessing individual broiler activity levels. To this end, we tested the UWB tracking system in small groups of broilers, from approximately 16 to 32 days old. We assessed the recorded distances from the UWB system and compared these to distances recorded on video, and studied general trends in activity levels over time (**chapter 2**). With the same setup, we also studied how activity and global gait classification of broilers are related (**chapter 3**). To assess activity in more detail and throughout the life of broilers, a passive HF RFID system was implemented for small groups of broilers from one-day-old up to slaughter age. The reliability of this system for activity tracking was studied in two respects: regarding location, comparing the RFID-recorded locations to the locations on video, and regarding distance moved, comparing the RFID-recorded distances to those observed on video and from UWB tracking (**chapter 4**). We then implemented the RFID system to record individual activity levels of multiple groups of broilers, to clarify the relationship between early activity and body weight gain in broilers (**chapter 5**). With the collected individual data on activity, we also studied the heritability of activity, to assess the potential of selecting for increased activity levels in broilers (**chapter 6**). In the General discussion (**chapter 7**), a synthesis of the results from this thesis is presented and future perspectives for implementing the tracking technologies are discussed.

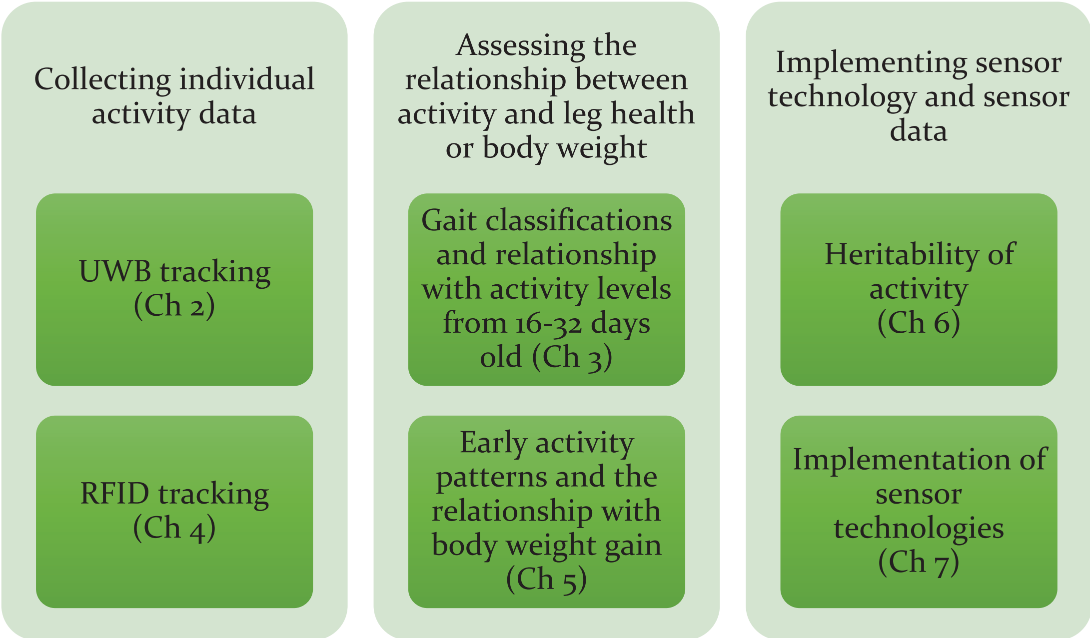

Figure 1.3. Schematic overview of the contents of this thesis.

Chapter 2:

Validation of an ultra-wideband tracking system for recording individual levels of activity in broilers

Malou van der Sluis[1,2], Britt de Klerk[3], Esther D. Ellen[1], Yvette de Haas[1], Thijme Hijink[4] and T. Bas Rodenburg[2,4]

[1] Animal Breeding and Genomics, Wageningen University & Research, 6700 AH Wageningen, The Netherlands;
[2] Department of Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, 3508 TD Utrecht, The Netherlands;
[3] Research & Development Department, Cobb Europe BV, 5831 GH Boxmeer, The Netherlands;
[4] Adaptation Physiology Group, Wageningen University & Research, 6700 AH Wageningen, The Netherlands

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Abstract

Individual data on activity of broilers is valuable, as activity may serve as a proxy for multiple health, welfare and performance indicators. However, broilers are often kept in large groups, which makes it difficult to identify and monitor them individually. Sensor technologies might offer solutions. Here, an ultra-wideband (**UWB**) tracking system was implemented with the goal of validating this system for individual tracking of activity of group-housed broilers. The implemented approaches were (1) a comparison of distances moved as recorded by the UWB system and on video and (2) a study recording individual levels of activity of broilers and assessing group-level trends in activity over time; that could be compared to activity trends from literature. There was a moderately strong positive correlation between the UWB system and video tracking. Using the UWB system, we detected reductions in activity over time and we found that lightweight birds were on average more active than heavier birds. Both findings match with reports in literature. Overall, the UWB system appears well-suited for activity monitoring in broilers, when the settings are kept the same for all individuals. The longitudinal information on differences in activity can potentially be used as proxy for health, welfare and performance; but further research into individual patterns in activity is required.

2.1 Introduction

In current husbandry systems, animals are often kept in large groups. This makes it difficult to identify and monitor animals at the individual level; especially when animals in a group are homogeneous in appearance, and automatic visual identification of animals is hampered. Often, video recordings are used to manually assess animal behaviour, but the manual analysis of these videos is time-consuming and may introduce human error (Catarinucci et al., 2014).

Even though monitoring of individuals is difficult, there is a growing interest in quantifying individual behaviours of group-housed animals, in order to study the link between individual behaviour and performance in more detail. Broiler chickens are an example of a livestock species for which data on individual behaviour could be valuable. A specific trait of interest in broilers is activity. Changes in activity in broilers appear to have potential as a proxy for multiple health, welfare and performance indicators. For example, ill animals may show a change in activity, as they generally increase their time spent sleeping (Gregory, 1998). Furthermore, it has been suggested that low activity levels, as well as higher body weights, are related to lameness or leg weakness (Haye and Simons, 1978; Kestin et al., 2001; Van Hertem et al., 2018a). Leg weakness is marked by an impaired walking ability, or abnormal gait, with a noninfectious cause and may negatively affect the welfare of the birds (Kestin et al., 1992). However, it has been indicated that increased locomotor activity can contribute to a lower prevalence or severity of leg weakness (Reiter, 2004). There might be potential to select for activity. For example, Bizeray et al. (2000) found differences in early life activity between two broiler stocks and noted that these differences were possibly due to genetic factors. Overall, individual levels of activity appear to be a key trait to monitor. However, as mentioned before, identifying animals and monitoring their behaviour in large groups is difficult.

Sensor technology systems, such as passive radio frequency identification (**RFID**), automated computer vision (**CV**) or ultra-wideband (**UWB**) tracking, might offer solutions for tracking individual animals that are housed in groups. These technologies have already been implemented in several studies focusing on poultry behaviour. In studies involving passive RFID, birds are generally fitted with tags, while antennas are placed in the location of interest to register presence of birds. RFID has been implemented for example to study range use (i.e., pop hole use) in laying hens (e.g., Gebhardt-Henrich et al. (2014b)), and to register nesting and feeding behaviour (e.g., L. Li et al. (2017)) or presence in a preference chamber (e.g., Sales et al. (2015)). In studies involving automated CV systems, a camera is often placed above a group to monitor behaviour. For example, Aydin (2017) used a 3D vision camera system with a depth sensor to study inactivity of broilers, by analysing the number of lying events and the latency to lie down of individual broilers walking through a test corridor. In studies involving UWB systems, animals are generally equipped with transmitters, while receivers, which receive the signals from the transmitters, are placed in the surroundings. Stadig et al. (2018a) implemented an UWB system to monitor free-range use of chickens, and observed in validation trials that the median error in the localisation of tags was 0.29 m. Rodenburg et al. (2017) compared UWB and automated video tracking to track individual laying hens and found that the results of both were very similar and that the UWB system could detect the bird's location with an accuracy of 85%.

Overall, it appears that these technologies all have potential for individual tracking (for a more elaborate review of these sensor technologies and their applicability for poultry, see Ellen et al. (2019)). However, to obtain information on activity levels in group-housed broilers, an UWB system appears most practical, as this allows for exact positioning of the animals and provides reliable identification of individuals. Furthermore, the UWB signals are noted to be relatively insensitive to reflections from the surroundings and do not require a direct line of sight (Alarifi et al., 2016; Nekoogar and Dowla, 2011). This makes the implementation of an UWB system a promising approach for identifying and locating individual group-housed broilers. Furthermore, using the longitudinal information on location that is provided by an UWB system, activity can potentially be assessed.

In the present study, an UWB tracking system was implemented to track individual activity in broilers, with the goal of validating the UWB system for individual tracking of activity of group-housed broilers. The two approaches implemented to validate the UWB system were (1) a comparison of the distances moved as recorded by the UWB system and the distances recorded on video and (2) a study recording individual levels of activity of broilers and assessing group-level trends in activity over time, which could be compared to known activity trends from literature. Literature on broiler activity indicates that age and weight affect activity in a general direction. Activity decreases are seen with increasing age of broilers (Tickle et al., 2018; Weeks et al., 2000). For example, Tickle et al. (2018) found changes over the growth period in the proportion of time spent actively moving and in walking speed, both of which declined with increasing age. Furthermore, the weight of birds is thought to affect the level of activity, with a lower level of activity in heavier birds. For example, Reiter and Bessei (2001) studied locomotor activity of 25% load-reduced and non-load-reduced birds and found that the average distance travelled by broilers was higher in load-reduced birds compared to non-load-reduced birds. Therefore, if the UWB system performs well, it would be expected that a decrease in activity over time and a lower level of activity in heavier birds are observed in this study, and that there is a strong correlation between distances recorded with the UWB system and on video.

2.2 Materials and methods

2.2.1 Ethical statement

Data were collected under control of Cobb Europe. Cobb Europe complies with the Dutch law on animal wellbeing. This study is not considered to be an animal experiment under the Law on Animal Experiments, as confirmed by the local Animal Welfare Body (20th of June, 2018, Lelystad, The Netherlands).

2.2.2 Location and housing

All trials were performed on a broiler farm in The Netherlands. The broilers were housed in groups and feed and water were provided ad libitum. Wood shavings were provided as bedding. The birds were kept under a commercial lighting and temperature schedule, and were vaccinated according to common practice (Cobb, 2018).

2.2.3 Ultra-wideband system

To track individual broilers and to determine their activity levels, an UWB system was implemented. Generally, UWB systems consist of transmitters, which can be fixed to the animal of interest, and receivers in the environment. UWB systems communicate between these transmitters and receivers using narrow radio frequency pulses (Nekoogar and Dowla, 2011) that are spread out over a spectrum of at least a 500 MHz bandwidth, or have a fractional bandwidth equal to or larger than 20% of the centre carrier frequency (FCC, 2002). These UWB signals allow for calculating the position of the transmitters using triangulation of the signal between the receivers. Here, a Ubisense UWB system with Series 7000 sensors and compact tags (Ubisense Limited, Cambridge, UK) was used, in combination with TrackLab software (Noldus Information Technology, Wageningen, The Netherlands). Broilers were fitted with a battery powered Ubisense tag with a size of approximately 3.8 by 3.9 cm and a weight of ~25 g on their backs, using elastic bands around their wing base. Every 6.91 s, these tags sent out a signal. The sampling rate could be set higher, but a pilot study showed that the current sampling rate was best suitable (Hijink, 2018). With the sampling rate of 6.91 s, the batteries of the tags could last for at least seventeen consecutive days, working continuously. Before each trial, all batteries were tested and only full batteries were used. The room in which the broilers were housed was fitted with four Ubisense beacons, in a square-like structure above the pen at a height of ~2.25 m that could receive these signals. Using triangulation of the signal from the tags, based on the time of arrival of the signal (Time Difference of Arrival (**TDoA**)), the location of the tags could be determined. When the signal did not meet the error threshold of the Ubisense tracking system, which could for example occur when the tag was in a partially hidden position, such as in a corner or underneath the drinking line, and the signal could not be picked up by a number of beacons, the sample was seen as invalid by the system and was not recorded. The recorded locations of the tags were sent to the TrackLab software. The output of the TrackLab software that was used in this study was the total distance moved in meters per individual per tracking period.

2.2.4 Distance validation study

To validate the distances moved as recorded by the UWB system (referred to as 'distance validation study' throughout this paper), 24 male broilers of two weeks of age from two genetic crosses were used. These birds were taken from a larger group, selecting the lowest and highest body weights. From each cross, three heavyweight and three lightweight birds were selected for UWB tracking. The average weight (±SEM) of the light birds was 0.42 ± 0.01 kg, while the heavy birds weighed on average 0.63 ± 0.01 kg, as measured on 15 days of age. These twelve birds were fitted with an UWB tag (n = 6 per cross) and were colour-marked for identification purposes. During the study, one tag stopped working and was replaced with another tag, resulting in thirteen tags being used. The 24 broilers were housed in a pen with a size of approximately 6 m^2 in an octagonal shape. The birds were tracked with the UWB system from day 15 to day 33 of life (n = 19 days), for approximately one hour each day, at different times. This one-hour sample per day was deemed sufficient as the main interest here was validating UWB recordings and not studying individual activity patterns over time. Video recordings were made from above, using a Zavio B6210 2MP (Zavio Inc., Hsinchu City, Taiwan) video camera, and were analysed using Kinovea video analysis software version 0.8.25. The length of one side of the octagonal pen (~1.15 m) was used for calibration of the distances for Kinovea. Manual corrections were applied where necessary, for example, when the bird was running, flapping its wings or was very close to other birds. To validate the UWB system, the distance moved according to the UWB system was compared to the distance moved as scored from video. One bird died, and consequently no data was available for day 29 to 33 for this animal, resulting in a total of 223 samples of recorded distances from both the UWB system and video tracking.

2.2.5 Activity trends study

To study individual levels of activity of broilers and assess group-level trends in activity over time (referred to as 'activity trends study' throughout this paper), 150 male broiler chickens from four crosses (A-D) were used, distributed over four consecutive trials (T1-T4; see **Table 2.1**). These birds were selected from a larger group of birds on day 13 or 14 of life, taking an equal sample of the lightest and heaviest birds for each cross and trial. During T1, T2 and T4, the broilers were housed in a pen with a size of around 6 m^2 in an octagonal shape. This pen was divided into two equally sized compartments, one for each cross. In T3, the broilers were housed in a rectangular pen with a size of approximately 8 m^2 in total. This pen was also divided into two equally sized compartments. In T3 and T4, extra birds that were not tracked were added to increase the density of birds to a level more comparable to commercial settings (generally up to 33 kg/m^2 in the EU (European Directive, 2007); **Table 2.1**). The birds were tracked from 00:00 to 23:30 each day. The data from day 16 to day 32 of life (n = 17 days) was used for all trials, with exception of the birds in T4 that were tracked from day 18 onwards (n = 15 days) and the birds in T3, where no data was available for day 26 and 27 of life

Table 2.1. Overview of sample sizes, densities, measurement days, weights (mean ± SEM; SW = start weight, EW = end weight) and average weight increase (mean ± SEM) for the activity trends study. The weights are separated for the two weight categories (L = light; H = heavy). Weights of individual birds were determined with five-gram precision and reported averages are rounded to five-grams. SEMs are rounded to round numbers.

Trial	Number of tagged birds (start)	Birds without tag added	Density (birds per m²)	Number of tagged birds (end)	SW day	EW day	Weight category	SW(g)	EW (g)	Average weight increase per day (g)
T1	36	No	~6	32	13	34	L (n=16)	420 ± 5	2435 ± 43	95 ± 2
							H (n=16)	520 ± 4	2635 ± 60	100 ± 3
T2	36	No	~6	35	13	33	L (n=18)	485 ± 7	2450 ± 34	100 ± 2
							H (n=17)	595 ± 6	2680 ± 45	105 ± 2
T3	40	Yes	~12	35	14	35	L (n=15)	480 ± 12	2500 ± 55	95 ± 2
							H (n=20)	630 ± 6	2715 ± 71	100 ± 3
T4	38	Yes	~12	35	13	35	L (n=17)	340 ± 17	2155 ± 78	85 ± 3
							H (n=18)	460 ± 5	2520 ± 32	95 ± 1
Total	150			137						

due to a system malfunction (n = 15 days). The tracking period was divided over five consecutive recording sessions of different durations. These sessions covered the periods between 00:00-03:30, 03:30-04:30, 04:30-07:00, 07:00-23:00 and 23:00-23:30. The half hour between 23:30 and 00:00 was not tracked in order to allow restarting the recordings each day. The UWB output was used to calculate the average distance moved per hour for each day and each bird. When over 10% of the samples were missing within a tracking session, which was true for approximately 1.4% of the tracking sessions, we considered that the tracking data was not complete for that day, and the average distance moved per hour was removed for this individual and day, resulting in a missing data point. The birds were weighed on day 13 or 14 and again on day 33, 34 or 35 of life (**Table 2.1**). Based on the start weight, individuals were categorised as lightweight or heavyweight within their respective trial and cross. Due to too much missing data (<75% of tracking days complete), death of birds (not related to the UWB tags) and mistakes in sexing, the final number of birds with tags was 32 in T1, and 35 in T2, T3 and T4 (**Table 2.1**). Overall, this resulted in a final sample size of 137 birds. For all trials, the mean weights of the broilers in the two weight categories are shown in **Table 2.1**.

2.2.6 Statistical analysis

All statistics were performed using R version 3.5.0 (R Foundation for Statistical Computing, Vienna, Austria; R Core Team, 2018). For the distance validation study, the data was not normally distributed. A square root transformation normalised the data, but the results for untransformed and transformed data were very similar. Therefore, the untransformed data was used for analysis and is presented here. The correlation between the recorded distances from UWB and from video was studied using a repeated measures correlation (package rmcorr (Bakdash and Marusich, 2018)), to correct for the repeated measures on the same tags, and a Pearson correlation. The Pearson correlation is presented here, as correcting for repeated measures only marginally affected the correlation. To study the relation between the recorded distances from the UWB system and video tracking in more detail, three groups (LD: low distance; MD: medium distance; HD: high distance) were created based on the distance moved on video, the boundaries for which were based on where the correlation line crossed the diagonal. For the activity trends study, the hourly activity data was not normally distributed, but a square root transformation did not improve the distribution. Therefore, untransformed data was used for the analysis and is presented here. To study how levels of activity are influenced by age and weight, a linear mixed-effects model was used (lme4 package (Bates et al., 2015); lmerTest package (Kuznetsova et al., 2017)). The effects tested were day of tracking, cross, trial, start weight category, weight change, and the random effect of animal by day. A backward stepwise approach without interactions was used to test the effects. All effects were significant except for weight change. Using the resulting terms left, all possible two-way interactions were included, with exception of the interaction between cross and trial as not all crosses were present in each trial, and backward selection was again performed. After removal of the interaction between trial and weight, which was not excluded in the backward selection but was not a significant effect in the resulting model, the final model was

$$Y_{ijklm} = \mu + \beta(DT)_i + C_j + T_k + SW_l + (\beta(DT) \times C)_{ij} + (\beta(DT) \times T)_{ik} + (\beta(DT) \times SW)_{il} + (1 + \beta(DT)_i | ID_m) + e_{ijklm}$$

where Y is the average distance moved per hour, μ is the overall mean, $\beta(DT)_i$ is the ith day of tracking (i = 1–17), C_j is the jth cross (j = A–D), T_k is the kth trial (k = 1–4), SW_l is the lth start weight category (l = light or heavy), $(\beta(DT) \times C)_{ij}$ is the interaction between day of tracking and cross, $(\beta(DT) \times T)_{ik}$ is the interaction between day of tracking and trial, $(\beta(DT) \times SW)_{il}$ is the interaction between day of tracking and start weight category, $(1 + \beta(DT)_i | ID_m)$ is the random effect of the mth animal by day and e_{ijklm} is the residual term. Visual inspection of the residuals indicated no obvious deviations from normality or homoscedasticity. *p*-values for the factors in the model were determined using the lmerTest package (Kuznetsova et al., 2017). The R^2 values of the model were determined using the MuMIn package (Barton, 2019). Additional contrasts were calculated using the emmeans package (Lenth, 2019). Figures were made using the ggpubr (Kassambara, 2018), ggplot2 (Wickham, 2016) and sjPlot (Lüdecke, 2019) packages. The level of statistical significance was set at 0.05. In the text reported results are rounded to two decimals and activity levels are given in meters moved per hour.

2.3 Results

2.3.1 Distance validation study

The recorded distance moved on video was positively correlated with the recorded distance as calculated from the UWB tracking data (Pearson correlation, r = 0.71 (95% CI: 0.64–0.77), df = 221, $p < 0.001$). **Figure 2.1** shows the resulting correlation. The diagonal is also indicated in this figure.

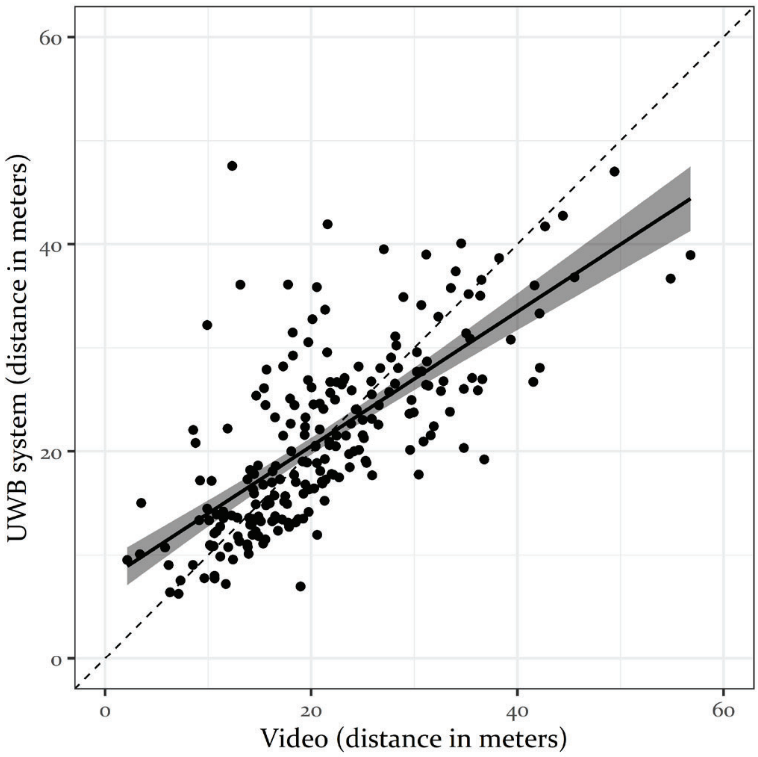

Figure 2.1. Plot of the correlation between the distances recorded from video observations using Kinovea and the distances recorded with the ultra-wideband (UWB) system. Dots represent individual data points. The solid black line shows the correlation coefficient, with the grey area representing the 95% confidence interval. The dashed line shows the diagonal where UWB and video distances would be exactly the same.

From **Figure 2.1** it can be seen that the spread of the individual data points around the diagonal is not equal for the different distances moved on video. The correlation line crosses the diagonal at about 22 m moved on video (**Figure 2.1**). Using this crossing as a reference point, the following three distance groups, based on the distance recorded on video, were created; low distance with distances below 15 m, medium distance ranging from 15 to 30 m and high distance with distances over 30 m. For the distance groups and the overall data set, the mean and median proportional differences between video and UWB tracking, as well as the largest under- and overestimations, can be found in **Table 2.2**. Overall, the UWB system on average overestimated the distance moved by 10% compared to the distance recorded on video, while the largest underestimation was 63% less than the distance recorded on video. The largest overestimation that was observed was 345% more than the distance recorded on video. When comparing the three distance groups, the MD group had the best average estimation (3% overestimation), but also had the largest underestimation (63%). The LD group had the largest mean deviation (40% overestimation) and the largest overestimation (345% overestimation), while in the HD group the UWB system on average underestimated the distance moved (15% underestimation).

Table 2.2. Under- and overestimations by the UWB system for the different distance groups and the complete data set. Proportional differences are calculated as ((UWB – Video)/Video).

Distance group	Low (<15 m)	Medium (15-30 m)	High (>30 m)	Total
n	59	122	42	223
Mean proportional difference	0.40	0.03	-0.15	0.10
Median proportional difference	0.10	-0.04	-0.16	-0.04
Largest proportional underestimation	-0.38	-0.63	-0.48	0.63
Largest proportional overestimation	3.45	1.03	0.25	3.45

2.3.2 Activity trends study

The results of the linear mixed-effects model for the predicted average activity are shown in **Table 2.3**. Clear effects of day and trial on activity levels were observed, as well as an interaction between these factors. Furthermore, an effect of weight category was observed. The activity model explained 56.93% of the variance when only fixed factors were included, while it explained 85.50% of the variance when both the fixed effects and the random effect of ID by day were included.

Table 2.3. Results of the linear mixed-effects model for the predicted average activity (meters moved per hour), including type III Analysis of Variance and estimates for the different factor levels.

Linear mixed-effects model					
Random effects					
Factor	**Variance**	**SD**	**Correlation**		
ID intercept	18.837	4.340	−0.72		
ID by Day	0.059	0.244			
Residual	5.707	2.389			
Fixed effects					
Factor[1]	**F-value**	***p*-value**	**Estimate**	**SE**	***p*-value**
Intercept			25.413	1.235	$<2 \times 10^{-16}$
Day	337.322	$<2.2 \times 10^{-16}$	−0.690	0.074	4.73×10^{-16}
Cross	2.313	0.079			
Cross B			−3.466	1.597	0.032
Cross C			−4.447	2.209	0.046
Cross D			−5.918	2.464	0.018
Trial	28.531	2.177×10^{-14}			
Trial 2			−2.366	1.525	0.123
Trial 3			10.728	2.096	1.05×10^{-6}
Trial 4			7.510	2.109	5.09×10^{-4}
Weight category	16.665	7.545×10^{-5}			
Heavyweight			−3.175	0.778	7.54×10^{-5}
Day-Cross	3.112	0.029			
Day-Cross B			−0.023	0.096	0.810
Day-Cross C			−0.053	0.133	0.688
Day-Cross D			−0.255	0.149	0.089
Day-Trial	19.052	2.273×10^{-10}			
Day-Trial 2			0.366	0.092	1.08×10^{-4}
Day-Trial 3			−0.021	0.126	0.868
Day-Trial4			0.372	0.127	0.004
Day-Weight category	6.810	0.010			
Day-Weight category heavy			0.123	0.047	0.010

[1] Interactions between factors are indicated with (-).

The overall average activity in the study was found to be 18.65 m per hour. The predicted average activity decreased over time, regardless of differences between trials and crosses (**Table 2.3**; **Figure 2.2**). From **Figure 2.2** it can be seen that in all trials the predicted average activity decreased over the tracking period. However, the activity was on average higher in T3 and T4, compared to T1 and T2. Furthermore, the degree of decrease in activity over time was higher in T1 and T3, compared to T2 and T4.

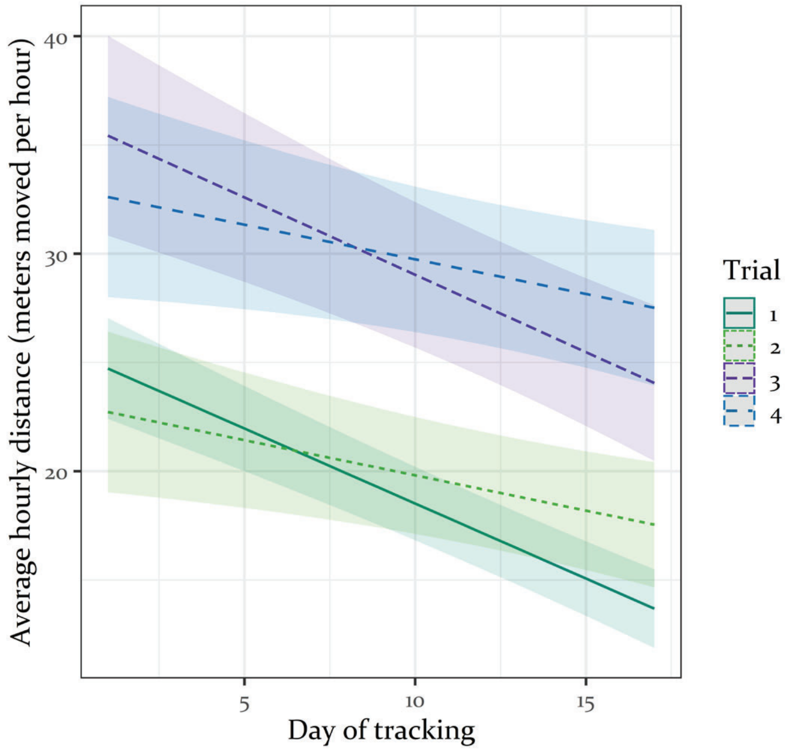

Figure 2.2. Predicted activity over the tracking period (day 1 to 17 of tracking, corresponding to day 16 to 32 of life) in the activity model, distinguishing between the different trials. Shaded areas represent 95% CIs.

The predicted average activity per hour also differed between the two weight categories, as well as the degree of decrease in activity over time (**Table 2.3**; **Figure 2.3**). From **Figure 2.3** it can be seen that heavyweight birds are on average less active and that the activity decreases faster in lightweight birds. Compared to heavyweight birds, lightweight birds are estimated to move on average 2.05 meters per hour more (averaged over time and the levels of trial and cross: day = 9.18, estimate = 2.05, SE = 0.55, df = 137, t = 3.74, p = 0.0003; **Figure 2.3**).

2.4 Discussion

In this study, the distance recorded with the UWB system was moderately strong positively correlated with the distance recorded from video. Furthermore, we found that the UWB system detected a decrease in activity

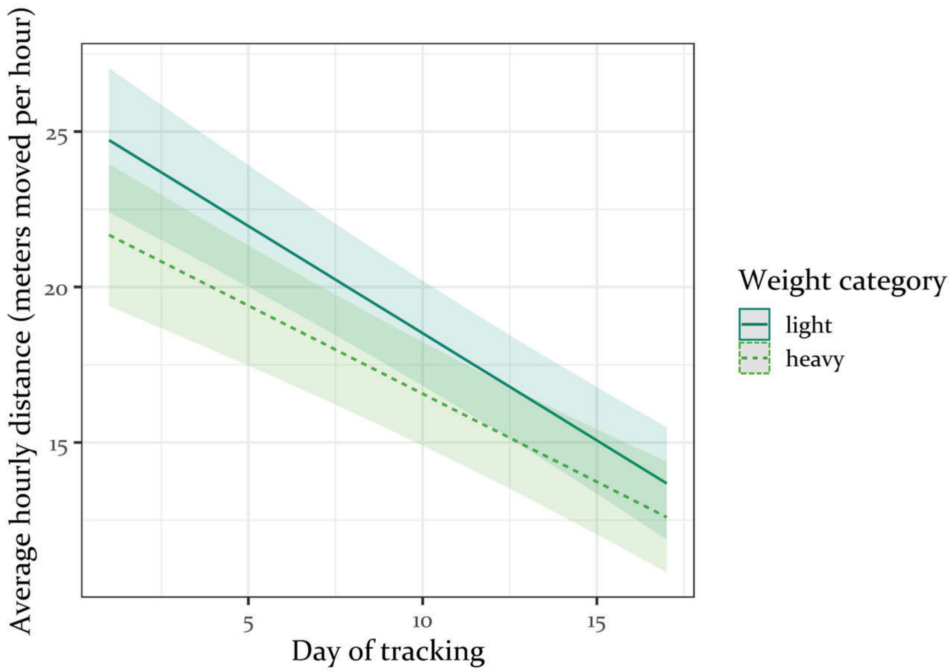

Figure 2.3. Predicted activity over the tracking period (day 1 to 17 of tracking, corresponding to day 16 to 32 of life) in the activity model, distinguishing between the different weight categories. Shaded areas represent 95% CIs.

over the duration of the trials and detected that lightweight birds on average moved longer distances per hour than heavier birds.

2.4.1 Distance validation study

The output of the UWB system was compared to video analysis using Kinovea to assess whether the UWB output was a reliable indicator of the actual distances moved. The moderately strong correlation between the output of the UWB system and video tracking using Kinovea indicates that the UWB system can provide reliable information on the distances moved by individual animals. Overall, the UWB system on average overestimates the 'true' distance moved by 10%. However, it does appear that the distance moved according to the UWB system is generally an overestimation of the actual distance moved as determined by video analysis when animals move less. When animals move more, the UWB system underestimates the distances moved (**Figure 2.1**). When looking at the different distance groups, this pattern is indeed observed. When broilers move low distances on video (<15 m), the distance recorded by the UWB system is on average an overestimation of 40%. However, when broilers move large distances on video (>30 m), the distance recorded by the UWB system is on average an underestimation of 15%. This could be due to the sampling rate that was implemented, i.e., 6.91 s in this study. With each sample that is received, there can be some noise; the triangulation-based location of the tag may deviate slightly from the actual location. Consequently, if an animal moves very little, this noise can make up a relatively large part of the total registered distance, which could explain the overestimation of distances moved by the UWB system when the actual distance moved is low. Alternatively, if an animal is very active, some of the movement of the animal might be missed by the system. For example, if an animal moves from position A to B and back to A within the specified sampling rate, the full distance covered is not registered. In this case, the sampling rate would result in the animal being located near position A two times in a row, while the movement to and from position B is (partly) missed. This could explain the underestimation by the UWB system of the distance moved by animals that covered larger distances on video. However, a pilot study (Hijink, 2018), in which different sampling rates were compared for agreement between distances moved with the UWB system and on video, indicated that the sampling rate used in the current study was the best fit for our implementation. Concluding, the distances moved as indicated by the UWB system may not fully represent the exact distances moved by the animals, but the moderately high correlation indicates that the UWB system is suitable for making comparisons between individual animals. However, because the sampling rate can result in over- or underestimation, it is pivotal to compare all groups or animals using the same sampling rate.

2.4.2 Activity trends study

2.4.2.1 Activity levels over time

This study showed a clear decrease in activity, measured here as average distance moved per hour, over time, as was expected from literature (e.g., Tickle et al. (2018)). A possible mechanism that may underlie the decrease in activity that is observed is the increasing weight of the birds over time. Tickle et al. (2018) showed that level of activity and metabolic costs are inversely related over development. Heavy birds are relatively inactive, and this is hypothesized to be the result of locomotion being more energetically expensive in heavier birds (Tickle et al., 2018). Furthermore, it has been shown that there is a relationship between body weight and gait score, with heavier birds often having a higher gait score (i.e., a worse gait; Kestin et al., 2001). Weeks et al. (2000) compared gait scores 0, 1, 2 and 3 (ranging from no detectable abnormality to an obvious gait defect (Kestin et al., 1992)) and found that the birds with higher gait scores spent less time walking. Possibly, the increasing weight of the birds over time could result in a decrease in distance moved as a consequence of these higher gait scores, but this cannot be concluded from the current study and the relationship between activity, gait and weight at the individual level requires further investigation. However, the finding that the UWB tracking system is able to detect these changes in activity over time supports the notion that the UWB tracking system is suitable for monitoring individual activity of broilers.

In the current study, the average distance moved per hour over the full tracking period was found to be ~18.7 m. When the birds were ~4.5 weeks old, the average distance moved per hour was approximately 15.1 m. However, the literature indicates that there is much variability between studies in distances moved by broilers. In a broiler study by Lewis and Hurnik (1990) lower distances moved were recorded. Their recorded distances varied between 8.1 and 10.0 m per hour, depending on the density at which the birds were housed (660–1320 cm^2 per bird, respectively). In their study, the activity was recorded at about five to six weeks of age, and, given the declining trend in activity over time mentioned earlier, these results may match the findings in the current study. However, in a study by Sherlock et al. (2010) an average distance moved per hour of 46.1 m was found at six weeks of age. This number was based on the number of gridlines crossed in their test and the average distance between them, and is higher than the distance found in the current study.

One possible explanation for the discrepancy in the results is that the added weight of the UWB tags decreased the distance moved by the broilers in our study. When broilers are fitted with tags, about 25 g of weight is added. Possibly, this increase in weight reduced the activity of the broilers. In this study, no comparison between birds with and without tags was made, but Stadig et al. (2018b) looked into the effects of fitting slow-growing broilers with UWB tags. Using backpacks, 35-day-old birds were fitted with UWB tags, with a weight of ~36 g, equal to about 1.8% of the birds' body weight at that time point. They compared birds with and without tags fitted and found an effect of the tag on walking behaviour in the first week after tagging (week six of life), with lower percentages of time spent walking for birds with tags, i.e., 5.8% for tagged birds and 8% for nontagged birds, respectively. This suggests that in the current study the tags may also have decreased the distance moved somewhat. However, there are several other possible explanations for the discrepancy in the results of the different studies. Activity in broilers has been noted to be influenced by numerous factors. For example, higher stocking densities are reported in literature to result in lower activity (Lewis and Hurnik, 1990). Another housing aspect that may influence broiler activity is the lighting. Blatchford et al. (2009) compared light intensities of 5, 50 and 200 lx, and found that broilers reared at 5 lx were less active during the day. The distance between feeders and drinkers may also affect the recorded activity levels. Reiter (2004) compared two feeder-drinker distances — 2 and 12 m — and found that the locomotor activity was higher when the distance was larger. Overall, differences in the surroundings of broilers may affect the recorded distances moved and cause discrepancies between studies.

2.4.2.2 Differences in activity between weight categories

This study showed that birds that are more lightweight at about two weeks of age are on average more active than heavier birds, and showed a faster decline in activity for lightweight birds compared to heavyweight birds. The difference in average activity is in agreement with the expectation from literature (e.g., Reiter and Bessei, 2001) and further supports the notion that the UWB system is suitable for tracking activity in broilers. As noted before, the decrease in activity that is seen over time is likely related to the increasing weight of broilers over time and this finding may also underlie the difference in activity that is observed between lightweight and heavyweight birds. Bokkers et al. (2007) studied high and low body weight groups (aiming at 90% and 50% of normal commercial conditions, respectively) from a fast-growing broiler hybrid in an operant runway test, where the broilers had to walk for a food reward. Birds with a lower body weight were found to walk a larger distance in the test than heavier birds (Bokkers et al., 2007). Reiter and Bessei (2001) studied locomotor activity of load-reduced and non-load-reduced birds. Load-reduced birds were fitted with a harness and suspension, to alleviate the weight load on the legs by 25 percent, while non-load-reduced birds received no weight alleviation (Reiter and Bessei, 2001). It was found that the average distance travelled by broilers over four weeks, determined from video observations made two days per week, was higher in load-reduced birds compared to non-load-reduced birds (Reiter and Bessei, 2001). Possibly, lower locomotor ability and pain related to a higher weight load on the legs underlie the lower activity of the non-load-reduced birds (Reiter and Bessei, 2001). Rutten et al. (2002) also performed a study where the weight load of broilers on their legs was alleviated, by 50 percent, with a suspension mechanism. The distance travelled by the broilers was found to be greater in the birds that received load reduction compared to birds that did not receive load reduction in the second week of the experiment, but not in the first week (day 6 to 12 of age (Rutten et al., 2002)). Possibly, in the first weeks all birds are sufficiently lightweight to not experience any locomotor consequences of the load-bearing on the legs. In the current study, all birds were tracked from two-weeks-old onwards, when the weight may have already limited the locomotor activity of the birds, as is supported by the difference in average activity of lightweight and heavyweight birds that was found.

Furthermore, in the current study, the degree of decrease in activity over time was larger for lightweight birds, and consequently the difference in average activity between lightweight and heavyweight birds became less pronounced over time. However, when looking at the weight of the birds at the start and end of tracking (**Table 2.1**), the relative difference in the weight of the lightweight and heavyweight categories is smaller at the end of tracking compared to at the start. Lightweight birds are approximately 22% lighter at the start compared to the heavyweight birds, while the difference is approximately 10% at the end. Possibly, as the weights of the two categories approach each other over time, the difference in activity level also becomes less, as is reflected in the difference in slope of activity over time. However, more detailed recordings of body weight over time are required in future studies to confirm this hypothesis.

2.4.2.3 Effects of trial and cross

Besides the overall effects over time and of different weight categories, effects of trial and cross in interaction with day were also observed. The four trials, over which this study was divided, differed in the average activity shown by the birds, as well as in the degree of decrease in activity over time. These differences between trials may have arisen from differences in the setup of these trials. First, the trials included different genetic crosses, which may have confounded cross and trial effects. It has been suggested in literature that broiler stocks may differ in activity due to genetic factors (Bizeray et al., 2000). Therefore, a difference between crosses in degree of decrease in activity over time, as was observed here, is not unexpected. However, the distribution of crosses over the trials was skewed and a small number of broilers per cross was available, which hinders drawing conclusions from these findings. Another difference in the setup of the trials was the pen size and stocking density. The size of the pen was different in T3, compared to the other trials. In T3, the pen had a size of approximately 8 m^2, while it had a size of approximately 6 m^2 in the other trials. Furthermore, in T3 and T4, the stocking density was higher than in T1 and T2 (approximately 12 birds/m^2 versus 6 birds/m^2). In the current study the birds housed at higher densities were more active. By contrast, as discussed before, the opposite has been reported in literature (Lewis and Hurnik, 1990). In the current study, however, the stocking densities were relatively low compared to those reported in literature. The densities reported in the study by Lewis and Hurnik (1990) correspond to ~7.6–15.2 birds per square meter. Blokhuis and van der Haar (1990) studied densities between 2 and 20 birds, and found a decrease in percentage of birds walking with increasing density in week seven of life. In our study, the number of birds per square meter varied between approximately 6 and 12. Therefore, the high density in the current study might actually be considered as moderate in comparison to literature and commercial situations. As a result, the restrictive element of a higher stocking density may not have applied here. Moreover, the higher stocking density in the current study may have resulted in increased activity, as a consequence of more disturbance by other birds. It has been noted that when broilers are housed at low densities, they preferentially remain close to feeders and drinkers, even though there is sufficient space available to move elsewhere (Arnould and Faure, 2004). However, when there is competition for space at the feeder and drinker, which might result at higher local densities, birds resting near the feeders and drinkers may be displaced, which may lead to higher distances moved. This might explain the higher activity found in T3 and T4 compared to T1 and T2. However, due to the differences between trials in the crosses studied, this cannot be conclusively determined with the current setup and requires more detailed investigation. Overall, however, it is evident that it is important to use a consistent study design when different trials are compared.

2.4.3 Activity as a predictor

In this study, individual data on activity of broilers was used to study group-level patterns for validation of the UWB system. However, in future work it would be interesting to study individual activity patterns in more detail, to determine the predictive value of activity for growth or gait problems, for example. Furthermore, data on the first two weeks of life would be interesting to add, to allow assessment of whether distances moved throughout the production period can be predicted using data from the first few days of life. A study by Bizeray et al. (2000) has shown correlations between activity early in life and at later ages. They found a positive correlation between activity at two to three days of age and at three weeks of age in fast-growing broilers. Such insights can be valuable for broiler breeding programmes. However, with the UWB tracking system used in this study, birds cannot be tracked in the first two weeks of life, due to the UWB tags being too large and heavy to wear at a young age. Possibly other tracking systems, such as passive RFID, have the potential to track activity of birds throughout life, but this requires further investigation.

2.5 Conclusions

In this study, it was shown that the implemented UWB system is suitable for tracking activity of individual broilers. There was a moderately strong positive correlation between the output of the UWB system and video tracking. Furthermore, the UWB system could detect reductions in activity over time and could detect that lightweight birds, as determined at about two weeks of life, are on average more active than heavier birds. However, it is important to keep all settings the same when comparing different birds and trials. The longitudinal information on differences in activity can potentially be used as a proxy for health, welfare and performance, but further research into individual patterns of activity is required.

2.6 Acknowledgements

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Chapter 3:
The relationship between gait and automated recordings of individual broiler activity levels

Malou van der Sluis[1,2], Esther D. Ellen[1], Britt de Klerk[3], T. Bas Rodenburg[2,4] and Yvette de Haas[1]

[1] Animal Breeding and Genomics, Wageningen University & Research, 6700 AH Wageningen, The Netherlands;
[2] Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, 3508 TD Utrecht, The Netherlands;
[3] Cobb Europe, 5831 GH Boxmeer, The Netherlands;
[4] Adaptation Physiology Group, Wageningen University & Research, 6700 AH Wageningen, The Netherlands

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Abstract

Gait, or walking ability, is an often-measured trait in broilers. Individual gait scores are generally determined manually, which can be time-consuming and subjective. Automated methods of scoring gait are available, but are often implemented at the group level. However, there is an interest in automated methods of scoring gait at the individual level. We hypothesized that locomotor activity could serve as a proxy for gait of individual broilers. Locomotor activity of 137 group-housed broilers from four crosses was recorded from approximately 16 to 32 days old, using an ultra-wideband tracking system. These birds were divided over four trials. Individual gait scores were determined at the end of the tracking period, on a scale from 0 to 5, with higher scores representing worse gait. Given the limited number of birds, birds were subsequently categorized as having a good gait (**GG**; scores 0–2) or a suboptimal gait (**SG**; scores 3–5). Relationships between activity and gait classification were studied to determine whether individual activity has the potential to serve as a proxy for gait. When comparing GG and SG birds using robust linear regression, SG birds showed a lower 1) activity around the start of tracking (estimate = -1.33 ± 0.56, $P = 0.019$), 2) activity near the end of tracking (estimate = -1.63 ± 0.38, $P < 0.001$), and 3) average activity (estimate = -1.12 ± 0.41, $P = 0.007$). When taking day of tracking, trial, cross and body weight category (heavy versus light at approximately 2 wk old) into account, a tendency was still observed for SG birds having lower activity levels within lightweight birds, but not within heavyweight birds. This study provides indications for activity differences between gait classifications. However, given that there was considerable overlap in activity levels between the gait classifications, future research implementing additional activity-related variables is required to allow a more complete distinction between birds with different gait classifications.

3.1 Introduction

Broiler chickens are often kept in large groups, of several thousands of birds (de Jong et al., 2015). With these large numbers of animals, it can be very complex to observe individual behavior, health, and welfare states. Therefore, there is an increasing interest in easy-to-measure traits that are related to health or welfare states, or to specific behaviors of individual broilers.

An often-measured trait in broilers is their walking ability, or gait, in order to examine leg weakness. Leg weakness is a general term to describe multiple pathological states resulting in impaired walking ability in broilers (Butterworth, 1999). The gait of birds is often classified according to a scoring system developed by Kestin et al. (1992), consisting of 6 categories, ranging from a score of zero that represents a normal gait with no detectable abnormalities to a score of five that represents birds that are incapable of sustained walking on their feet. Side effects of genetic selection, growth rate, body conformation, exercise, stocking density, and other factors have been suggested to be involved in causing leg weakness (reviewed in Bradshaw et al., 2002). Leg weakness has had a considerable prevalence in the conventional broiler industry. In a UK survey by Knowles et al. (2008) it was reported that 27.6% of the birds represented in the survey had a gait score of 3 (i.e., obvious gait defect which affects the ability to move about (Kestin et al., 1992)) or higher at an average age of 40 d, although there was considerable variation between flocks. Leg weakness may negatively affect the birds' welfare, as there are indications that leg weakness might be painful for the affected birds (Danbury et al., 2000) and, in severe cases, birds may have difficulties competing with others for resources and may be limited in performing specific behaviors (Kestin et al., 1992), such as dustbathing or preening while standing (Vestergaard and Sanotra, 1999; Weeks et al., 2000). Furthermore, lameness can have economic consequences for farmers. For example, in some studies, associations between gait score and footpad dermatitis have been observed in broilers, for example, with high odds of footpad dermatitis becoming more severe as the gait score increases, that is, with a worse gait (Opengart et al., 2018). In the Netherlands, if a threshold of a percentage of birds showing footpad dermatitis is crossed, farmers have to temporarily reduce their stocking densities (Afsprakenkader Implementatie Vleeskuikenrichtlijn, 2009), hereby affecting the farm's economics. It has been suggested that increased locomotor activity can contribute to a lower prevalence of leg weakness (Reiter and Bessei, 2009). Furthermore, different leg health traits have been shown to be heritable (Kapell et al., 2012). For example, tibial dyschondroplasia has been estimated to have a heritability of 0.10 to 0.27 (Kapell et al., 2012). Therefore, information on gait at the level of the individual can be of great value for breeding programs.

Currently, gait scores of individual birds are often determined manually and require an experienced scorer to observe individual birds and grade their walking ability, for example according to the earlier-mentioned 6-scale scoring system from Kestin et al. (1992). However, this manual scoring can be time-consuming and subjective. Therefore, automated ways of estimating, or even predicting, gait scores are desired. Several studies have tested automated ways of scoring gait or expected correlated traits, for example using image technology (e.g., Aydin, 2017; Aydin et al., 2010; Dawkins et al., 2012; Nääs et al., 2018; Silvera et al., 2017; Van Hertem et al., 2018a) or inertial measurement units (**IMUs**; e.g., in turkeys; Bouwman et al., 2020). However, the main focus appears to have been on measurements at a group-level. For example, Aydin et al. (2010) implemented an automatic image monitoring system to study activity levels of small groups of birds, clustered based on their manually determined gait score, and observed a relationship between the activity level and the manually determined gait score. They observed that broilers with gait scores 4 and 5 showed the lowest activity levels, although they note that more experiments are needed to assess the repeatability of these findings. Van Hertem et al. (2018a) implemented a camera-based automatic animal behavior monitoring tool, to assess, among other things, bird activity levels of flocks, and observed a negative correlation between gait score and flock activity. On the other hand, some automated measurements of individual locomotion have been performed, for example using IMUs (Bouwman et al., 2020). However, although steps could be detected in turkeys with this approach, the relationship with gait score was not studied (Bouwman et al., 2020). Another approach was implemented by Aydin (2017), who manually placed single birds in a test setup with a 3D vision camera system to record the number of lying events and the latency to lie down. Although this has potential to make gait scoring more objective, it was only tested on single birds and in the current setup likely remains a time-consuming and labor-intensive method, as it still requires handling of individual birds for each observation. Therefore, there is a need for a proxy trait that can be used as an indicator for gait score that can be recorded on multiple birds while they are housed in their normal environment. The relationship between gait and the level of locomotor activity of broilers that was reported in some studies (e.g., Aydin et al., 2010; Van Hertem et al., 2018a) indicates that the level of locomotor activity at group- or flock-level is correlated with gait and may even have potential as a proxy for gait scores. However, to study the relationship between gait and activity of broilers at the individual level in more detail, individual recordings of gait score and activity are required.

Previous work has shown that the measurement of activity, recorded as distances moved, in broilers can be automated at the individual level (van der Sluis et al., 2019). By tracking activity of individual birds automatically, one can potentially obtain insight into the relationship between activity and gait score of individual birds while they are in a more normal, group-housed situation. If a strong relationship between activity and gait score at the individual level would be observed, activity could potentially be used as a proxy for gait, thereby making scoring of individual birds' gait more time-efficient and objective. Furthermore, information on activity levels might at the same time be informative for other reasons. For example, activity levels could serve as an indicator of illness, as ill animals often spend more time resting (Gregory, 1998). This renders the collection of activity data at the individual level a potentially fruitful investment.

In this research, data on activity levels, recorded as distances moved, of individual broilers were collected using an ultra-wideband (**UWB**) tracking system and were studied to determine the relationship between individual locomotor activity and gait. Different aspects of individual activity were studied in relation to gait: 1) the activity level at different time points, 2) the overall average activity level, and 3) the slope of activity over time. Furthermore, it was studied whether gait and activity over time were related while accounting for other potentially influential factors, including for example genetic background and body weight of the birds.

3.2 Materials and methods

3.2.1 Ethical statement

Data were collected under control of Cobb Europe. Cobb Europe complies with the Dutch law on animal well-being. This study is not considered to be an animal experiment under the Law on Animal Experiments, as confirmed by the local Animal Welfare Body (June 20, 2018, Lelystad, the Netherlands).

3.2.2 Location and housing

All data were collected on a broiler farm in the Netherlands. The broilers were group-housed, with feed and water provided ad libitum and wood shavings as bedding. No perches or other additional enrichments were provided. Commercial lighting and temperature schedules were used, and all birds were vaccinated according to common practice (Cobb, 2018).

3.2.3 Ultra-wideband tracking system

A Ubisense UWB system with Series 7000 sensors and compact tags (Ubisense Limited, Cambridge, UK) was used, in combination with TrackLab software (version 1.4, Noldus Information Technology, Wageningen, the Netherlands), to collect data on activity of broilers. The system is described in more detail in van der Sluis et al. (2019). All broilers were fitted with battery-powered UWB tags on their backs, with a size of approximately 3.8 by 3.9 cm and a weight of approximately 25 g, using elastic bands around their wing base. This system recorded the locations of the birds over time, with a frequency of one sample per bird approximately every 6.9 s, and the resulting calculated distances moved of the broilers were used as a measure of individual activity.

3.2.4 Activity data collection

Four consecutive UWB tracking trials (T1–T4) were performed, that is using four production rounds, and activity data were collected on a total of 150 commercial male broiler chickens from 4 different crosses. Not all crosses were present in each trial, as each trial included birds from only 2 crosses, and not all crosses were equally represented in the study (**Table 3.1**). At approximately 2-wk-old, the focal birds were selected from a larger group, based on their body weight. This was done to obtain approximately equal samples of lightweight and heavyweight birds within the respective cross and trial. The birds were tracked in a pen with a size of approximately 6 m^2 in T1, T2, and T4, and in a pen with a size of approximately 8 m^2 in T3. In all trials,

Table 3.1. Overview of the weights of the birds in the respective weight categories for the different trials.

T	SW day	EW day	Weight category	Included crosses	SW (g)	EW (g)	Average increase per day (g)
T1	13	34	L (n = 16)	C1 (n = 7); C2 (n = 9)	420 (SD 21)	2435 (SD 165)	95 (SD 7)
			H (n = 16)	C1 (n = 8); C2 (n = 8)	520 (SD 14)	2635 (SD 233)	100 (SD 11)
T2	14	33	L (n = 18)	C2 (n = 9); C3 (n = 9)	485 (SD 29)	2450 (SD 141)	105 (SD 7)
			H (n = 17)	C2 (n = 9); C3 (n = 8)	595 (SD 24)	2680 (SD 181)	110 (SD 10)
T3	14	35	L (n = 15)	C3 (n = 8); C4 (n = 7)	480 (SD 45)	2500 (SD 205)	95 (SD 8)
			H (n = 20)	C3 (n = 10); C4 (n = 10)	630 (SD 25)	2715 (SD 307)	100 (SD 15)
T4	13	35	L (n = 17)	C3 (n = 8); C4 (n = 9)	340 (SD 68)	2155 (SD 313)	85 (SD 12)
			H (n = 18)	C3 (n = 9); C4 (n = 9)	460 (SD 22)	2520 (SD 132)	95 (SD 6)

Abbreviations: C1, cross 1; C2, cross 2; C3, cross 3; C4, cross 4; EW, end weight; H, heavyweight; L, lightweight; SW, start weight; T, trial. Weights of individual birds were determined with 5-gram precision and reported averages are rounded to five-grams. SDs are rounded to whole numbers.

the pen was divided into 2 equal-sized compartments, each housing a single cross. Additional birds from the same line without UWB tags were added before the tracking started in T3 and T4 to increase the housing density to approximately 12 birds/m^2, compared to a density of approximately 6 birds/m^2 in T1 and T2. UWB recordings were made from 00:00 to 23:30 each day and the data from 16 to 32 days old (n = 17 d) were used in this study. For T4 there were no data available before 18 d old (n = 15 days of data included for this trial) and in T3 there was a technical issue resulting in no data for 26 and 27 days old (n = 15 days of data included for this trial). Due to too much missing data (see van der Sluis et al. (2019) for details on the data filtering), death of birds and mistakes in sexing, a total sample size of 137 birds was available for analysis. **Table 3.1** shows the weights of the birds in the different weight categories and trials. For these 137 birds, the average distance moved in meters per hour was calculated per day and animal, and was used as the measure of locomotor activity.

3.2.5 Gait scoring

For the gait scores, the data on gait that are routinely collected on this farm were used. Individual gait was determined at 33, 34 or 35 days old, depending on the trial. The gait was determined at 34 days old in T1, at 33 days old in T2, and at 35 days old in T3 and T4. The birds were observed while walking and given a gait score by an experienced human observer. For the different trials, this was not always the same observer, as two observers scored gait during this study. However, scoring within a trial was performed by a single observer. No data on inter-observer reliability was available, but both observers were trained in the same manner, that is, by scoring gait together with an experienced observer until sufficient experience and confidence were developed to start scoring individually. The gait scoring was performed in the pen, but was combined with individual weighing of the birds. Therefore, all birds were handled immediately before gait was scored. Upon placing the birds back in the pen after weighing, their gait was assessed. It must be noted that, as the birds were handled immediately before their gait was scored, stress from the handling may have impacted their gait. However, given that all birds were handled, this potential influence on gait is assumed to be similar for all birds. For the gait scoring, the scoring system shown in **Table 3.2** was used, which is the commonly used system at the farm where the study was

Table 3.2. Gait scoring system used to determine gait scores in this study.

Score	Description	Criteria
0	Walks very well	
1	Walks good / supple	• Controlled • Stands straight on legs
2	Walks relatively well	• Oriented
3	Walks mediocre	• More out of balance • Sits down quickly • Can translocate well but sits down quickly
4	Walks poorly	• Walks with bent legs and waddles • Walks with spread legs • Legs outwards • Wings often hang down
5	Barely walks	• Can only move by also using wings

conducted. Although this scoring system is not exactly the same as the commonly implemented scoring system from Kestin et al. (1992), the overall idea is similar and for comparing purposes the gait score categories from both scoring systems are assumed to represent similar gaits. The distribution of gait scores is shown in **Table 3.3**, where it is also indicated into which weight category the birds were categorized. Given the small sample sizes for some of the gait score categories, a further classification into a 'good gait' (**GG**) vs. a 'suboptimal gait' (**SG**) was made that was used in the subsequent analyses. The gait score categories 0 to 2 were classified as GG, whereas 3 and higher were classified as SG. This cut-off value was based on the general assumption that with gait score 3 and higher the welfare of the birds is potentially impaired (Kestin et al., 1992). As can be seen from **Table 3.3**, this resulted in 79 GG birds and 58 SG birds.

3.2.6 Statistics

For all statistics, R version 4.0.2 was used (R Core Team, 2020). The hourly average activity data were not normally distributed and untransformed data were used for the analyses. The slope of individual activity was calculated by means of linear regression, using the hourly average activity per day over the trial per individual. Linear regression models with sum-to-zero contrasts were

Table 3.3. Gait score distribution, shown for the two weight categories (see Table 3.1).

Gait score / classification	Lightweight	Heavyweight	Total
0	0	0	0
1	9	3	12
2	37	30	67
3	11	24	35
4	8	8	16
5	1	6	7
Good gait (gait scores 0-2)	46	33	79
Suboptimal gait (gait scores 3-5)	20	38	58

implemented to study the relationship between gait classification as GG or SG and the following activity measures:

1) Activity at 18 to 20 days old, representing early activity with all trials having data available; average activity over the three days per animal and only including individuals with all three days available (i.e., no days with too many missing samples for an animal, threshold was set at 90% of samples present within each tracking session, see van der Sluis et al. (2019) for details on data filtering), n = 131.
2) Activity at 30 to 32 days old, representing late activity; average activity over the 3 d per animal and only including individuals with all 3 d available, n = 134.
3) Overall average activity level; only including individuals with all days of the respective trial available, n = 120.
4) The slope of activity over time; all animals included regardless of some missing data, n = 137.

Here, each of the activity measures was separately modeled as a linear function of the gait classification only. This was done to gain insight into whether gait classification alone can be linked to activity levels, regardless of differences in genetic background of the birds, their body weight, or the trial in which the birds were recorded. Given that there appeared to be some outliers in the data, robust linear regression models from the robustbase package (Maechler et al., 2020) were used, which are less sensitive to outliers than common linear regression models. To study how gait classification was related to activity levels while accounting for other potentially influential factors, a linear mixed-effects model with sum-to-zero contrasts was implemented, using the lme4

(Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages. For this analysis, a total of 2,160 observations for 137 animals were used. The fixed effects tested were day of tracking, trial, cross, gait classification, start weight category and weight change. The distribution of crosses and start weight categories across trials is indicated in **Table 3.1**. Correlated random intercepts and slopes for individual animals were included in the model as random effects. To test the fixed effects, a backward stepwise approach without interactions was used that included all these effects. The resulting terms that were left were all included in 2-way interactions, except for the interaction between cross and trial, as not all crosses were present in multiple trials. Backward selection was then again performed, and both significant effects ($P < 0.05$) and effects showing a tendency ($P < 0.1$) were kept in the model. The resulting final model was

$$Y_{ijklmn} = \mu + \beta(DT)_i + C_j + T_k + SW_l + GSC_m + (\beta(DT) \text{ x } C)_{ij} + (\beta(DT) \text{ x } T)_{ik} + (\beta(DT) \text{ x } SW)_{il} + (SW \text{ x } GSC)_{lm} + (1 + \beta(DT)_i|ID_n) + e_{ijklmn}$$

where Y is the average distance moved per hour, μ is the overall mean, $\beta(DT)_i$ is the ith day of tracking (i = 1 to 17), C_j is the jth cross (j = 1 to 4), T_k is the kth trial (k = 1 to 4), SW_l is the lth start weight category (l = light or heavy), GSC_m is the classification of gait (m = GG or SG), $(\beta(DT) \text{ x } C)_{ij}$ is the interaction between day of tracking and cross, $(\beta(DT) \text{ x } T)_{ik}$ is the interaction between day of tracking and trial, $(\beta(DT) \text{ x } SW)_{il}$ is the interaction between day of tracking and start weight category, $(SW \text{ x } GSC)_{lm}$ is the interaction between start weight category and the classification of gait, $(1 + \beta(DT)_i|ID_n)$ is the random effect of the nth animal's intercept and correlated slope, and e_{ijklmn} is the residual term. Given that the 2 crosses within a trial were housed in 2 separate compartments in the tracking pen, there was a possible influence of side of the pen. However, including side of the pen as a fixed effect did not lead to different conclusions regarding the relationship between activity and gait and side of the pen was therefore not included as a fixed effect. No obvious deviations from normality or homoscedasticity were observed upon visual inspection of the residuals of the model. Reported *P*-values for the model estimates were obtained using the lmerTest package (Kuznetsova et al., 2017). The MuMIn package (Barton, 2020) was used to determine the R^2 values for the model. The ggplot2 (Wickham, 2016) and sjPlot (Ludecke, 2020) packages were used to make the visualizations. The level of statistical significance was set at 0.05 and results that are reported in the text are rounded to two decimals.

3.3 Results

3.3.1 Relationship between gait classification and activity

The start activity, as measured at 18 to 20 days old, differed between GG and SG birds, with a higher activity level for GG birds (estimate = 1.33 ± 0.56, *P* = 0.019; **Table 3.4**; **Figure 3.1A**). This means that on average, GG birds moved 1.33 meters per hour more than the overall average distance recorded at 18 to 20 days old in the study, and thus 2.66 m more than SG birds. The end activity, as measured at 30 to 32 days old, also differed between GG and SG birds, again with a higher activity for GG birds (estimate = 1.63 ± 0.38, *P* < 0.001; **Table 3.4**; **Figure 3.1A**). The average activity was also higher for GG birds (estimate = 1.12 ± 0.41, *P* = 0.007; **Table 3.4**; **Figure 3.1A**). No relationship between slope of activity and gait classification was observed (**Table 3.4**; **Figure 3.1B**).

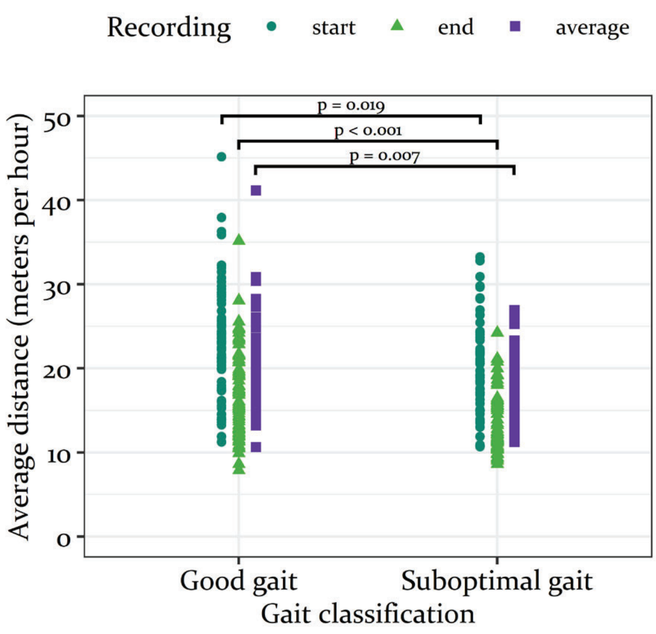
Table 3.4. Results of the robust linear regression models for the relationship between gait classification and 1) start activity, 2) end activity, 3) average activity, and 4) slope of activity.

Coefficients	Estimate	SE	t-value	Pr(>|t|)
Start activity (Adjusted R2 = 0.037)				
Intercept	21.094	0.576	36.650	<0.001
Gait classification: GG	1.331	0.562	2.369	0.019
End activity (Adjusted R2 = 0.118)				
Intercept	14.888	0.414	35.949	<0.001
Gait classification: GG	1.626	0.378	4.302	<0.001
Average activity (Adjusted R2 = 0.056)				
Intercept	18.000	0.437	41.157	<0.001
Gait classification: GG	1.121	0.412	2.722	0.007
Slope of activity (Adjusted R2 = -0.007)				
Intercept	-0.528	0.032	-16.398	<0.001
Gait classification: GG	0.010	0.032	0.305	0.761

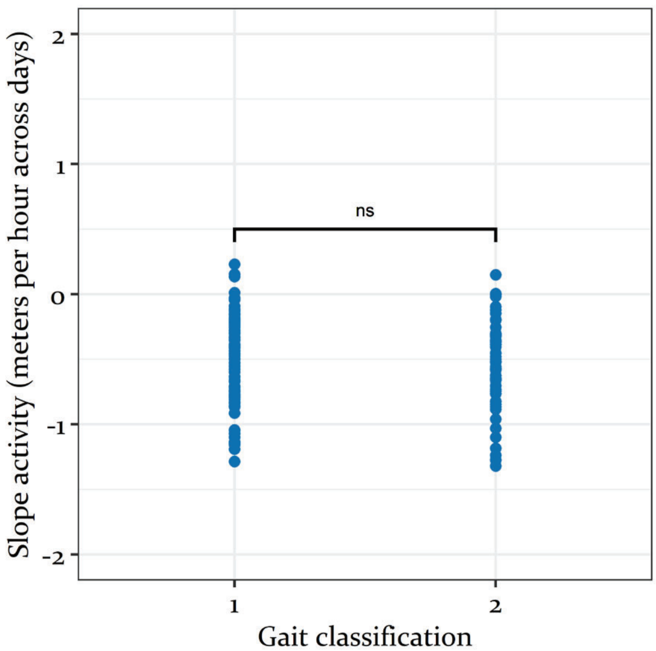
Abbreviation: GG, good gait.

3.3.2 Relationship between gait classification and activity in the presence of other influential factors

To study the effect of GG versus SG on activity levels while taking other possibly influential factors into account, a linear mixed-effects model was implemented (**Table 3.5**). This model explained 57.80% of the variance when only fixed effects were included and explained 85.58% of the variance when



(A)



(B)

Figure 3.1. Average distance moved in meters per hour or slope of activity over time for individuals from the different gait classifications. Dots, triangles and squares represent individual data points. (A) start activity as measured at 18 to 20 days old, end activity as measured at 30 to 32 days old, and average activity over the trial; (B) slope of activity over time.

random effects were included as well. The model showed a tendency for an interaction between gait classification and weight category (**Table 3.5**). Within lightweight birds, SG birds appeared to have a lower level of activity than GG birds (**Figure 3.2**). This difference between SG and GG birds was not observed within the heavyweight category (**Figure 3.2**). Furthermore, a decrease in activity over time was observed, as well as an effect of trial. The degree of the decrease in activity over time differed between trials, crosses, and weight categories.

Table 3.5. Results of the linear mixed-effects model for the predicted average activity (meters moved per hour).

Linear mixed-effects model					
Fixed effects					
Factor	**F-value**	**Pr(>F)**	**Estimate**	**SE**	**Pr(>|t|)**
Intercept			24.085	0.495	<2e-16
Day	337.834	<2.2e-16	-0.532	0.029	<2e-16
Trial	27.012	9.66e-14			
- Trial 1			-3.736	1.343	0.006
- Trial 2			-6.218	0.779	6.30e-13
- Trial 3			6.680	0.956	1.21e-10
Cross	2.059	0.109			
- Cross A			3.256	1.465	0.028
- Cross B			0.090	0.913	0.922
- Cross C			-0.907	0.914	0.323
Weight Category	11.329	9.86e-04			
- Light			1.353	0.402	9.86e-04
Gait	5.025	0.027			
- Good Gait			0.637	0.284	0.027
Day x Trial	19.091	2.18e-10			
- Day x Trial 1			-0.179	0.080	0.026
- Day x Trial 2			0.187	0.046	9.56e-05
- Day x Trial 3			-0.200	0.057	5.46e-04
Day x Cross	3.127	0.028			
- Day x Cross A			0.083	0.087	0.342
- Day x Cross B			0.060	0.054	0.274
- Day x Cross C			0.029	0.054	0.588
Day x Weight Category	6.800	0.010			
- Light			-0.061	0.024	0.010
Weight Category x Gait	3.047	0.083			
- Light x Good Gait			0.484	0.277	0.083
Random effects					
Factor	**Variance**	**SD**	**Correlation**		
ID intercept	19.245	4.387			
ID by Day	0.059	0.244	-0.75		
Residual	5.707	2.389			

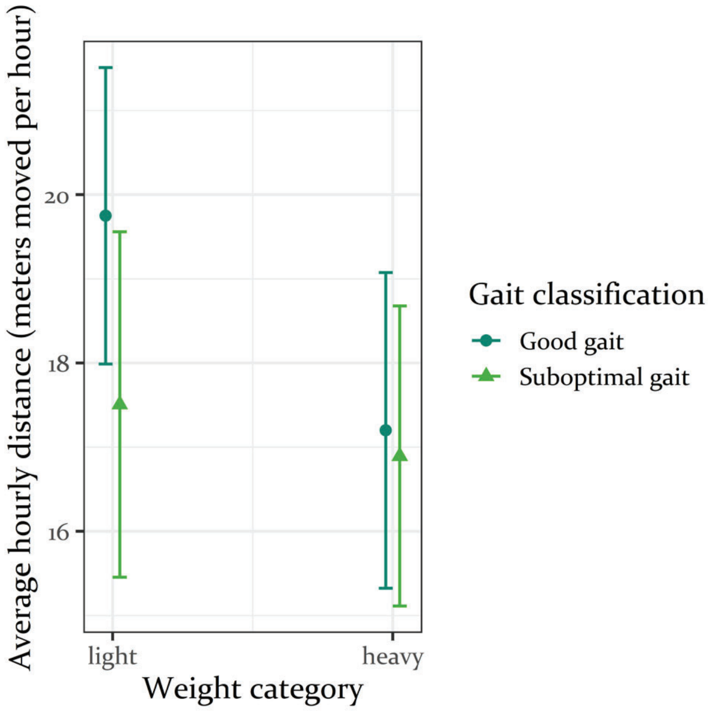


Figure 3.2. Linear mixed-effects model estimated average hourly distance for good gait and suboptimal gait birds, in interaction with weight category. Bars represent 95% CIs.

3.4 Discussion

In this research, it was studied whether individual levels of activity were related to gait. To this end, the relationships between the individuals' gait classification and different measures of activity levels were analyzed. Indications for relationships between gait classification and different measures of activity were observed, but gait explained little of the variation in these activity measurements, as R^2 ranged from 0.04 to 0.12. When taking other possibly influential factors, like day, trial, cross and weight category, into account, a larger part of the variance in activity was explained and a tendency for an interaction between gait classification and weight category was observed. In this interaction, a difference in level of activity was observed between GG and SG in lightweight birds, but not in heavyweight birds.

3.4.1 Relationship between gait classification and activity

In this research, a difference between GG and SG birds was observed for several activity measurements. The relationships between gait classification and start activity (18–20 days old), end activity (30–32 days old) and average activity, respectively, indicated that birds with a suboptimal gait showed a lower locomotor activity compared to birds with a good gait. This decrease in activity levels for SG birds, that is, birds with gait score 3 or higher, matches reports in literature in which lower activity levels for birds with higher gait scores were reported (e.g., Van Hertem et al., 2018a; Weeks et al., 2000). This often-reported negative relationship between activity level and gait score can have different underlying causes that are difficult to separate from each other. First, it could be the case that the gait itself resulted in the birds limiting their locomotor activity. Several studies have indicated that gait problems might be painful for birds (e.g., Danbury et al., 2000; McGeown et al., 1999) and therefore lame birds might reduce their locomotor activity. On the other hand, it has been suggested that increased locomotor activity may contribute to preventing the development of gait problems (e.g,. Reiter and Bessei, 1998; Reiter and Bessei, 2009). For example, Reiter and Bessei (2009) compared 2 distances between feeders and drinkers, that is, 2 and 12 meters, in broilers. It was observed that the groups of birds with the larger distance had fewer cases of leg weakness and that the locomotor activity in this treatment was higher. Vasdal et al. (2019) performed a pilot study on broiler activity in enriched environments, including peat, bales of lucerne hay, and elevated platforms, and control environments. They observed that the birds in an enriched environment showed higher levels of several activities, for example, ground scratching and ground pecking while standing, and a tendency for a lower gait score than birds in a control environment. Kaukonen et al. (2017) also studied environmental enrichment, as a way to increase activity and thereby improve gait in broilers. They implemented perches and elevated platforms and observed, among other things, lower mean gait scores for the birds in the platform-equipped houses. It was hypothesized that the platform access increased walking to reach the platforms and enabled more versatile movement, which could have positively impacted gait (Kaukonen et al., 2017). Adding elevated platforms or perches, or potentially other types of environmental enrichment, seems a practical approach to improve activity and gait. However, it must be noted that several other studies did not observe a positive effect of perches or platforms on gait (e.g., Bailie and O'Connell, 2015, Baxter et al., 2020). Altogether, birds with higher activity levels might be less prone to gait problems. Moreover, increased activity early in the growing period has been suggested to reduce leg disorders (reviewed in Bradshaw et al., 2002). Unfortunately, in the current study it was not possible to study activity early in life, due to the weight of the tags being a limitation for smaller birds. For future work it would be interesting to look into activity in the first few days of life as well, to gain more insight into the causal relationship between activity level and gait score.

No association between the slope of activity over time and gait classification was observed, suggesting that the difference in activity level between the gait classifications remained relatively constant over time, that is, from 16 to 32 days old. In other words, based on the current data, the activity does not appear to decrease faster over time for SG birds compared to GG birds. It is important to note, however, that the slope values were approached using linear regression, which may have masked some of the nuances in the patterns over time. In a study by Weeks et al. (2000), birds with gaits ranging from gait score 0 to 3 were observed on 6 d between 39 and 49 days old. Exactly which 6 d these were, was not specified further. Although not discussed in detail in their study, when comparing gait score 0 and 1 birds to gait score 2 and 3 birds, it appeared that on the first observation day, the absolute difference in percentage of time spent allocated to walking was smaller compared to d 2 to 5 of observation. However, on d 6 of observation, the absolute difference again appeared to be relatively small. During these 6 recording days, the gait score 2 and 3 birds initially showed a steep decline in the percentage of time that was allocated to walking, but seemed to stabilize over the remainder of the observation period. The gait score 0 and 1 birds showed a more constant decline over these 6 recording days. This suggests that there might be a difference in the activity pattern over time, at least in the period ranging from 39 to 49 days old, which was outside the range of our study period. More research is required to clarify this relationship, preferably over the full life span of broilers and with gait recordings at different time points.

3.4.2 Relationship between gait classification and activity in the presence of other influential factors

In the abovementioned discussion of the relationship between gait classification and activity, other possibly influential factors were not accounted for. Research has indicated that there are relationships between activity and age of the birds (Weeks et al., 2000), weight of the birds (Tickle et al., 2018) and possibly genetics of the birds (Bizeray et al., 2000), respectively. Therefore, in the analysis implementing a linear mixed-effects model, other factors besides gait classification were taken into account. These included time (i.e., age), trial, cross and weight category effects, as well as the interactions between them. Only the main findings related to gait will be discussed here. Results for the other factors have been reported earlier (van der Sluis et al., 2019). Overall, taking the other factors into account still resulted in a tendency for an effect of gait classification being observed, in interaction with weight category. A difference in activity between GG and SG birds appeared to not be present in heavyweight birds, only in lightweight birds (**Figure 3.2**). Earlier studies have indicated that birds with higher body weights often walk shorter distances compared to lighter birds, for example in an operant runway test (Bokkers et al., 2007). Also voluntarily, that is, when not necessarily walking for a reward, lightweight birds have been observed to walk longer distances. This was studied for example using weight load reduction, where the weight load on birds' legs was reduced by partially lifting the birds' weight using a suspension device (Rutten et al., 2002). A possible explanation for this finding is that as body weight increases, the energetic cost of standing becomes larger than for sitting (Tickle et al., 2018). If heavy birds already limit their activity to the minimally required level to obtain sufficient water and feed, it could be that a suboptimal gait does not decrease the activity level further. Lightweight birds, however, might show activity levels that are higher than required solely for obtaining water and feed. If lightweight birds show a suboptimal gait, this may reduce their activity levels to the level required for solely obtaining water and feed, resulting in an overall decrease in activity. The effect of gait on feeder visits was studied by Weeks et al. (2000). They compared gait score 0 to gait score 3 birds, and observed that gait score 3 broilers visited the feeder less often per day, but increased the visit duration accordingly, resulting in an overall time spent feeding that was similar to that of gait score 0 birds. However, by reducing the number of feeder visits, the distance walked would decrease as well, which could explain the finding in the current study that lightweight birds with a suboptimal gait showed lower distances moved compared to lightweight birds with a good gait.

3.4.3 Gait score and consequences for welfare

In this research, gait scores of birds were assigned using a 6-scale scoring system. However, given the relatively small sample size, the different gait score categories were later on combined into GG (gait scores 0 to 2) and SG (gait scores 3 to 5) classes for analysis. In this classification of GG versus SG, the cut-off point was positioned between gait scores 2 and 3. This was based on the general assumption that the welfare of birds is potentially impaired at gait score 3 and higher (Kestin et al., 1992). However, it is debatable whether this indeed is a very clear cut-off point. Skinner-Noble and Teeter (2009) compared gait score 2 and gait score 3 birds, and observed among other things that gait score 3 birds stood less and rested more, compared to gait score 2 birds. However, they also studied for example heterophil:lymphocyte ratios as a measure of long-term physiological stress and observed no difference between the 2 groups. Overall, they conclude that there are no indications in their study that the 2 gait score groups differ in their welfare (Skinner- Noble and Teeter, 2009). These findings make it difficult to state where a potential cut-off value may truly lie in terms of welfare. Therefore, if additional research indicates a different cut-off value, it would be advisable for future research to study the relationship between activity levels and the classification GG versus SG based on this new cut-off value.

Furthermore, the different gait scores that comprise each gait classification may differ from each other. For example, gait score 0 is generally described as "[..] walked normally with no detectable abnormality; it was dexterous and agile. [..]", whereas gait score 2 is generally described as "[..] had a definite and identifiable defect in its gait but the lesion did not hinder it from moving or competing for resources [..]" (Kestin et al., 1992). These 2 gait scores are both classified as GG in this study, but the birds' behavior and well-being may differ as a consequence of their gait. In our research, it was not possible to study differences between the six gait score categories, due to the limited sample size, but future research with sufficient data on animals from all gait score categories could look into whether it is possible to distinguish each of the 6 gait scores individually, based on activity recordings. This would allow us to assess individual birds' gait and well-being at a more detailed level.

3.4.4 Predicting individual broiler gait using activity levels

In this research, we studied the relationship between individual activity and gait classification. Insight into this relationship could, for example, aid in assessing gait of individual birds based on their individual level of activity, which can be recorded in an automated manner. One example of a benefit of this approach for assessing gait is that the possibly confounding effect of stress induced by handling birds, to assess their gait, could be removed. Individual data on broilers' gait could be informative for many purposes, including for broiler management and for research into the development of gait problems. Furthermore, it has been suggested that some gait problems can be alleviated by selective breeding (reviewed in Bradshaw et al., 2002), which requires data on individual broilers' gait. It has been reported that out of 3 major broiler breeding companies, at least one implements walking ability, that is, gait, as a trait subject to genetic selection and all select for leg strength (Hiemstra and ten Napel, 2013). A fast way of obtaining gait scores would therefore be beneficial. Moreover, given that it is not feasible for breeding companies to have a single-observer score for all birds, automated gait scoring using activity levels could aid in making gait scoring more objective. However, this study shows that it is difficult to predict the gait score of individual broilers based solely on the here-present activity information, as individual broilers within a gait classification were observed to show quite different activity levels. Furthermore, the observed activity levels within one gait classification showed quite a large overlap with that of the other gait classification, making it difficult to distinguish between gait classifications, and in these models the proportion of the variance in activity that was explained by gait classification was very small. When taking other influential factors into account, a tendency for an interaction between gait classification and weight category was observed. This interaction suggests that activity recordings have the potential to aid in predicting gait of individual birds, when taking other influences on activity levels into account, but that this is only feasible for lightweight birds, as heavyweight birds might already have relatively low activity levels. Overall, it remains difficult to distinguish individual birds' gait based on distances moved during the period from 16 to 32 days old. Future research could focus on a longer period of time, preferably throughout the entire production period with manual gait recordings periodically implemented, to further study the development of gait problems and the relationship with (early life) activity.

Furthermore, additional variables could be studied that are potentially related to gait problems, including, for example, feeder visits (based on findings in Weeks et al., 2000), acceleration and speed of movement (Kestin et al., 1992) and use of the available area. With these additions, automated scoring of individual gait may be feasible, but this remains to be investigated.

In the current setup, the birds were housed in a small pen compared to common broiler housing systems. This potentially resulted in relatively low recorded distances, as activity levels can, for example, be influenced by the distance between feed and water (Reiter and Bessei, 2009), which is likely to be larger in common broiler housing systems. However, in the current study, the focus was on relative activity levels and the differences in activity between GG and SG birds. Therefore, the exact distances moved were not directly of interest. However, Baxter and O'Connell (2020) implemented an UWB system for broiler tracking under commercial conditions and concluded that this was an accurate method for tracking indoor locations of broilers and that, even though absolute distances were generally overestimated, the system can be used to study differences between groups. This suggests that the approach implemented in the current study also has potential for recording activity in larger areas.

3.5 Conclusions

In this research, it was studied whether individual levels of activity were related to gait of broilers. Indications for relationships between gait classification and different measures of activity were observed, with lower activity levels for birds with a suboptimal gait, but gait explained little of the variation in activity. When taking other possibly influential factors, including day, trial, cross, and weight category into account, a larger part of the variation in activity was explained and a tendency for an interaction between gait classification and weight category was observed. In this interaction, a difference in level of activity was observed between gait classifications in lightweight birds, but not in heavyweight birds. It has to be further investigated if this is a consequence of higher body weight already limiting activity levels. Overall, the differences in activity levels of birds with different gait classifications were not very clear and therefore it remains difficult to distinguish gait classifications based on distances moved during the period from 16 to 32 days old. It is recommended for future studies to look into the relationship between gait and multiple activity-related variables in more detail, throughout the life of broilers, to assess whether automated measures of activity have potential to serve as a proxy for gait at the individual level.

3.6 Acknowledgments

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Chapter 4:

Assessing the activity of individual group-housed broilers throughout life using a passive radio frequency identification system – A validation study

Malou van der Sluis[1,2], Yvette de Haas[1], Britt de Klerk[3], T. Bas Rodenburg[2,4] and Esther D. Ellen[1]

[1] Animal Breeding and Genomics, Wageningen University & Research, 6700 AH Wageningen, The Netherlands;
[2] Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, 3508 TD Utrecht, The Netherlands;
[3] Cobb Europe, 5831 GH Boxmeer, The Netherlands;
[4] Adaptation Physiology Group, Wageningen University & Research, 6700 AH Wageningen, The Netherlands

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Abstract

Individual data are valuable for assessing the health, welfare and performance of broilers. In particular, data on the first few days of life are needed to study the predictive value of traits recorded early in life for later life performance. However, broilers are generally kept in groups, which hampers individual identification and monitoring of animals. Sensor technologies may aid in identifying and monitoring individual animals. In this study, a passive radio frequency identification (**RFID**) system was implemented to record broiler activity, in combination with traditional video recordings. The two main objectives were (1) to validate the output of the RFID system by comparing it to the recorded locations on video, and (2) to assess whether the number of antennas visited per unit time could serve as a measure of activity, by comparing it to the distance recorded on video and to the distance moved as recorded using a validated ultra-wideband (**UWB**) tracking system. The locations recorded by the RFID system exactly matched the video in 62.5% of the cases, and in 99.2% of the cases when allowing for a deviation of one antenna grid cell. There were moderately strong Spearman rank correlations between the distance recorded with the RFID system and the distance recorded from video ($r_s = 0.82$) and between UWB and RFID ($r_s = 0.70$) in approximately one-hour recordings, indicating that the RFID system can adequately track relative individual broiler activity, i.e., the activity level of a broiler in comparison to its group members. As the RFID tags are small and lightweight, the RFID system is well suited for monitoring the individual activity of group-housed broilers throughout life.

4.1 Introduction

Knowledge of the relationships between behaviour and health, welfare and performance indicators can be used to assess overall welfare in production animals. Broiler chickens are an example of a livestock species for which insight into these relationships can be valuable. Broilers are, however, often kept in intensive systems, where large numbers of birds (~75,000 per farm in The Netherlands on average; Centraal Bureau voor de Statistiek, 2020) are housed. This makes it difficult and time consuming to observe the animals and to measure the health, welfare and performance of individual birds. The use of a proxy could potentially help improve the ease with which the health, welfare and performance of the animals can be assessed. A promising trait that could serve as a proxy for multiple indicators of health, welfare and performance is locomotor activity, given the relationship between (changes in) activity and, for example, disease (Gregory, 1998), gait problems (Van Hertem et al., 2018a), or body weight (Reiter and Bessei, 2001).

Specifically, information at the individual level is desired to obtain a good view of an individual's state. Furthermore, when individual data can be collected in a group setting with pen mates present, a more realistic image of an animal's performance in a production environment can be obtained. In particular, data on individual activity levels of young broilers could be very valuable. For example, it has been established that the activity level of broilers from a fast-growing stock early in life positively correlates with activity later in life (Bizeray et al., 2000). Furthermore, it has been reported that increased activity can result in fewer leg problems (Reiter, 2004; Reiter and Bessei, 1998). Moreover, it has been suggested that increased activity, specifically at an early stage in the growing period, may reduce leg disorders, as leg bone development might be mostly affected in the first days of life (reviewed in Bradshaw et al. (2002); van der Pol et al. (2015)). Overall, activity levels in the first days of life are potentially very informative for the health, welfare and production of animals later in life and therefore an activity tracking system that allows tracking of individual broilers from the first day of life is desired.

Given that broilers are generally housed in large groups in production systems, identification and activity tracking of individual broilers is a challenge. Sensor technologies, such as computer vision (**CV**), ultra-wideband (**UWB**) tracking and passive radio frequency identification (**RFID**) may offer solutions (see Ellen et al. (2019) for a review of the applicability of sensor technologies for poultry). In particular, passive RFID seems to have potential for tracking individual broilers from the first day of life, as passive RFID tags do not require batteries and can therefore be small and lightweight (Finkenzeller, 2010). Therefore, in the current study, the suitability of a passive RFID system for tracking individual broiler activity throughout life was investigated.

The implementation of a passive RFID system for tracking individual broiler activity can have added value in comparison to existing tracking systems. For example, several studies on automated activity recording of broilers and its relationship with different health, welfare and performance traits have been performed (e.g., Aydin et al. (2010)), but many of these studies focused on group-level patterns. For example, Van Hertem et al. (2018a) implemented a camera-based automatic animal behaviour monitoring tool (eYeNamic^{TM}, Fancom BV, The Netherlands) to study flock activity. They reported a linear trend between flock activity and the average gait score of the flock, where a lower activity level was linked to a worse gait in the flock. Another study, using optical flow patterns, reported, among other things, that a lower average level of flock movement was related to a higher mortality percentage in the flock and that skewness and kurtosis of flock movement were correlated with the percentage of birds in the flock having hock burns (Dawkins et al., 2012). Although such automated flock-level monitoring can be a useful tool for monitoring the overall welfare of a flock, it does not provide information at the individual level and may hereby overlook animals that deviate, which, for example, remain more active than the flock mates and do not show gait problems. Therefore, an automated passive RFID tracking system that provides information on activity levels of individual broilers can have added value. Furthermore, passive RFID has more potential for tracking young broilers in comparison to some other tracking technologies. For example, in our previous study (van der Sluis et al., 2019), the suitability of an UWB system for tracking individual levels of activity in group-housed broilers was determined. This system was concluded to be suitable for tracking broiler activity, with, among other things, a correlation of 0.71 between video and UWB recorded distances, but it has to be noted that the UWB tags were quite large and heavy, rendering tracking of young broilers, i.e., of less than two weeks old, not feasible (van der Sluis et al., 2019). Given the small and lightweight tags of passive RFID systems, passive RFID does have potential for studying birds younger than two weeks old. Passive RFID has already been used for poultry—for example, to study range use, nest box use and feeding behaviour (e.g., Chien and Chen (2018), L. Li et al. (2017), Richards et al. (2011); reviewed in Ellen et al. (2019)). Passive RFID has also been used to study general locomotor activity (Kjaer, 2017). In a study by Kjaer (2017), RFID antennas were placed inside the central area of the pen of the birds in order to record the locomotor activity of laying hens. These antennas covered an area equal to 33% of the total pen area. Possibly, a more detailed image of activity can be obtained using more antennas, covering a larger percentage of the pen.

In this study, the suitability of a passive RFID system, covering the full pen, for tracking individual broiler activity throughout life was investigated. The two main objectives of this study were (1) to validate the RFID recorded locations by comparing these to the locations recorded on video, and (2) to assess whether the moving distances calculated from the RFID data, using the recorded antenna positions over time and the distances between antennas, could serve as a measure of activity. To this end, the RFID recorded distances were compared to the total distance moved as recorded on video and using a validated UWB tracking system.

4.2 Materials and methods

4.2.1 Ethical statement

Data were collected under control of Cobb Europe. Cobb Europe complies with the Dutch legislation on animal welfare. This study is considered not to be an animal experiment under the Law on Animal Experiments, as confirmed by the local Animal Welfare Body (20th of June, 2018, Lelystad, The Netherlands).

4.2.2 Location and subjects

This study was performed on a broiler farm in The Netherlands. In total, 40 male broiler chickens from two genetic crosses were used. The birds were fitted with RFID tags on the day of hatching and four birds, two from each cross, were marked visually using marker spray in four different colours. Near-continuous RFID recordings were made from day of hatching to 34 days old.

At 15 days old, birds were also fitted with an UWB tag. Due to limitations in the number of tags, a total of 34 out of 40 birds were fitted with an UWB tag.

4.2.3 Housing

The broilers were housed in a rectangular pen, with a size of approximately 1.8 by 2.6 m and an area of approximately 4.7 m². In this pen, feed and water were provided ad libitum. Wood shavings were provided as bedding. The birds were kept under a commercial lighting and temperature schedule and were vaccinated according to common practice (Cobb, 2018).

4.2.4 Radio frequency identification (RFID) system

To track individual broilers and monitor their activity throughout life, a passive RFID system from Dorset Identification (Dorset Identification B.V., Aalten, The Netherlands) was used. All 40 broilers were fitted with a high frequency (**HF**) tag operating at 13.56 MHz. These tags had a size of approximately 15 × 3.7 mm and a weight of less than one gram. The tags were fitted to the legs of the birds using rubber bands and tape (**Figure 4.1**). The rubber bands were changed to a larger size once during this study in order to fit the birds' legs, and checked every couple of days.

The pen in which the broilers were housed was fitted with 30 HF antennas of 32 by 41 cm, in a grid covering the full pen (**Figure 4.2**). The antennas were located on the underside of a false floor in order to protect them from water, moisture and dirt. The antennas were connected to one of two readers (HF RFID reader DSLR1000, freaquent froschelectronics GmbH,

Figure 4.1. Radio frequency identification (RFID) tag fitted to a broiler's leg using a rubber band (shown in the larger size) and tape.

Figure 4.2. Top view of the tracking pen during this study, with bedding on the false floor underneath which the RFID antennas are situated. Lines on the pen walls indicate the position of the antenna grid.

Graz, Austria) that read the tags at the antennas and sent the information to the custom-made RFID software from the same company. The tags could generally be read in the 32 by 41 cm rectangle that each antenna covered and up to a height of approximately ten centimetres. A log file was stored which included the time of registration, the unique hexadecimal ID code of the tag and the location, i.e., antenna, at which the tag was registered. Antenna 7 of the RFID system (see **Section 4.2.6.1** 'RFID distance calculations') was not recording birds due to a technical issue. The recording frequency used here was generally one registration per second for each antenna. In the instances in which antenna switches occurred within the same second, two different registrations could be observed. RFID recordings were made continuously for 24 h per day, with exception of the periods in which the leg bands were being checked or the birds were being weighed. Weighing was done four times during the trial and took approximately two to four hours. This included checks of the leg bands, and UWB tags when applicable. Additional leg band and UWB tag checks were performed three times during the growing period and took up to two hours each time. Two broilers died during this study and for two broilers there were too much missing RFID data (<75% of data available) due to loss of their leg tags during this study. The final sample size was 36 broilers—of which, 33 had an UWB tag. The weights of the broilers over time are shown in **Table 4.1**.

Table 4.1. Overview of bodyweights of the subjects in this study (n = 36) at different ages. SEMs are rounded to round numbers.

Age	Weight (mean ± SEM)
8 days [1]	250 g ± 4 g
15 days [1]	620 g ± 9 g
22 days [1,2]	1160 g ± 17 g
29 days [1,2]	1840 g ± 25 g
34 days [3]	2414 g ± 27 g

[1] Ten-gram precision; leg tags included. [2] Weighed including UWB tags; 25 g in weight subtracted for birds with UWB tags. [3] Different scale used with two-gram precision; leg tags and UWB tags not included.

4.2.5 Study A: RFID location validation

To study whether the RFID system correctly registered the location of individual birds over time, the RFID log file was compared to video recordings. The implemented approach is visualised in **Figure 4.3**.

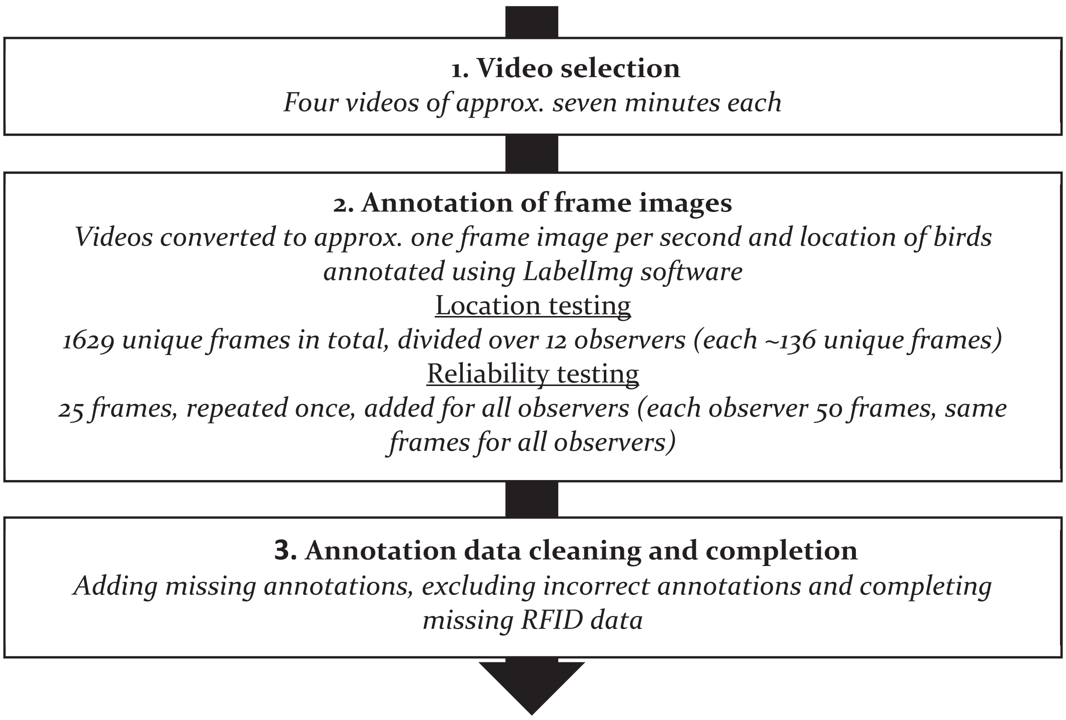

Figure 4.3. Graphical overview of the implemented approach for the RFID location validation study.

4.2.5.1 Video selection

Top-view video recordings were made for approximately two hours per day, using a Zavio B6210 2MP video camera (Zavio Inc., Hsinchu City, Taiwan) with a frame rate of 25 frames per second. From these recordings, four videos with a duration of approximately 7 min each were selected at random: one from 0 to 6 days old, one from 7 to 12 days old, one from 17 to 26 days old and one from 28 to 34 days old. These periods were selected because of the differences in activity, body size and absence/presence of UWB tags between these periods, and excluded days closely after fitting the UWB tags or when not all data were available. The details of the four videos are shown in **Table 4.2**. Before placing bedding and birds in the pen, an image showing the RFID grid was taken. This image was used as a reference for the location of the birds in order to score coordinates and associated areas of the different antennas.

Table 4.2. Overview of the characteristics of the videos that were selected for the location validation study. UWB = ultra-wideband.

Video	Age of the broilers (days)	Time of day (start of video)	Duration (mm:ss)	Average expected activity level	UWB tags present
Video 1	1	09:48:49	06:47	Low	No
Video 2	11	10:43:32	06:49	High	No
Video 3	18	11:53:42	06:55	High	Yes
Video 4	34	11:05:11	06:59	Low	Yes

4.2.5.2 Annotation of frame images

The randomly selected video recordings were converted to frame images, with a rate of approximately one frame per second, using VLC media player (VideoLAN, Paris, France). In these frame images, four colour-marked birds were annotated using LabelImg software (Tzutalin, 2015). The frames were annotated by in total twelve different observers, all scoring a unique set of one-twelfth of the total number of frames (i.e., ~136 out of 1629 frames), as well as an overlapping set of frames. This overlapping set of frames consisted of 25 frames, randomly taken from the overall 1629 frames available, that were repeated once, resulting in 50 frames that were the same for every observer. This overlapping data set allowed for calculation of inter- and intra-observer reliability, in which 100 annotations, i.e., four animals from 25 frames, could be compared and missing annotations were deleted row-wise. For the inter-observer reliability, 85 annotations were left for comparison between all observers after removal of missing annotations. The number of annotations available for intra-observer calculations after removal of missing annotations differed per scorer and ranged between 83 and 98.

4.2.5.3 Annotation data cleaning and completion

The annotations of the unique sets of frames from all observers provided coordinates of the location of the birds in the image. These coordinates were compared to the location of the different antennas from the reference image in order to assign an antenna number to the location of the birds. Sometimes, a scorer missed a bird — for example, because it was hidden or simply overseen, — or labelled two different birds with the same ID. In these instances, the correct annotation was added later on, by a single scorer who was already included in the group of annotators. It is important to note that this step was only performed for the frames included in the location validation set and not for the frames used for determination of inter- and intra-observer reliability, where only raw data were used. When a bird was positioned at the side of the pen, leaning against the pen wall, it could happen that the bird was annotated outside the antenna range. In these instances, a missing value was given and this observation was excluded from the calculations. Upon visual inspection of the coordinate data, some incorrect annotations were observed, where birds were located for only a single frame at a non-adjacent antenna. These incorrect annotations were removed, resulting in a missing data point. Furthermore, if location 7, where the antenna was not functioning, was scored on video, this value was replaced with a missing value and not included in the analysis. The final output data from the videos that were used here included a time stamp, the animal ID and the antenna at which the animal was located. This output was then compared to the output of the RFID system, which included the same variables. For this location validation, the output of the RFID system was manually completed after recording, as the RFID system sometimes did not register birds when they were lying down, resulting in missing recordings. The RFID system functions best when the tags are in a vertical position, i.e., at a 90 degree angle in comparison to the antenna floor, which is the case when birds stand. When birds are lying down, the tags are in a position parallel to the antenna floor and are often not registered. It was assumed that as long as a bird was not registered elsewhere, the bird was still located at the antenna position where the bird was last registered. This assumption was used to fill in the missing rows in the RFID data for the location comparison. In later analyses of distances moved, and future implementations, this is not necessary, as the main factor of interest in those cases are likely the antenna switches, and when studying these the last recorded position can be used, regardless of the time between the last recorded position and the next recorded position. However, for the location validation this would result in missing data, and therefore missing rows were filled in based on this assumption. Both the overall registrations and the trajectory walked by the birds were compared between the RFID system and video recordings. For the overall registrations, the percentage of (dis)agreement was calculated, while for the trajectory analysis the path of the animals was visually compared.

4.2.6 Study B: Moving distance validation

To study whether the distance moved as recorded with the RFID system was a good indicator of activity, the RFID output, in terms of antenna switches per unit of time, was compared to the distance moved that was recorded (1) from video and (2) using an UWB system. The implemented approach is visualised in **Figure 4.4**.

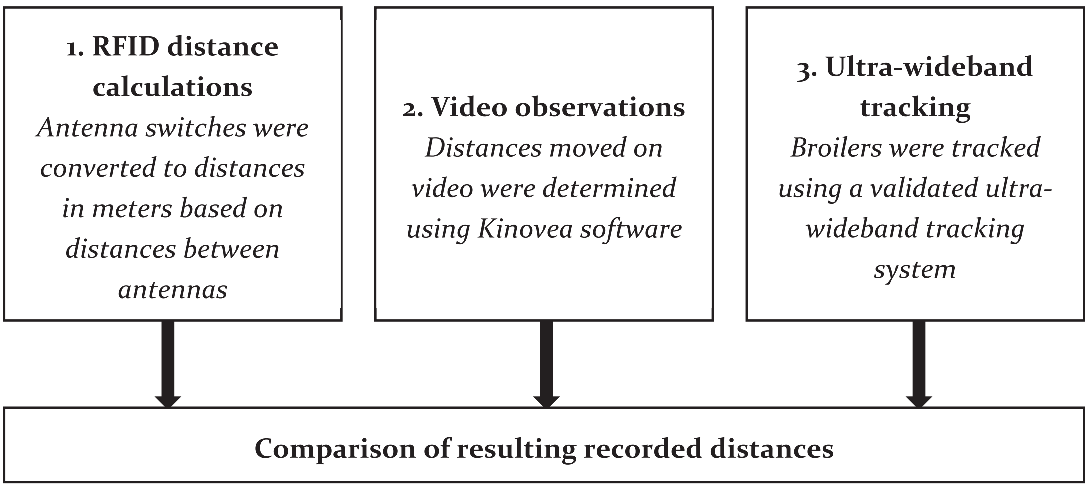

Figure 4.4. Graphical overview of the implemented approach for the RFID distance validation study.

4.2.6.1 RFID distance calculations

The RFID recordings required conversion to metrical distances. The number of switches between antennas was used as an initial measure of activity for the

RFID system. The RFID antennas were all the same size (32 × 41 cm), but were not exact squares, so a correction was made in the RFID output to account for the difference in distance between centre points of antennas in the x and y direction. Furthermore, antennas were mounted on a total of fifteen PVC panels, in sets of two with identical configurations and the short sides of the antennas adjacent to each other, resulting in a difference in distance between two antennas on the same panel and between adjacent antennas on different panels. This was also accounted for in the RFID distance calculations. **Figure 4.5** shows a schematic top view of the antenna grid. The shortest distance between two centre points was 36 cm, and this distance was set as 'one switch'. All other switches were calculated in relation to this distance, e.g., a distance of 45 cm was recorded as 1.25 switch. For each antenna switch, the distance between the last recorded and next recorded antenna was calculated using the shortest possible route to this antenna, i.e., a straight line. False switches could occur when an animal was on the boundary between two antennas, which could result in alternating registrations at the two adjacent antennas. Therefore, if an animal was registered at a new antenna and this same new antenna was also registered less than five seconds before, the switch in between was not included in the calculation of the total number of antenna

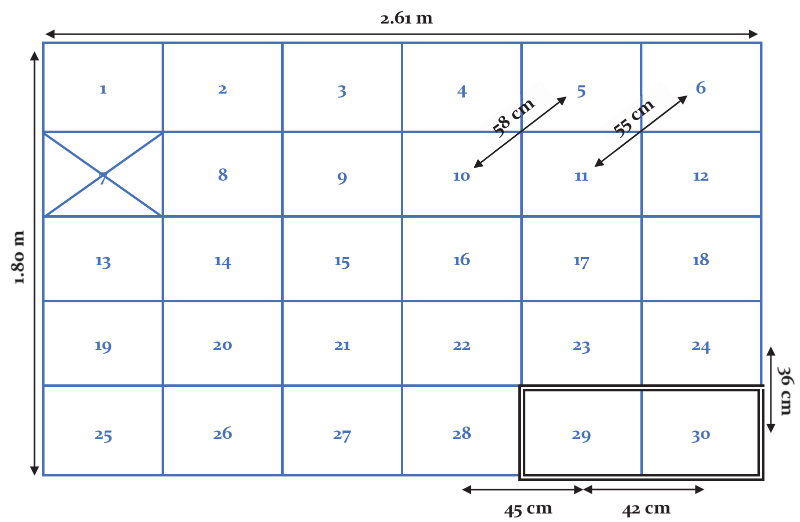

Figure 4.5. Schematic top view of the RFID grid. One of the fifteen PVC antenna panels that includes two RFID antennas is outlined as an example (antenna 29 and 30). Distances between centre points of antennas are indicated, as well as the total size of the grid. The non-functioning antenna is marked with an X.

switches. Per tracking period, the antenna switches were added up as an indicator of the activity level of that animal in that period and could be multiplied by 0.36 to convert this to a distance in meters.

4.2.6.2 Video observations

For the video comparison, Kinovea software (version 0.8.15; Charmant and contributors, 2020) was used to calculate the distances moved by four colour-marked birds. The length of one side of the tracking pen was used for calibrating the distances. The positions of the birds were marked in the video by drawing a circle around each bird, closely matching the outline of the lateral sides of the bird. This was done to make it easier to see when a bird changed position and to set a threshold for movement. In this study, movement was only included if the bird shifted at least half a body width, i.e., the centre of the bird moved outside the circle. When this criterion was met, the bird was marked with a tracker in the video and the movement of the broiler was tracked automatically. Manual corrections were applied when the tracking algorithm did not recognise the movement of the bird or tracked the path incorrectly. When a bird stopped moving, the recording was stopped and the distance covered was noted, before placing the circle around the bird again and resuming the video analysis. After completion of the video analyses, the distances per animal and per time period were summed in order to provide the full distance moved by each animal in the desired timeframe. Of the two-hour video recordings each day, approximately one hour of video was analysed per day for a total of eight days, ranging from 25 to 33 days old. Day 27 was excluded as no RFID data were available due to a technical error. In these videos, the distances moved of the four colour-marked birds were analysed, resulting in four data points per one-hour video. Due to one colour marking fading away, three one-hour videos were analysed for three instead of four animals. A total of 29 one-hour records was available for analysis. The distances recorded in these videos were compared to the distances recorded with the RFID system during the same periods.

4.2.6.3 Ultra-wideband tracking

For the comparison with UWB tracking, a Ubisense UWB system with Series 7000 sensors and compact tags (Ubisense Limited, Cambridge, UK) was used, in combination with TrackLab software version 1.4 (Noldus Information Technology, Wageningen, The Netherlands). In total, 34 out of 40 birds were fitted with an UWB tag, due to a limitation in the number of tags available during this study. These tags had a size of 3.8 by 3.9 cm and a weight of approximately 25 g, and were fitted to the birds using elastic bands around their wing base. This UWB system, the setup and validation are described in more detail in van der Sluis et al. (2019). A sampling rate of one sample approximately every 6.91 s was implemented. The UWB system recorded the activity of the birds near continuously from 15 days old, with exception of the periods in which the leg bands were being checked or the birds were being weighed. The UWB output that was used here was the total distance moved in meters per individual per tracking period, where the tracking periods were selected to match the duration of the analysed videos and corresponding RFID recordings, with a possible shift of several seconds due to the sampling rate of the UWB system. Furthermore, to study whether longer RFID and UWB recordings would average out any shorter-term differences, the average hourly activity levels from the RFID and UWB systems were calculated, per day and for each animal, and were corrected for the recording duration. Because we ran out of batteries during the experiment and because of too much missing UWB data (<75% of data available), near-complete UWB data was available for 25 animals. In total, approximately 18 days with UWB and RFID data were included in the comparison between UWB and RFID recorded distances.

4.2.7 Statistical analyses

All statistics were performed using R version 3.5.0 (R Core Team, 2018). To validate the location output of the RFID system, a comparison with the locations recorded on video was made. To study the inter-observer reliability of the annotated video recordings, a Fleiss' Kappa calculation was performed on one-half of the data (i.e., no repetitions of the same frames within an observer), using the irr package (Gamer et al., 2019). To determine the intra-observer reliability of the annotation of the video recordings, Cohen's Kappa tests from the irr package were performed. Descriptive statistics were used to study the number of matches and mismatches between video and RFID in terms of location where the birds were observed. Visual comparisons were made of the trajectories walked by the birds. To assess whether the antenna switches recorded per unit time could serve as a measure of activity, Spearman rank correlations were determined between the distances recorded by the RFID system, on video and as recorded with the UWB system, as the data were not normally distributed. The 95% confidence intervals for the Spearman rank correlations were calculated by bootstrapping using the RVAideMemoire package (Hervé, 2019). Strictly speaking, the data points in the correlations are non-independent, as the same animals have been repeatedly measured across days. To determine the common within-day or within-individual correlation, repeated-measures correlations were implemented (Bakdash and Marusich, 2017) using the rmcorr package (Bakdash and Marusich, 2018). This analysis assumes, among other things, linearity of the relationship between the two variables and normal distribution of the errors, and the assumptions were not fully met here. Therefore, interpretation of these results requires some caution. However, the resulting correlational values fell within the 95% confidence intervals of the overall correlations and are therefore not reported in the results. A Spearman rank correlation was also used to determine the correlation between the hourly average activity as recorded by the RFID and UWB system. Furthermore, for the analysis of the hourly average activity as recorded by the RFID and UWB system, repeated-measures correlations were again implemented to determine the common within-day or within-individual correlation, and are reported in the results. Again, the assumptions were not fully met and the interpretation of these results requires some caution. Visualisations were made using the trajr (McLean and Skowron Volponi, 2018), ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2019) and rmcorr (Bakdash and Marusich, 2018) packages. The level of statistical significance was set at 0.05. In the text, reported results are rounded to two decimals.

4.3 Results

4.3.1 Study A: RFID location validation

The overall inter-observer reliability for the annotated antennas was 0.90 (Fleiss' Kappa, subjects = 85, raters = 12, Z = 209, $p < 0.001$). The intra-observer reliability for the annotated antennas was on average 0.92, ranging from a minimum agreement of 0.75 to a maximum agreement of 0.99 (see **Table S4.1** in the supplementary data). Comparison of the locations on video and from the RFID system showed that video 2 had the highest exact agreement with RFID (80.9% matches), while video 3 had the lowest exact agreement (47.4% matches; **Table 4.3**). Overall, 62.5% of the registrations exactly matched between RFID and video. This percentage increased to 99.2% when allowing for a deviation of one antenna, i.e., a registration at one of the maximum eight neighbouring antennas.

Table 4.3. Overview of the percentage of exact matches and near matches between video and RFID registrations for the four videos.

Video	Bird age (days)	Expected activity	UWB	Registrations included[1]	Exact matches	Near matches[2]
Video 1	1	Low	No	1319	72.1%	100.0%
Video 2	11	High	No	1576	80.9%	99.9%
Video 3	18	High	Yes	1463	47.4%	97.0%
Video 4	34	Low	Yes	1575	50.5%	99.9%
Total				5933	62.5%	99.2%

[1] I.e., the number of available RFID and video locations to compare in the respective video. [2] Allowing for a deviation of one antenna, i.e., a registration at one of the maximum eight neighbouring antennas.

Although there appeared to be more detail, or positional changes, in the video data compared to the RFID data, the trajectories walked by the birds according to the RFID system and as observed on video showed good agreement (**Figure 4.6**). Moreover, the RFID location of the individuals did not deviate strongly from the location on video. In other words, no RFID registrations were observed in regions where the animals were not near. However, although the trajectories from video and RFID matched well, the timing of the recorded location switches sometimes differed between video and RFID. To illustrate this, **Figure 4.7** shows the video and RFID locations over time of two example tracks, one from video 2 (high percentage of matches; animal ID 2) and one from video 3 (low percentage of matches; animal ID 4). In both these examples, the animals were not very active and the RFID and video trajectories matched completely (**Figure 4.6**). However, **Figure 4.7A** shows that the video and RFID matched almost completely for the first of the two tracks, while **Figure 4.7B** shows that the video registered many switches between the two recorded antennas for the second track, causing mismatches between RFID and video in terms of location for part of the recording.

4.3.2 Study B: Moving distance validation

There were moderately strong Spearman rank correlations in terms of distances moved between RFID and video (r_s = 0.82 (95% CI 0.61–0.92), S =

730, $p < 0.001$; **Figure 4.8A**), between RFID and UWB ($r_s = 0.70$ (95% CI 0.41–0.86), S = 1224, $p < 0.001$, **Figure 4.8B**) and between video and UWB ($r_s = 0.79$ (95% CI 0.53–0.92), S = 854, $p < 0.001$; **Figure 4.8C**).

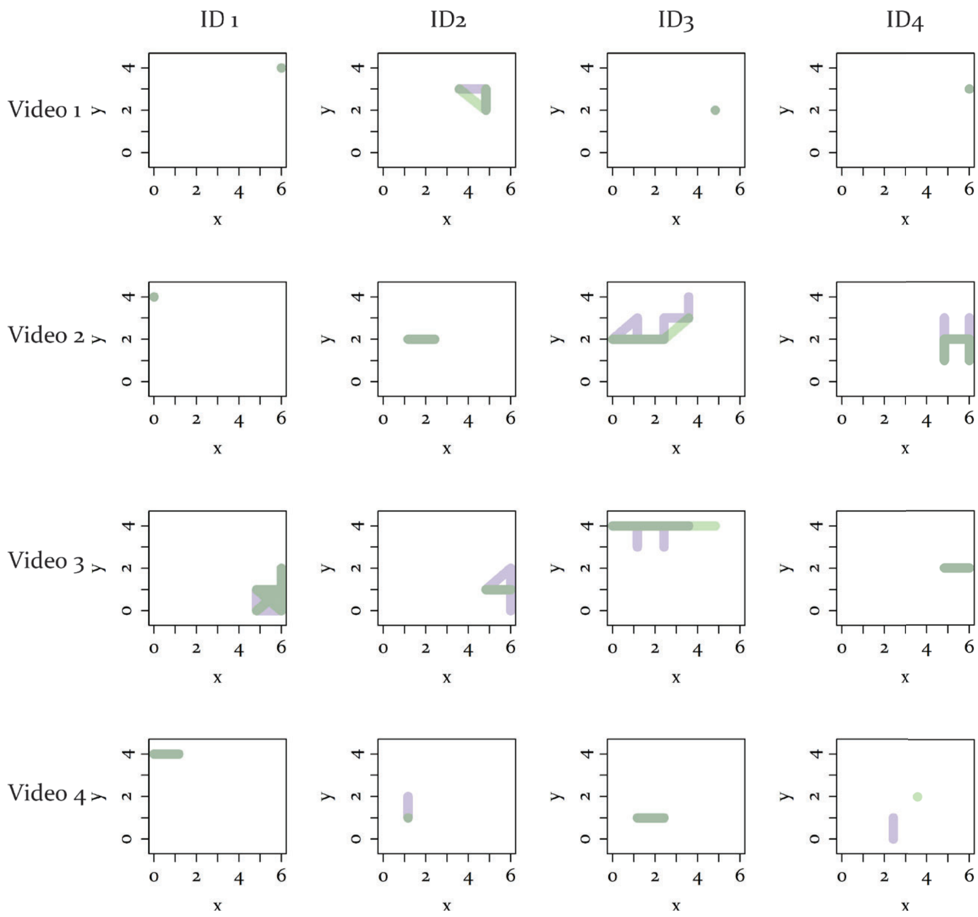

Figure 4.6. Trajectories walked by individual birds in the four videos. Each square represents the pen from above. The four videos (see Table 4.2) are on separate rows, while different columns represent different individuals in these videos. The purple line represents the video track, while the green line represents the RFID track. Overlapping parts of the track are depicted in dark green.

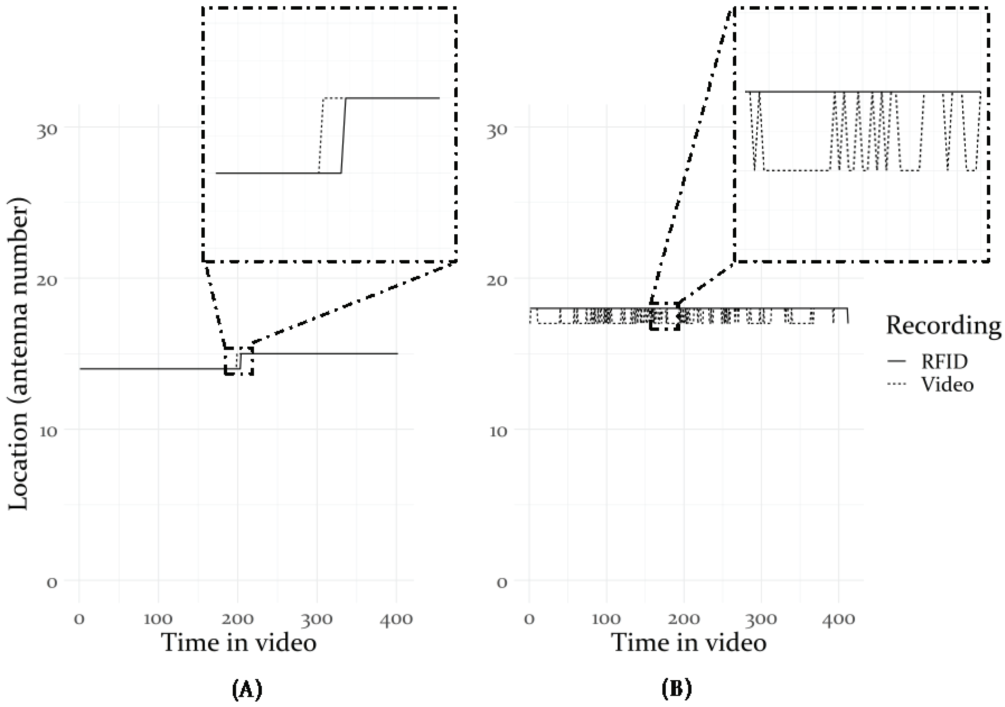

Figure 4.7. Locations over time for two individuals in two videos. The solid black line represents the RFID location, while the dashed black line represents the video location. In the boxes, zoomed-in parts of the graph are shown. (A) Almost fully matching track from animal ID 2 from video 2; (B) track with many mismatches occurring from animal ID 4 from video 3.

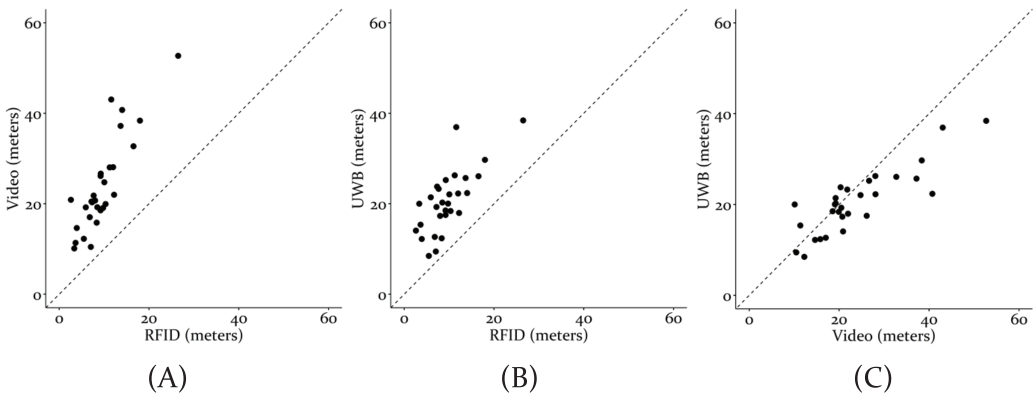

Figure 4.8. Scatter plots of the relationships between two measuring techniques. Distances are depicted in meters on both axes. The dashed line indicates the diagonal. Dots represent individual data points. (A) RFID versus video; (B) RFID versus UWB; (C) video versus UWB.

There was a moderately strong Spearman rank correlation between RFID and UWB when using the average distance moved per hour, calculated per day and animal (r_s =0.71 (95% CI 0.65–0.75), S = 4041800, p < 0.001; **Figure 4.9**). However, the UWB system nearly always recorded higher distances than the RFID system (**Figure 4.9**). When looking within days, a correlation of 0.65 was observed (repeated-measures correlation, r = 0.65 (95% CI 0.59–0.70), df = 417, p < 0.001; **Figure 4.10A**). When looking within individuals, or tags, a correlation of 0.73 was observed (repeated-measures correlation, r = 0.73 (95% CI 0.69–0.78), df = 410, p < 0.001; **Figure 4.10B**). The repeated-measures correlations strongly resembled the overall correlation without taking repeated-measures into account (**Figure 4.10**).

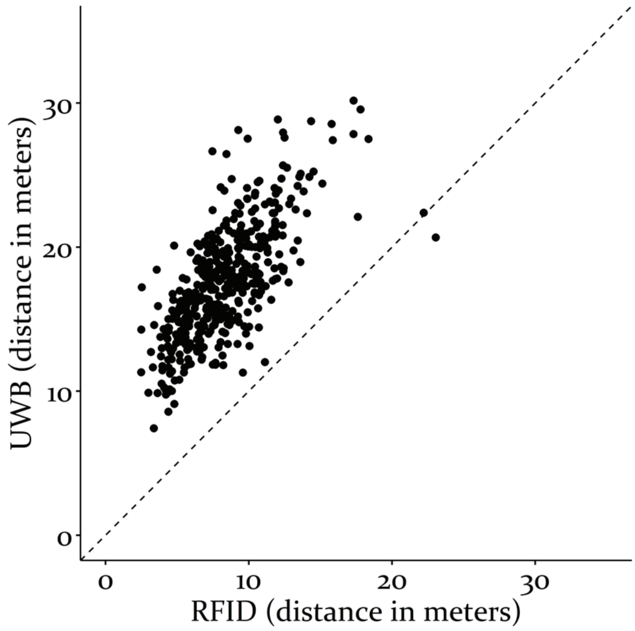

Figure 4.9. Scatter plot of the relationship between RFID and UWB over a longer period of time, as average distances moved per hour for each day and animal. Distances are depicted in meters. The dashed line indicates the diagonal. Dots represent individual data points.

4.4 Discussion

In this research, it was studied whether a passive high frequency RFID tracking system was suitable for monitoring the individual levels of activity of broilers throughout life. On average, 62.5% of the RFID recordings matched exactly with video in terms of the location of the animals and 99.2% of the

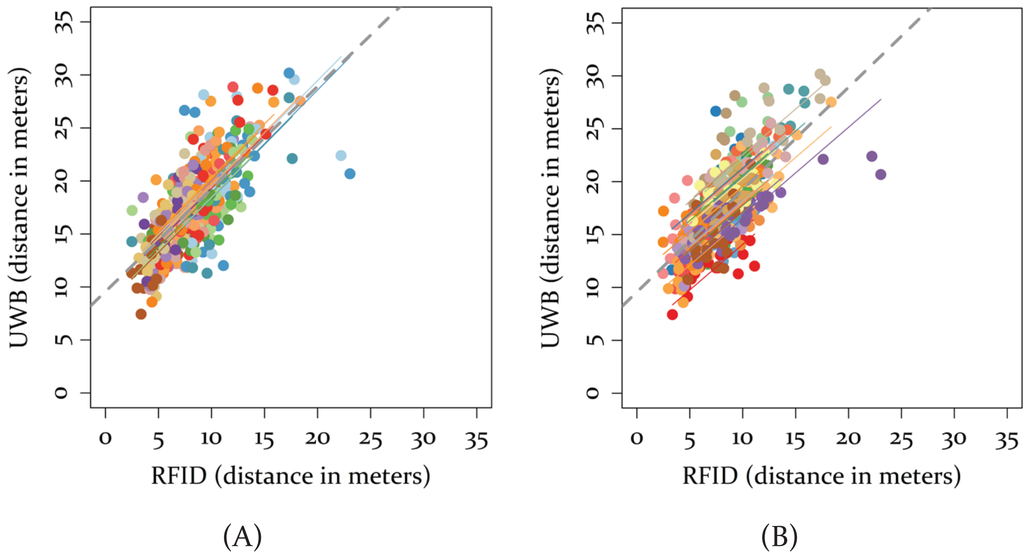

Figure 4.10. Scatter plot of the relationship between RFID and UWB over a longer period of time, as average distances moved per hour for each day and animal, looking at the correlation within days (A) or individuals (B). Different colours represent different days (A) or individuals (B). Distances are depicted in meters. The dashed line indicates the regression when ignoring the repeated-measures variable, i.e., indicating the overall correlation. Dots represent individual data points. (A) Scatter plot of the relationship between RFID and UWB for different days; (B) scatter plot of the relationship between RFID and UWB for different individuals.

registrations matched when allowing for a deviation of one antenna, indicating that the RFID system can give a good approximation of the location of individual birds. Furthermore, distances recorded with the RFID system correlated well with both video and UWB measurements. These correlations indicate that the RFID system can provide good estimations of the activity levels of individual broilers in relation to their pen mates.

4.4.1 Study A: RFID location validation

Overall, almost two-thirds of the location registrations exactly matched between RFID and video. Furthermore, the difference in position detected with the RFID system and on video was rarely larger than one antenna, i.e., birds were rarely registered further away than an adjacent antenna compared to the position on video, as indicated by the 99.2% near matches. This indicates that the RFID system can give a good approximation of the location of individual birds and has potential to be implemented to, for example, study locational patterns of individual birds over time. However, there was some disagreement between video and RFID in terms of exact registered locations, which has several possible explanations that are related to how RFID and video differ in the way they can be used to detect the birds. These include the reference point used for location determination, the level of detail of the recordings, the effect of intermittent tag registrations, and the tag orientation, and are outlined below.

4.4.1.1 Reference point for location determination

On video the centre point of the birds is used to determine the location of the bird, while the RFID tag is attached to a single leg. This could result in a bird being registered at a different antenna on video than with the RFID system, as the centre point of a bird might be situated at a different antenna than the leg of the bird. It has been reported that broilers generally place their feet in a lateral position relative to the hip (Paxton et al., 2013). In particular, when birds are larger, this may affect the results of the position recordings on video and using RFID. A positive relationship between body weight and pelvis width, measured across the back of a standing chicken as the distance between the exterior of the thighs, has been observed in chickens (Latshaw and Bishop, 2001). Therefore, the absolute distance between the centre point of the birds and their legs may increase further as the birds grow, which could explain why more mismatches were seen in video 3 and 4, in which the birds were larger. However, it must also be noted that video 3 and 4 included birds with UWB tags, while video 1 and 2 did not. With the current data, it cannot be completely ruled out that the UWB tags affected the RFID recordings, but given that the two systems communicate on different radio frequencies, this is not expected. It is therefore hypothesised that the difference in size of the birds was the main cause of the higher number of mismatches in video 3 and 4 and likely, if on video the leg of the bird that was fitted with an RFID tag was annotated, fewer mismatches between RFID and video would be observed. However, with the current setup using top-view video recordings, it was not feasible to annotate single legs of birds as these were not always visible. Therefore, the centre point of the birds was used as an approximation, likely resulting in an increase in mismatches between RFID and video.

4.4.1.2 Level of detail of the recordings

As was shown in **Figure 4.7B**, there were more location switches registered on video, compared to with the RFID tracking. One possible explanation for this higher number of switches on video is that the video recorded more detailed changes than the RFID system. For example, when a bird was situated on the edge between two antennas, even a slight shift of a few pixels could result in the bird being registered at a different antenna than before. This shift might be registered on video due to a slight movement of the birds, but can also be caused by an inaccuracy in the annotations of the birds. Moreover, at the very edges of the antenna panels, there was not always full antenna coverage, as there was some uncovered space between the individual antennas. Therefore, when the video registered a bird at the edge of a new antenna, the RFID system was not always able to 'see' this bird and did not register a new location for this bird.

4.4.1.3 Intermittent tag registrations

In some cases, the RFID system did see the tag when it was situated between two antennas. This could result in the bird being registered in two positions intermittently, while the bird was not moving on video, resulting in approximately half of the registrations being a mismatch. In the distance validation, this was filtered out in order to exclude false positives for locomotor movement between antennas, but in the location analysis these switches were kept to allow a full second-by-second comparison of the positions of the animals using the raw RFID data as a basis. Such intermittent registrations at two antennas were observed in the current study and have also been reported in other studies using RFID technology to track positions of animals (e.g., low frequency RFID in rats (Redfern et al., 2017); ultra-high frequency RFID in mice (Catarinucci et al., 2014)).

4.4.1.4 Tag orientation

The RFID system sometimes could not register birds when they were lying down. The RFID system functions best when the tags are in a vertical position, i.e., at a 90 degree angle in relation to the antenna floor. This is often the case when birds stand upright. When birds are lying down, the tags are in a position parallel to the antenna floor. Therefore, when a bird shifts position without fully rising to stand, the RFID system may not register this movement. In the analysis performed here, missing RFID registrations were completed using the last recorded position of the animal, because no 'new' information was available from the RFID system and therefore the last registered antenna was the most likely current position of the animal. However, this position may potentially no longer have been the correct one, for the reason mentioned above. For practice, the notion that birds are often not registered when lying down is not a cause for concern. On the contrary, when one is interested in recording locomotor activity, it might even be preferable to not register movement of birds that are not standing up, as this may not be considered as true locomotor movement of the bird. By not registering birds while they are lying down, likely no relevant information is lost and it might reduce noise in the data that could be caused by birds slightly shifting position, for example, because they are preening, while lying down.

Altogether, the differences in the way RFID and video register the birds may underlie the mismatches that are observed. However, the trajectory plots (**Figure 4.6**) show that when RFID and video do not agree on the trajectory, the deviation is generally not larger than one antenna. In other words, birds were, at least in these videos, never registered in locations where they really could not be located near, i.e., at far-away antennas in relation to the position on video. It appears that the RFID system might not always register all changes that are happening on video, because these changes might be too subtle for the RFID system to detect or are not actually caused by birds walking. However, the RFID system also does not register birds in locations where they are not located near based on the video observations. Overall, it appears that the RFID system is not very sensitive to small changes, but does provide a good approximation of the location of birds over time.

4.4.2 Study B: Moving distance validation

4.4.2.1 RFID versus video

In this study, it was assumed that the distance moved as recorded from video is the true distance moved by the animals. There was a moderately strong rank correlation (0.82) between video and RFID in terms of distance moved. This indicates that the RFID system is suitable for comparison of activity levels between animals, and can be implemented to study individual differences in activity levels in a more time-efficient and objective manner compared to visual observations from video recordings. In terms of absolute distances moved, the distance as recorded from video was generally somewhat higher than the distance registered by the RFID system. There are several explanations for this finding.

First, the RFID and video recordings could differ in the exact starting point of the distance recording. In theory, the RFID and video recordings spanned the same time period, but, as explained earlier, the RFID system did not always register birds, especially when they were lying down. Consequently, when a recording started, the RFID system might only have registered a bird when something in the position of the bird changed — for example, when it moved to a new antenna, as it was possibly no longer registering the previous position of the bird. This could result in the first 'switch' between antennas being missed, as only the second antenna might be registered, while on video, all movements from the start of the recording can be observed. Consequently, the RFID system might show a slightly lower recorded distance compared to video.

Second, as discussed earlier, the video can capture much smaller changes than the RFID system. On video, all changes can be observed, while the RFID system only registers movement between antennas, and not within antennas, which can cause the distance recorded with the RFID system to be lower. The absolute difference between the recorded distances on video and using RFID appeared larger when birds moved longer distances (**Figure 4.8A**). Redfern et al. (2017) used a low frequency RFID tracking system in rats, with a setup similar to the setup in the current study, and compared RFID and manual distance tracking. They found a good correlation between the two (ICC = 0.83), although it depended on the position of their implanted RFID tag, but noted that the RFID distance was lower than the manual tracking for higher activity levels. One of their explanations for this discrepancy was that faster locomotion could have impacted the tracking accuracy (Redfern et al., 2017). Indeed, other studies have reported that when tags move faster, their probability of registration can decline (Gebhardt-Henrich et al., 2014a). In the current study, this may have impacted the recorded distances in two ways. First, some movement to and from antennas might be missed due to unrecorded antenna visits, resulting in a lower recorded RFID movement, and second, via the way the RFID distances were calculated. The RFID moving distances were based on the shortest possible straight line from one antenna to the other (see **Figure 4.5**). It is, however, unlikely that animals always walk in a straight line from one antenna to another. Therefore, a part of the true distances moved may be missed by using the shortest possible route in the calculations. This influences the recorded distances between antennas that are not adjacent. Specifically, when an animal is registered at two subsequent antennas that are far apart, the absolute distance underestimation can become large, as the absolute difference between the calculated shortest route and possible true routes is especially large here. However, although in the current study the absolute difference between RFID and video was observed to be larger at higher distances, the relative difference appeared to remain relatively constant. Furthermore, the earlier-mentioned studies reporting an effect of movement speed of the tag on recording reliability implemented low frequency RFID, while in the current study, high frequency RFID was implemented, which has a faster read rate (Ruiz-Garcia and Lunadei, 2011). Therefore, it is less likely that fast movements may have been missed in a similar way, and it appears more likely that the distances moved within the antennas are the main cause of the difference between RFID and video, as opposed to the moving speed of the birds.

Third, during this study, antenna 7 of the RFID system was not functioning and therefore not recording birds. Moreover, after this study, it was discovered that the antennas close to the drinker were no longer recording birds near the end of the tracking period, likely due to the moisture in the bedding underneath the drinker blocking the RFID signal. This could have resulted in some missed registrations, and hereby could have lowered the recorded RFID distance in comparison to video.

Overall, the distance moved calculated from the RFID data may not exactly represent true distances moved. However, the moderately strong correlation between video and RFID does allow comparison of activity levels between animals tracked with the same RFID setup.

4.4.2.2 RFID versus UWB

There were moderately strong rank correlations between UWB and RFID in the one-hour sessions (0.70) and in the longer recordings (0.71). This indicates that the RFID system and the UWB system generally agree on the relative ranking of animals in terms of distances moved. Whether animals are tracked using the RFID system or the UWB system, the same animals will likely be identified as relatively active or inactive by both systems. In terms of absolute distances, the distance recorded with the UWB system was higher than the distance recorded with the RFID system for nearly all data points. This could again be explained by the RFID system not registering movement within antennas, while the UWB system can theoretically pick up on all movements. However, the difference between RFID and UWB may be enlarged by the localisation error or noise that may occur with the UWB system. There may be some noise in the localisation of the UWB system, which could result in slight deviations from the actual position of the bird over time, hereby increasing the recorded distance moved (van der Sluis et al., 2019). The repeated-measures correlations on the longer-period UWB and RFID data showed that the distribution of data points differed somewhat between days and individuals. The differences in distribution of data points between individuals can be explained by differences in activity between birds, i.e., it can be expected that some birds are consistently more active than others or move faster, or in a different way, compared to others, which may affect the RFID and UWB recordings to some extent. The difference in distribution of data points between days is less intuitive. Although differences in overall activity levels are expected across days, theoretically the UWB and RFID systems function in a consistent manner and should therefore not show differences in the relationship between them. However, the systems can be affected by the surroundings. For example, metals or water in the environment may affect RFID systems (Ruiz-Garcia and Lunadei, 2011), while UWB systems may miss registrations when tags are in a (partially) covered position (e.g., Stadig et al. (2018a); also for a discussion on how to deal with missing UWB registrations). In this study the surroundings were kept the same as much as possible, so it would be expected that the influence of water, metals and coverage of tags was the same across the entire trial and therefore the differences between days may simply reflect random noise. However, as mentioned earlier, the antennas close to the drinker were no longer recording birds near the end of the tracking period, likely due to the moisture in the bedding underneath the drinker blocking the RFID signal. This could have reduced the RFID recorded distances over time, and hereby the correlation between UWB and RFID. Regardless, as is shown in **Figure 4.10**, the observed correlations did not differ much between the repeated-measures and assumed-independent data and the direction of the relationship between RFID and UWB appeared to be the same. In practice, the overall correlation across days and individuals seems the most relevant, as this is the correlation that is representative of comparing animals that are tracked at different timepoints, regardless of whether these same animals have been tracked before and on what specific day. It is important to note, however, that an approach like this does not take changes in activity over time within animals into account, so it would be advisable to only compare the activity of animals that are approximately the same age at the time of measurement. Overall, although there are differences between the two tracking systems in observed absolute distance moved, it appears that the RFID and UWB systems show good agreement on which animals are relatively active or inactive.

4.4.2.3 Video versus UWB

There was also a moderately strong correlation between video and UWB, with $r_s = 0.79$. This indicates that the UWB recorded activity reflected the ranking of the animals in terms of distances moved well. In our previous study (van der Sluis et al., 2019), a similar approach was used and a correlation between video and UWB of 0.71 was reported. The correlation in the current study was somewhat higher, but it must be noted that the confidence interval for the correlation of 0.79 ranges from 0.53 to 0.92 and thus includes the earlier reported correlational value. Therefore, no strong conclusions can be drawn from this difference between studies. It is noteworthy, however, that in the current study the observations were made later in the life of the birds. In our earlier study (van der Sluis et al., 2019), the observations were made from 15 to 33 days old, while in the current study the observations were made from 25 to 33 days old. Given the decreasing trend in activity that is often reported as broilers age (e.g., Tickle et al. (2018), van der Sluis et al. (2019), Weeks et al. (2000)), it was likely that in the current study somewhat lower average activity levels were included in the comparison between video and UWB, compared to the earlier study. However, the median distance moved on video, assumed to be the true distance moved, observed in the earlier study was approximately 20 m (ranging from approximately 2 to 57 m in this study), while in the current study this was approximately 21 m (ranging from approximately 10 to 53 m). The setup of the current study did, however, differ in comparison to our earlier study in terms of, among other things, the placement of water and food, the density of the birds and the human scorer of the videos. Possibly, this may have influenced the observed correlation, but this could not be confirmed with the data from the current study. Generally, however, it is important to note that the distance comparisons in this study, with exception of the RFID-UWB comparison over longer periods of time, were made using data from 25 to 33 days old, and therefore the observed correlations are representative only of the later part of broiler production. Although it is not expected that the relationships between systems are very different at different timepoints, additional work involving RFID early in life is needed to confirm equal performance of the RFID system at younger ages.

Overall, the moderately strong correlations between the different observation methods suggest that the RFID system and the UWB system are both suitable for estimating differences in activity levels between individual birds. However, as the RFID system allows for monitoring activity from hatching onward, due to its small and lightweight tags, it is here deemed to be the preferable method for tracking individual activity in group-housed broilers throughout life.

4.4.3 Future prospects for implementation of the RFID system

The RFID system that was implemented here shows great potential for recording individual activity levels of broilers throughout life, on multiple animals simultaneously, in a time-efficient and objective manner. The current setup provided a high level of detail, using antennas with a size of 32 by 41 cm, and can be of great value in research into behaviour of group-housed birds. However, as mentioned earlier, some difficulties were experienced with the RFID system. One antenna was not recording animals due to a technical error that was related to a faulty contact in the antenna. Further, several antennas were not recording birds at the end of the trial due to moisture in the bedding underneath the drinker. In general, the RFID system can be sensitive to the environment, which is important to take into account when implementing the system in practice. Furthermore, the RFID system used here is relatively easy to dismantle and move to a different location, but it would be advisable to setup the system in a location where it can remain for longer periods of time, as the transport and change in surroundings can result in recalibration of antennas being required for the system to work as desired and cleaning of the antennas is difficult as no water can be used.

The intended purpose of implementing an RFID system is important to consider when selecting a system, as this may affect the requirements for the RFID system. For example, the current setup implemented high frequency RFID, among other things, to allow registrations of multiple animals at an antenna and avoid data collision. However, the radio frequency also has consequences for how much the system is affected by water and metal in the environment and what communication ranges can be achieved (Ruiz-Garcia and Lunadei, 2011), and it is therefore important to weigh up the benefits and downsides when selecting a system. The level of spatial resolution can also vary, depending on the antenna size and configuration. For more detailed position recording, more and smaller antennas may be desired. However, for larger scale implementations in practice, a high spatial resolution may not be realistic. Given that it is not feasible to fit large housings with a full grid of antennas of the current size, an alternative could be to fit specific areas in the housing with antennas — for example, the feeders. This would not provide the detail in the activity level that could be recorded in the present study, but does allow one to keep track of feeder visits. Another option would be to use several line antennas to divide the overall area into different zones, and to study how often birds switch between zones. Future research could look into how well these crude measurements correlate with detailed data on activity levels in order to determine whether these crude measurements can serve as a proxy for activity in large scale production environments.

Future research should furthermore look into alternative methods for attaching the RFID tags to the legs. The rubber bands used in this study were effective, i.e., the tags were well secured and not often lost, and evoked little reaction from the broilers after a short habituation period in which some pecking at the leg bands was observed, suggesting that the leg tags did not interfere with the normal behaviour of the broilers. Furthermore, no issues were observed with the rubber bands catching on objects in the environment, although it must be noted that all the objects in the pen and the pen walls had smooth surfaces. However, the leg bands had to be checked every couple of days and had to be switched to a larger size during the trial to avoid the rubber bands becoming too tight. Therefore, there would be added value to leg bands that can keep up with the growth of the broilers' legs in order to reduce the required manual labour.

Future research should also look into the potential of using RFID recordings to determine true distances moved in more detail. The relationship between RFID and video, which seems to show a linear pattern, indicates that there is potential to use RFID recordings to estimate true distances moved. Although not studied here, the RFID system could possibly be implemented to study true distances moved of group-housed birds, which could be a valuable tool in, for example, determining threshold levels of activity for reducing leg problems.

4.5 Conclusions

It was studied here whether a passive high frequency RFID tracking system was suitable for monitoring the individual levels of activity of broilers throughout life. In 62.5% of the cases, the RFID system was in full agreement with video in terms of the location of the animals. In total, 99.2% near matches, allowing for a deviation of one antenna, were observed between RFID and video in terms of location. There were moderately strong correlations in terms of distances moved between RFID and video (r_s = 0.82) and between RFID and UWB (r_s = 0.70). This indicates that the RFID system can accurately register among-individual variation in activity. However, the absolute values of the RFID recorded distances are generally an underestimation of the true distances moved. Overall, the RFID system appears suitable for monitoring the relative activity of individual group-housed broilers and can contribute to obtaining activity measures at the individual level early, and later, in life. Main benefits of the RFID system for tracking activity, compared to, for example, UWB tracking or video observations, include its small, lightweight tags that allow tracking of broilers from hatching onwards, limited manual labour and reliable identification of individuals. Data on activity levels early in life can potentially aid in identifying and predicting health, welfare and production parameters, but more research in this area is required.

4.6 Acknowledgements

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4.7 Supplementary information

Table S4.1 Intra-observer reliability scores for every observer.

Observer	Subjects	Raters	Kappa	Z	*p*-value
1	95	2	0.988	29.3	<0.001
2	96	2	0.977	29.3	<0.001
3	95	2	0.929	27.5	<0.001
4	94	2	0.753	23.6	<0.001
5	83	2	0.879	24.9	<0.001
6	95	2	0.953	28.5	<0.001
7	95	2	0.929	27.7	<0.001
8	92	2	0.951	27.5	<0.001
9	96	2	0.907	27.4	<0.001
10	93	2	0.952	27.8	<0.001
11	95	2	0.976	29.1	<0.001
12	98	2	0.887	27.9	<0.001

Chapter 5:
Early activity patterns and the relationship with body weight gain in broilers

Malou van der Sluis[1,2], Lucy Asher[3], T. Bas Rodenburg[2,4], Yvette de Haas[1], Britt de Klerk[5] and Esther D. Ellen[1]

[1] Animal Breeding and Genomics, Wageningen University & Research, 6700 AH Wageningen, The Netherlands;
[2] Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, 3508 TD Utrecht, The Netherlands;
[3] School of Natural and Environmental Sciences, Newcastle University, NE1 7RU, Newcastle upon Tyne, United Kingdom;
[4] Adaptation Physiology Group, Wageningen University & Research, 6700 AH Wageningen, The Netherlands;
[5] Cobb Europe, 5831 GH Boxmeer, The Netherlands

Abstract

Broilers show a relatively large decline in activity with age and this is thought to be a result of selection for high growth rates. This reduced activity level is considered a major cause of leg weakness and associated leg health problems. Increased activity, especially in the first weeks of life, is suggested to have positive effects on leg health, but the relationship between early activity and growth is unclear. A clearer understanding of the relationship between activity early in life and body weight gain could help to determine how selecting on increased early activity could affect body weight gain in broilers. Here, a radio frequency identification (**RFID**) tracking system was implemented to record daily individual broiler activity throughout life, in five production rounds. As mean activity levels alone do not capture the variation in activity over time, multiple (dynamic) descriptors of activity were determined based on the individual birds' daily distances moved, focussing on the period from 0 to 15 days old. The mean, skewness, root mean square error (**RMSE**), autocorrelation and entropy of (deviations in) activity were determined at the individual level, as well as the average daily gain (**ADG**). Relationships between activity descriptors and ADG were determined for 318 birds. Both when combining the data from the different production rounds and when taking production round and start weight into account, a negative relationship between ADG and RMSE was observed, indicating that birds that were more variable in their activity levels had a lower ADG. However, the activity descriptors, in combination with recording round and start weight, explained only a small part (8%) of the variation in ADG. Therefore, it is recommended for future research to also record other factors affecting ADG (e.g., type of feed provided and feed intake) and to model growth curves. Overall, this study suggests that increasing early activity does not necessarily negatively affect body weight gain. This could contribute to improved broiler health and welfare if selecting for increased activity has the expected positive effects on leg health.

5.1 Introduction

The growth rate of broilers has changed considerably over the years, as a consequence of selection for an increased growth rate and feed conversion, as well as changes in the diet. Havenstein et al. (2003) compared broiler strains from 1957 and 2001 on diets representative of those times, and estimated that the 2001 strain on the 2001 diet would reach a body weight of 1815 grams at 32 days of age, while the 1957 strain on the 1957 diet would only have reached this body weight at 101 days of age. The selection for high body weight gain is suggested to have resulted in a reduced activity level (Bizeray et al., 2000; Weeks et al., 2000). Dixon (2020) compared three faster growing broiler breeds to a slower growing breed, at the same age, and observed that the faster growing breeds spent more time sitting and less time in locomotion, mainly later in life. Similar observations were reported by Bokkers and Koene (2003), indicating that fast-growing broilers are relatively inactive.

Not only compared to slow-growing broilers, but also within fast-growing broilers, differences in activity have been observed that have been hypothesised to be related to body weight. For example, it has been observed that birds categorised as lightweight at approximately two-weeks-old showed a higher mean activity level over the period from 16 to 32 days old, than birds categorised as heavyweight (van der Sluis et al., 2019). Bokkers et al. (2007) tested fast-growing broilers in an operant runway test and observed that lightweight birds walked longer distances than heavyweight birds. These studies suggest that higher body weights are linked to lower activity levels.

Low activity levels are considered to be one of the main causes of leg weakness (Bessei, 2006), which is a general term to describe multiple pathological states that result in impaired walking ability in broilers (Butterworth, 1999). Leg weakness might be painful for the affected birds (Danbury et al., 2000). Moreover, in severe cases, birds may have difficulties competing with others for resources and may be restricted in performing specific behaviours, like dustbathing (Kestin et al., 1992; Vestergaard and Sanotra, 1999). Increasing locomotor activity levels may contribute to the prevention of leg problems (Reiter and Bessei, 2009). Different approaches for increasing activity, and hereby reducing leg problems, have been studied, for example, using different types of environmental enrichment (Vasdal et al., 2019), sequential feeding (Bizeray et al., 2002), or elevated platforms

(Kaukonen et al., 2017). It has been suggested that specifically early in life increased activity can improve leg health (e.g., Bizeray et al., 2000)), as the first weeks after hatching form a critical period in terms of bone development (Sanchez-Rodriguez et al., 2019), and a recent study has shown that early life activity is heritable in broilers (Ellen et al., 2021). However, it is unclear how early activity relates to body weight gain.

A clearer understanding of the relationship between activity early in life and body weight later in life would be valuable, as this would help to gain insight into how selecting on increased early activity affects growth in broilers. However, as emphasized in a review by Asher et al. (2009), behaviour is complex and multi-dimensional, and consequently the use of multi-disciplinary approaches for behaviour analyses is encouraged. Mean activity levels alone may provide insufficient insight to detect early differences in activity and therefore additional dynamic activity descriptors can have great added value. For example, Dawkins et al. (2012) studied optical flow patterns in broiler flocks, in relation to welfare measurements such as gait. They observed no correlation between the mean gait score and the mean optical flow, but did observe correlations between the mean gait score and the skew and kurtosis of optical flow. This shows that dynamic descriptors of activity can be more sensitive than mean activity levels alone.

In this study, dynamic descriptors of activity were studied in relation to body weight gain. We implemented an earlier-validated radio frequency identification (**RFID**) tracking system to record the activity of individual broilers throughout life (van der Sluis et al., 2020). From these activity recordings, individual activity levels, here calculated as distances moved, were determined for the first two weeks after hatching. We calculated the mean, skewness, root mean square error (**RMSE**) and autocorrelation of (deviations in) activity, as these descriptors of traits have been suggested to provide indications for resilience (Berghof et al., 2019b; van der Zande et al., 2020) and have potential to be implemented on repeatedly measured traits. For example, several of these indicators were calculated for body weight deviations in layer chickens (Berghof et al. 2019a) and for activity levels in pigs (van der Zande et al., 2020). We furthermore included entropy of activity, to assess the regularity of activity within days. Existing implementations of entropy in animal behaviour are limited, but it has for example been observed that spontaneously hypertensive rats displayed a higher complexity of movement time series, that is, a higher entropy, than control rats (Fasmer and Johansen, 2016). To examine whether these descriptors are related to broiler growth, we recorded the body weight of the birds every week and calculated the average daily gain (**ADG**) across the full production period. The overall aim of this study was to investigate the relationship between early activity and body weight gain, by determining dynamic descriptors of activity and combining the activity and body weight records. The broilers were kept under commercial conditions and no additional challenges were implemented to achieve contrast between individuals. Therefore, we expected only small effects in terms of activity descriptors. Under the assumption that a lower ADG is indicative of reduced well-being, the expectation was that a lower ADG would be linked to: 1) a lower mean activity; 2) a reduced (due to consistently low activity) or increased (due to some uncharacteristically inactive days) skewness of activity; 3) an increased RMSE due to more deviations in activity levels; 4) an increased autocorrelation of activity deviations due to activity deviations on subsequent days becoming more related; and 5) an increased entropy of activity due to a less regular daily activity pattern.

5.2 Materials and methods

5.2.1 Ethical statement

Data were collected on a broiler farm in the Netherlands, under control of Cobb Europe. Cobb Europe complies with the Dutch legislation on animal welfare. This study is considered not to be an animal experiment under the Law on Animal Experiments, as confirmed by the local Animal Welfare Body (20th of June, 2018, Lelystad, the Netherlands).

5.2.2 Location and subjects

In total, the activity of 402 broiler chickens was tracked on-farm with an RFID system. These 402 broilers were divided over five consecutive production rounds. The initial number of birds and the recording length differed per round and are shown in **Table 5.1**. Only male broilers were aimed to be included in this study, and therefore females that were included due to errors in sexing were excluded from the data, as well as birds that died during the study (**Figure 5.1**).

Table 5.1. Overview of the number of broilers at the start of the round and the age of the birds at the start and end of the round, per recorded production round.

Round	Age at start (days)	Age at end (days)	n_{birds} start
1	1	36	80
2	1	33	82
3	0	35	82
4	1	35	78
5	1	33	80
Total			402

5.2.3 Housing

In all five production rounds, the broilers were housed in a rectangular pen with a size of approximately 1.8 × 2.6 m (i.e., 4.7 m^2), which was fitted with a passive RFID system (van der Sluis et al., 2020). During the production period, the weight of the birds was monitored closely. Before the density in the pen would reach approximately 33 kg/m^2, some birds were removed from the pen and housed elsewhere, to avoid reduced activity due to high densities in the pen. Birds that were removed were excluded from the analyses, as no end weight was available for these birds (**Figure 5.1**). In the pen, feed and water

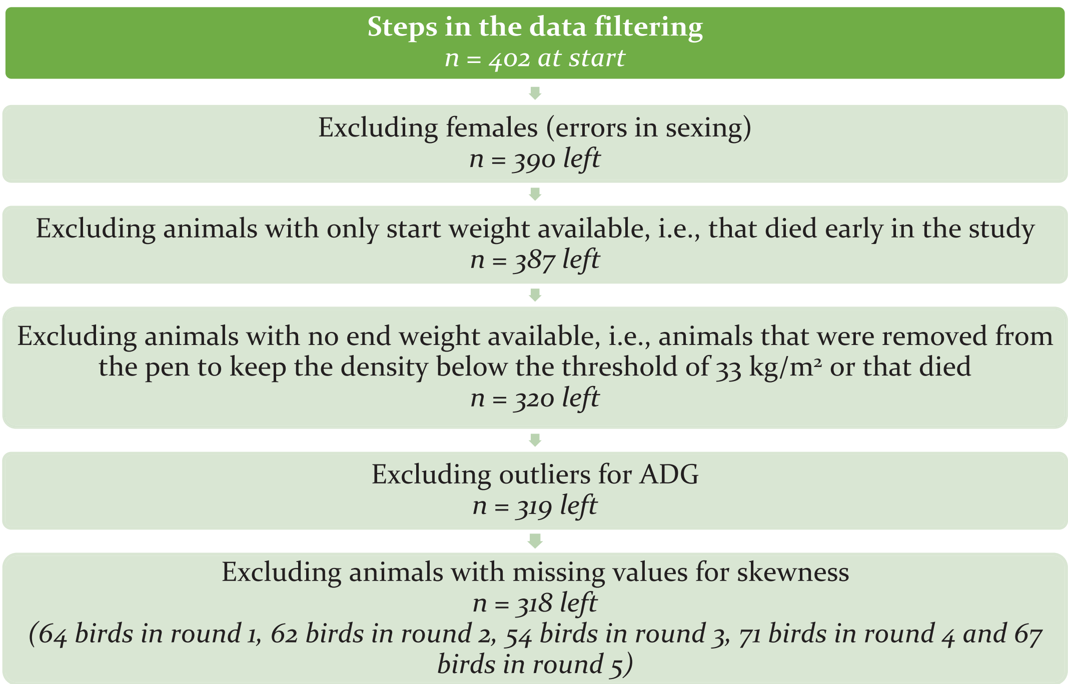

Figure 5.1. Overview of the steps in the data filtering and resulting sample sizes.

were provided *ad libitum* and wood shavings were provided as bedding. Starter, grower and finisher feed types were provided according to the common Cobb broiler feeding scheme (Cobb, 2018). The birds were kept under a commercial lighting and temperature schedule and were vaccinated according to common practice (Cobb, 2018).

5.2.4 Body weight records

Weighing of the birds was performed weekly, starting on the day of hatching and ending on the last day of the respective round (see **Table 5.1**). Weights were determined with five-gram precision, except for the start weight, which was determined with two-gram precision in rounds 1 and 3 and with one-gram precision in rounds 2, 4 and 5. The ADG was calculated as:

$$ADG = \frac{(body\ weight_{end} - body\ weight_{start})}{(age\ in\ days_{end} - age\ in\ days_{start})}$$

The age at which the end weight was recorded differed per round due to unequal lengths of rounds. By looking at ADG instead of end weight, a correction for this unequal length of rounds was made. The ADG across the period from 0-1 to 33-36 days old was used as the descriptor of body weight gain in this study. Extreme outliers for ADG were identified using a threshold of four times the standard deviation, and were determined within rounds. The outliers were identified using all animals in that round and ADG outliers were removed row-wise, meaning these animals were completely removed from the data (**Figure 5.1**). At this point, data for a total of 319 birds were available for analysis.

5.2.5 Activity records

To record the activity levels of the broilers, a passive RFID system from Dorset ID (Dorset Identification B.V., Aalten, the Netherlands) was implemented. All birds were fitted with an RFID tag, with a size of approximately 15 x 3.7 mm and a weight of less than one gram. These tags were attached to the birds' legs using rubber bands and tape, and were changed to a larger size once during the study and checked every couple of days. The broilers' pen was fitted with 30 high frequency antennas, with a size of 32 by 41 cm each, in a grid on the underside of a false floor. The RFID system could register the presence of RFID tags at these antennas and stored a log file, that included a timestamp, the ID code of the tag and the location, that is, antenna, at which the tag was registered. The recording frequency used here was generally one sample per second for all antennas, with exception of the instances in which antenna switches occurred and two different registrations within the same second could be observed. The RFID system and its validation, showing among other things a rank correlation between video and RFID of 0.82 in terms of recorded distances moved, are described in more detail in van der Sluis et al. (2020). In this study, RFID recordings were generally made continuously for 24 hours per day, but the periods in which the birds were not all in the pen, for example due to weighing or switching the leg bands of the birds, were excluded. Furthermore, corrections for missing data, for example due to technical problems, were made at the group-level when no RFID data was recorded for more than five consecutive minutes. Very occasionally, this resulted in a day without data available. Due to technical difficulties in round 1, there were relatively many short periods of missing data in this round. Furthermore, due to wet bedding in round 1, which was avoided in later rounds, the antennas underneath the drinker could not detect the presence of tags well, resulting in lower recorded activity levels in this round.

5.2.6 Activity calculations

The activity calculations in this study were made using only the data on the main light period from 07.00-23.00 each day, since broilers are observed to be mainly active during the light periods and relatively inactive during the dark periods (e.g., Nielsen et al. (2004)), and we observed a strong correlation between the total distances moved across 24 hours and across the main light period in the full dataset (tau = 0.953, (95% CI 0.952 – 0.954), $p < 0.001$). Given the interest in activity early in the production period, only data from the first two weeks after hatching (up to and including 15 days old) were studied here. From the RFID log, multiple activity indicators were calculated, for which the mean distance moved per hour formed the basis. Using the average distance moved per hour the skewness, RMSE and autocorrelation of (deviations in) activity were calculated. Furthermore, the entropy of activity was calculated using the raw RFID data. Each of these activity calculations is described in more detail below. Extreme outliers in the data were identified using a threshold of four times the standard deviation. Outliers were determined within rounds for the five activity descriptors. All outliers were identified using all animals in that round and any outliers observed were set to missing.

5.2.6.1 Mean distance

The distance calculations were based on the registered antennas over time for each individual and day in the RFID log file. The centre points of the antennas were used as an approximation of the location of birds within the antenna range, to calculate approximate distances moved. For a more elaborate description of how distances were calculated from the recorded antenna positions over time, see van der Sluis et al. (2020). The total distance recorded was then divided by the recording duration between 07.00 and 23.00 for that specific day, to obtain a daily average distance moved per hour (**DADM**). This was done to allow for comparisons between days and rounds, even when data were missing for part of a day due to for example weighing of the birds. The mean distance moved (**MD**; presented in meters per hour) was calculated for each animal by taking the mean of the DADM in the first two weeks after hatching, excluding days when data were missing for an individual.

5.2.6.2 Skewness

Skewness is a measure of the asymmetry of a distribution, with a positive skew meaning that the right tail of the distribution is longer than the left tail, and a negative skew meaning that the left tail is longer than the right tail (Legendre and Legendre, 2012). For each individual, the skewness of the activity level, based on the DADM, was calculated using the e1071 package (D. Meyer et al., 2019) in R. Days when data were missing were excluded on an individual basis. For one bird, there were too few days available to calculate skewness and this bird was therefore excluded (**Figure 5.1**). The resulting output was a skewness value per individual (**Skew**).

5.2.6.3 RMSE

The RMSE is a measure of the differences between model-predicted values and observed values. Given the expected decreasing trend in activity over time (e.g., van der Sluis et al. (2019)), linear regression models were used to obtain predicted activity levels over time for each individual's own linear pattern. To this end, linear models were fitted for each individual, with DADM modelled as a function of the day in the trial (i.e., age). Missing days of data were excluded on an individual basis. The predicted activity levels were then compared to the observed activity levels to obtain the RMSE of activity for each individual, with exclusion of missing values. The resulting output was an RMSE value per individual.

5.2.6.4 Autocorrelation

Using the residuals from the linear models described for the RMSE calculations, the lag-1 autocorrelation of deviations in activity was calculated for every individual. The lag-1 autocorrelation is the degree of correlation between the time series and the same time series set off by one time unit. Missing values for activity deviations were excluded, only complete cases per individual (that is, both the current deviation and the one-time-unit-offset deviation were available) were included in the calculation. The resulting output was an autocorrelation (**AC**) value per individual.

5.2.6.5 Entropy

Entropy is a measure of predictability and there are different statistical approaches to entropy. Here, sample entropy (**SampEn**) was used, which is a measure of the randomness or regularity of time series based on the existence of patterns (Delgado-Bonal and Marshak, 2019). Lower SampEn values indicate regularity and higher SampEn values indicate randomness. Here, entropy values were calculated for each individual and day, between 07.00 and 23.00 hours. To this end, it was first determined for every minute whether an antenna switch was registered within this minute for this animal. Then, for each 15-minute bin the number of minutes in which an antenna switch was registered was calculated. These values were then categorized into four classes based on the quantiles observed in the data, with 1 = very inactive (0-2 minutes), 2 = inactive (3-4 minutes), 3 = active (5-7 minutes), and 4 = very active (8-15 minutes). These classes for the 15-minute bins across the day were then used as the underlying time series for the entropy calculation. The entropy values can therefore be interpreted as a measure of regularity in activity within days. However, entropy could only be accurately calculated for days without missing data. Therefore, entropy was only calculated on a subset of data of all birds in round 2, 3, 4 and 5. Round 1 (n = 64) was excluded, because of the earlier-mentioned missing data due to technical difficulties. Furthermore, days on which the birds were weighed or their leg bands were checked, were excluded. This resulted in 1827 days of data available, from a total of 254 birds, for entropy calculation. The SampEn values were determined using the pracma package (Borchers, 2021) in R. The mean entropy per individual was then calculated for the two-week period (excluding missing values) and the resulting output was an entropy value per individual (**ENT**).

5.2.7 Statistical analyses

All analyses were performed using R version 4.0.2 (R Core Team, 2020). Descriptive statistics were used to examine the ADG and activity descriptor values for each of the tracking rounds. Correlations between activity descriptors and ADG were determined using Kendall rank correlations, as ADG and several of the activity descriptors were not normally distributed and there were ties in the data, i.e., multiple observations with the same value. For these correlations 95% CIs were determined using bootstrapping with the NSM3 package (Schneider et al., 2021). To examine whether activity descriptors were correlated, pairwise Kendall's rank correlations were determined between descriptors (**Table S5.1**). To take into account the confounding effects of 1) round in which the birds were tracked and 2) start body weight of the birds, a linear model with sum-to-zero contrasts was implemented. As no entropy data were available for round 1, the full model only included rounds 2 to 5. Furthermore, as it became apparent that there was a strong correlation between MD and RMSE (tau = 0.32 (95% CI 0.26 – 0.38), $p < 0.001$; **Table S5.1**) and RMSE was already observed to be correlated with ADG (see results section), RMSE was included in the model for estimating ADG, and MD was not. Round, start body weight and the activity descriptors apart from MD were included as fixed effects. To test the fixed effects, a backward stepwise approach without interactions was used that included all these effects. The resulting terms that were left were all included in two-way interactions. Backward selection was then again performed. The resulting final model was

$$Y_{ijk} = \mu + Round_i + \ \beta(SW)_j + +\beta(RMSE)_k + e_{ijk}$$

Where Y_{ijk} is the average daily gain, μ is the overall mean, $Round_i$ is the round of tracking (i = 2-5), $\beta(SW)_j$ is the start weight, $\beta(RMSE)_k$ is the RMSE and e_{ijk} is the residual term. No obvious deviations from normality or homoscedasticity were observed upon visual inspection of the residuals of the model. The ggplot2 (Wickham, 2016) package was used to make the visualizations. The level of statistical significance was set at 0.05 and in the text reported results are rounded to two decimals.

5.3 Results

5.3.1 Average daily gain

The mean ADG across all rounds was 77.46 (SD 10.48) grams. The ADG was very similar for the different rounds, with 78.04 (SD 7.80) grams for round 1; 74.81 (11.13) grams for round 2, 79.93 (SD 10.89) grams for round 3, 78.86 (SD 8.24) grams for round 4, and 75.91 (SD 13.08) grams for round 5.

5.3.2 Activity descriptors

The daily activity levels across the first two weeks after hatching are shown in **Figure 5.2**. Even though the rounds differed in their average activity level and exact pattern, a decrease in activity over time was observed in all rounds. The mean recorded distance was relatively low in round 1, likely due to the earlier-mentioned wet bedding. The entropy of daily activity over time for each of the four included rounds is shown in **Figure 5.3**. The average entropy remained relatively stable over time and the different rounds did not show large differences. However, there was quite some variation in individual entropy values within days and rounds. The mean values for all activity descriptors are shown in **Table 5.2**. The Skew, RMSE, AC and ENT values did not show large differences between rounds. There was variation in MD values between

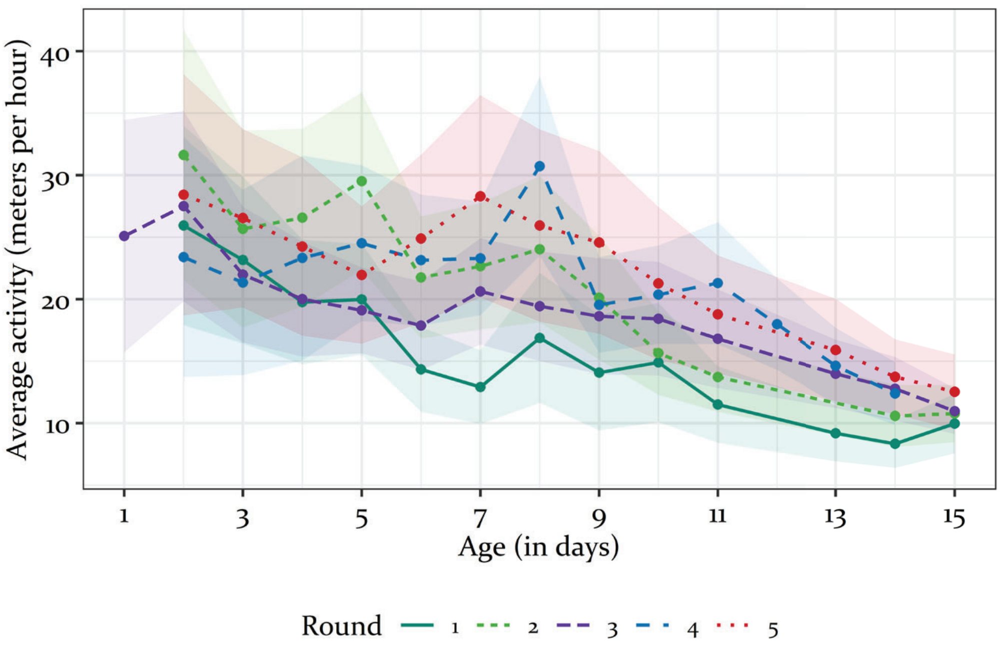

Figure 5.2. Average activity level over time for the different rounds. Shaded areas indicate SD ranges.

rounds, as was already shown in **Figure 5.2**. The average MD ranged between 15.21 (round 1) and 22.11 (round 5) meters per hour.

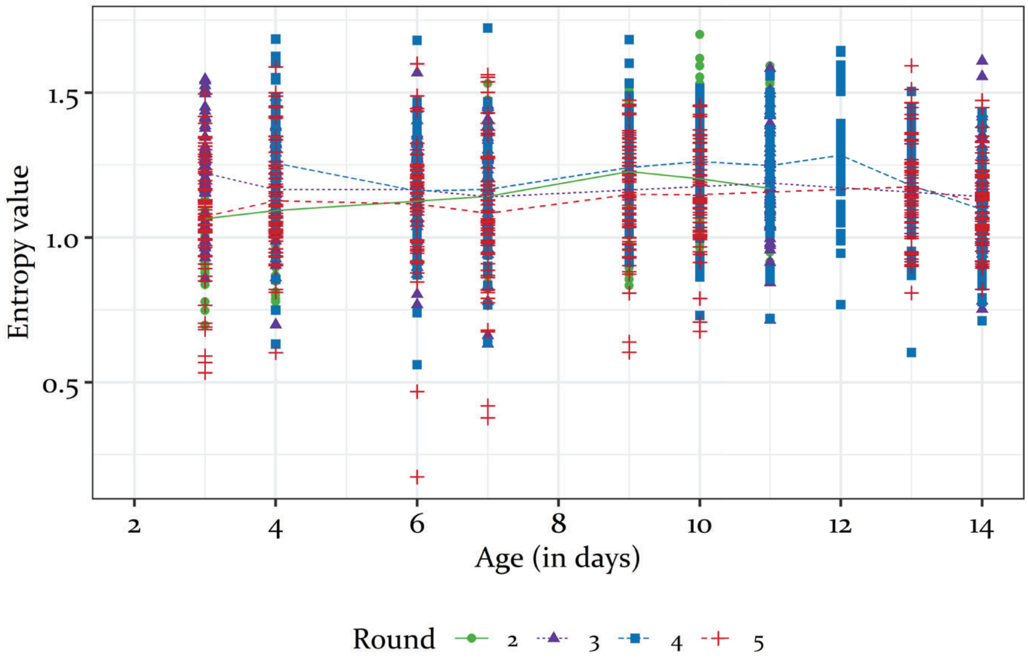

Figure 5.3. Entropy of daily activity over time for each of the four included rounds. Lines represent the average entropy values per day for the different rounds. Shapes represent individual entropy values for birds within days for different rounds.

Table 5.2. Mean values for the activity descriptors, both per round and across all rounds. Standard deviations are indicated between parentheses. RMSE = root mean square error.

Round	Mean distance	Skewness	RMSE	Autocorrelation	Entropy
1	15.21 (3.21)	0.75 (0.58)	3.00 (1.21)	0.16 (0.32)	NA[1]
2	21.00 (3.81)	0.17 (0.63)	3.74 (1.42)	0.10 (0.26)	1.15 (0.08)
3	18.78 (2.98)	0.52 (0.65)	3.16 (1.66)	0.25 (0.32)	1.17 (0.07)
4	21.24 (3.91)	0.35 (0.65)	4.48 (1.58)	0.14 (0.24)	1.21 (0.07)
5	22.11 (5.11)	0.08 (0.58)	3.71 (1.76)	0.26 (0.35)	1.12 (0.09)
Overall	19.74 (4.63)	0.37 (0.66)	3.65 (1.62)	0.18 (0.31)	1.16 (0.09)

[1] Entropy was not calculated for round 1 due to too many missing data.

5.3.3 Correlations between activity descriptors and ADG

Table 5.3 shows the rank correlations between the activity descriptors and ADG, when combining the data from all five rounds, with exception of entropy where round 1 was excluded. A negative correlation between RMSE and ADG was observed, indicating that broilers with a higher RMSE have a lower ADG. A trend for a negative correlation between MD and ADG was also observed, suggesting that birds that walk longer distances have a lower ADG.

Table 5.3. Rank correlations between the activity descriptors and ADG.

Activity descriptor	tau	95% CI	z value	*p* value
Mean distance	-0.065	-0.131 – 0.004	-1.724	0.085
Skewness	0.016	-0.069 – 0.097	0.426	0.670
Root mean square error	-0.105	-0.176 – -0.034	-2.787	0.005
Autocorrelation	-0.018	-0.092 – 0.055	-0.486	0.627
Entropy[1]	0.024	-0.064 – 0.117	0.564	0.573

1 Entropy was not calculated for round 1 due to too many missing data and was therefore excluded here.

5.3.4 Estimating ADG using activity descriptors

To take into account the possible confounding effects of round and start body weight, a linear model was implemented. The results from this model are presented in **Table 5.4**. The model had an adjusted R^2 of 0.08. Overall, there were differences between rounds in ADG and birds with higher start weights showed a higher ADG (estimate = 0.41, p = 0.005). The ADG decreased when the RMSE increased (estimate = -1.08, p = 0.011). An increase in RMSE means that the activity of an individual deviates more, in both directions (higher and lower), from a linear pattern over time.

Table 5.4. Results of the linear model for the predicted average daily gain (grams per day).

Factor	df	F-value	Pr(>F)	Estimate	SE	t-value	Pr(>|t|)
Intercept	1	51.480	<0.001	59.481	8.290	7.175	<0.001
Round[1]	3	3.874	0.010				
Round 2				-2.594	1.165	-2.228	0.027
Round 3				3.024	1.325	2.282	0.023
Round 4				1.532	1.192	1.285	0.200
Bodyweight start	1	7.876	0.005	0.414	0.147	2.806	0.005
RMSE	1	6.493	0.011	-1.078	0.423	-2.548	0.011

[1] Round 1 was not included in the model.

5.4 Discussion

In this study, the relationship between early activity and body weight gain was investigated. Activity records, collected using an RFID system and converted to several dynamic descriptors of activity, and body weight records were combined. Overall, only a few of the descriptors of activity were observed to be linked to ADG and these did not explain a large amount of the variance in ADG. The main findings will be elaborated on in the remainder of this discussion.

5.4.1 Average daily gain

The ADG was similar in all five production rounds. The overall mean ADG observed in this study was 77.46 (SD 10.48) grams, which is slightly higher than the common commercial ADG level. This difference is likely caused by the fact that pure lines and only males were studied here and that the housing density in this study was relatively low compared to commercial practice. However, our mean ADG does not differ substantially from other reports in literature, as for example M. Meyer et al. (2019) reported ADGs of 66.6 and 72.3 grams for broilers in a non-enriched or laser-enriched environment.

5.4.2 Activity descriptors

Only RMSE showed a statistically significant correlation with ADG, all other activity descriptors did not. The correlation between RMSE and ADG was not strong, but RMSE was still shown to be linked to ADG even when taking production round and start weight into account. Each of the activity descriptors is discussed in more detail here.

5.4.2.1 Mean distance

Overall, a decrease in mean distance moved over time was observed in this study. This decrease in activity over time matches with earlier observations and reports in literature (van der Sluis et al., 2019; Weeks et al., 2000), and is likely linked to the birds' increasing body weights (Tickle et al., 2018). At the same time, there appeared to be more variation in activity in the first days compared to later in the two-week period under study (**Figure 5.2**). This highlights the importance of activity records early in life, as later in life there might be too little variation in activity to really be able to distinguish individuals (see also Ellen et al. (2021), who used a dataset partly overlapping with the current study).

No statistically significant correlation between mean distance and ADG was observed, but there was a trend suggesting that the more active birds had a lower ADG. This indication fits with our earlier observations of lower activity levels for heavier birds (van der Sluis et al., 2019), as we assumed that birds with higher body weights later in life would have a higher ADG than lighter birds. This is supported by the observation of a strong positive correlation between ADG and final body weight in this study (tau = 0.80 (95% CI 0.78-0.83)). However, other studies have observed that differences in body weight are more pronounced later in life. For example, Dixon (2020) observed that broilers with different growth rates showed no difference in proportion of observations in which locomotion was observed when the birds were young and did not differ significantly in their body weight at two-weeks-old, but a difference in locomotory activity was observed at 30-days-old-and-over and in body weight from four-weeks-old onwards. One potential hypothesis for why we see this relationship between ADG and activity already early in life is that the overall ADG is strongly affected by the weight gain in the first two weeks. This weight gain might be related to early activity, and this could for example be the case when the ADG later in life is very similar for all birds and differences in ADG occur mainly early in the growing period. However, given that only a trend was observed, the interpretation of this suggested relationship requires some caution. Furthermore, a limitation of this study is that only ADG across the full growing period was examined, as a starting point for examining the relationship between early activity and body weight gain, and thus approaching the growth of birds as linear over time. In reality, ADG is not constant across the growing period (e.g., Zuidhof et al. (2014)) and there might be nuances to this growth pattern that other curves describe better (Topal and Bolukbasi, 2008). Individual growth curves would therefore be interesting to consider in future research, to examine which parts of the growth curve are related to different activity descriptors.

5.4.2.2 Skewness

For all rounds the average skewness of activity was close to zero, indicating fairly symmetrical distributions of daily activity levels during the two-week period under study. No correlations between skewness of activity and ADG were observed. For animals that are resilient, a skewness around zero would be expected and indeed for pigs it was observed that a decrease in skewness, towards zero, after a health challenge (that is, an infection with the Porcine Reproductive and Respiratory Syndrome Virus; **PRRSV**) lowered the risk of mortality (van der Zande et al., 2020). It must however be noted that skewness was not found to be related to morbidity. In accordance, other studies have concluded that skewness is not the most promising dynamic descriptor, for example for body weight deviations of layer chickens in relation to resilience (Berghof et al., 2019a). Here it appears that also for ADG in broilers the skewness, in this case of the activity level, is not informative.

5.4.2.3 Root mean square error

Across the different rounds, the average RMSE of activity was quite similar. A negative relationship with ADG was observed, also when taking production round and start weight into account. This relationship indicated that broilers with a higher RMSE, that is, more deviations from the expected linear trend in activity, had a lower ADG. In other words, it appears that when broilers strongly fluctuate in their activity level, instead of showing a steady, and generally declining, activity level over time, their growth is reduced. Other studies examining the RMSE of activity are limited, but van der Zande et al. (2020) observed that a higher RMSE after a PRRSV challenge tended to increase the risk of morbidity in pigs. In a study examining the RMSE of feed intake or feeding duration in pigs under a natural disease challenge, Putz et al. (2019) observed positive genetic correlations between both RMSEs and mortality, and a negative genetic correlation between the RMSE of feed intake and finishing ADG, which is in line with our observation of an increased RMSE (albeit of activity) being linked to a reduced performance. Although RMSE was observed to positively correlate with MD (**Table S5.1**), RMSE might be a more sensitive indicator of differences in activity patterns than mean activity levels, as mean activity levels alone cannot distinguish between a generally very active individual with several short severe activity decreases and a consistently moderately active individual. However, a difficulty with RMSE values is that they do not distinguish between different directions of deviations: high RMSE values could indicate 1) some days with higher activity levels than expected or predicted, 2) some days with lower activity levels than expected or predicted, or 3) a combination of both. Consequently, it is difficult to interpret the behaviour underlying the association between RMSE and reduced ADG. We hypothesize that one possibility could be that activity levels are linked to the number of feeder visits in broilers, and hereby potentially to feed intake. If there are fluctuations in activity, this may be indicative of fluctuations in feeding motivation (or feeding) and this may negatively affect the ADG, in line with the earlier-mentioned observation of a negative genetic correlation between the RMSE of feed intake and finishing ADG in pigs by Putz et al. (2019). However, more research is required to test this hypothesis through, for example, provisioning of fluctuating amounts of feed to broilers, or to examine whether other factors are at play that affect both ADG and activity.

5.4.2.4 Autocorrelation

The mean observed (lag-1 day) autocorrelations for the different rounds were all close to zero. This suggests that deviations in activity on subsequent days are unrelated (Berghof et al., 2019b). As discussed in Berghof et al. (2019b), an autocorrelation around zero is expected for individuals that show no disturbances or that recover fast from disturbances. It appears that, on average, the animals in our study showed few deviations in their daily activity level or, if they did, those changes did not last for prolonged periods of time. We observed no correlation with ADG. Generally, it is expected that less resilient animals show a positive autocorrelation (Berghof et al., 2019b). This has also been observed in practice, for example by van Dixhoorn et al. (2018) who observed that the autocorrelation in lying time in dairy cows could serve as a short-term predictor of disease severity. However, other studies have observed that autocorrelation was not informative, for example for morbidity or mortality in pigs, where autocorrelation in activity was calculated (van der Zande et al., 2020). It appears that also for ADG in broilers autocorrelation in activity is not informative.

5.4.2.5 Entropy

Studies have indicated that entropy can be informative, or even predictive, of human behaviour, health and well-being (Glenn et al., 2006; Montirosso et al., 2010; Okamoto et al., 2021). Entropy in non-human animal behaviour has not been extensively studied, but there are indications that entropy can be an informative measure of non-human animal behaviour as well (e.g., Guerrero-Bosagna et al. (2020), Stamps et al. (2013)). For example, McVey et al. (2020) studied milking order in dairy cattle using entropy, and observed that cows at the front and rear of the queue were more consistent in their entry position than individuals in the middle of the queue. Eguiraun et al. (2014) studied the collective response in groups of fish to a stochastic event (sudden hit in the tank) through entropy and observed that a group of fish exposed to a contaminant (methylmercury) showed a lower entropy compared to control groups. In the current study, the different production rounds were observed to show similar entropy means. No correlation between entropy and ADG was observed, indicating that more or less regular patterns in daily activity early in life are not associated with differences in ADG. However, it would be worth exploring different ways of applying entropy in the future to animal behaviour data to understand how to best capture the behaviour feature of interest.

5.4.3 Predicting ADG using activity descriptors and future directions

This study provides indications that deviations in early activity, represented in the RMSE of activity, are linked to a decreased ADG. However, the relationships were not strong and the implemented model explained little of the total variation in ADG in broilers (8%). This was expected, as ADG in broilers is known to be affected by many factors that likely play a larger role in the observed ADG than activity does. For example, body weight gain can be influenced by the type of feed that is provided, the amount of feed consumed and the feed conversion ratio (Havenstein et al., 2003; Marchesi et al., 2021). For practice this means that the RMSE of activity is insufficient to fully distinguish between birds with high or low ADG, as these other factors also play (likely even larger) roles in the observed ADG. For future research, it is recommended to also record these factors at the individual level, and to model growth curves instead of overall ADG to obtain a more complete picture of broiler growth and the factors that play a (predictive) role. In this way, more subtle patterns in growth can perhaps be detected and linked to activity in broilers. Besides the observations of RMSE and production round being linked to ADG, start weight was also observed to affect ADG. Broilers with a higher start weight showed a higher ADG, and the ADG was strongly correlated with the end weight in our study. Other studies have also observed a correlation between start weight and final body weight. For example, Willemsen et al. (2008) examined the predictive value of several chick quality measurements for slaughter-age body weight in different breeder lines and observed positive correlations between the body weight at one-day-old and the body weight on day 42. It must be noted, however, that several other studies observed no correlation between start weight and body weight later in life (e.g., Pinchasov (1991)).

There was no strong and statistically significant correlation between the distance moved early in life and ADG. Similar results have been reported by Ruiz-Feria et al. (2014), who observed that an increased distance between water and feed did not reduce the body weight of broilers, and similar observations have been reported by Reiter and Bessei (2009). However, addition of a ramp between water and feed did reduce the body weight of broilers, and this was suggested to be due to avoidance of the ramp as the broilers grew heavier, as the birds with a ramp ate less (Ruiz-Feria et al., 2014). This highlights the importance of examining the method for increasing activity closely, to assess whether there are no unintentional side effects of increasing the activity level of broilers. Overall, the current study suggests that it is possible to increase early-life activity without necessarily negatively affecting broiler growth. Increased activity can positively affect leg health in broilers (Kaukonen et al., 2017; Reiter and Bessei, 2009), and hereby contribute to improved broiler welfare and broiler production economics.

Not all activity descriptors examined in this study were linked to ADG in broilers. However, the continuous data on activity and subsequently calculated activity descriptors can provide us with more insight into the activity patterns of individual broilers over time. Possibly, this type of information can be informative or predictive for other traits in broilers, such as leg health. Given the individual variation that was observed in for example entropy of activity, there appears to be potential for further examination of whether and how such differences relate to different traits in broilers, to in the future be better able to monitor or even predict broiler health and welfare. Especially when the conditions in which the broilers are kept in future studies are more challenging than they were here, differences in activity patterns may be more pronounced.

5.5 Conclusions

This study examined the relationship between RFID-recorded early-life activity patterns and body weight gain in broilers. The RMSE of activity was correlated with ADG, and suggested that broilers with a higher RMSE had a lower ADG, but currently explained only a small part of the variation in ADG. Overall, this study suggests that increasing early-life activity without negatively affecting body weight gain in broilers is feasible, as there were no strong and statistically significant correlations between ADG and distances moved early in life. Through the expected positive effects of increased activity on leg health, this may in the future contribute to improved broiler health and welfare. Moreover, the activity descriptors studied here can provide more insight into the activity patterns of individual broilers over time, and allow for further examination of whether and how such patterns relate to different traits in broilers, to in the future be better able to monitor or even predict broiler health and welfare.

5.6 Acknowledgements

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5.7 Supplementary information

Supplementary table S5.1. Kendall rank correlations between activity descriptors. 95% CIs are indicated between parentheses. MD = mean distance, Skew = skewness, RMSE = root mean square error, AC = autocorrelation, ENT = entropy.

	MD	Skew	RMSE	AC	ENT[1]
MD		-0.17 (-0.24 – -0.10)	0.32 (0.26 – 0.38)	0.02 (-0.05 – 0.10)	-0.09 (-0.19 – -0.00)
Skew			0.11 (0.04 – 0.19)	-0.05 (-0.13 – 0.02)	0.03 (-0.07 – 0.11)
RMSE				0.20 (0.12 – 0.28)	-0.04 (-0.13 – 0.05)
AC					-0.04 (-0.13 – 0.05)
ENT					

Legend: ns | $p < 0.05$ | $p < 0.01$ | $p < 0.001$

[1] Entropy was not calculated for round 1 due to too many missing data and was therefore excluded here.

Chapter 6:

Genetic parameters for activity recorded using RFID throughout life in broilers

Esther D. Ellen[1], Malou van der Sluis[1,2], Britt de Klerk[3], John Henshall[3], Yvette de Haas[1] and T. Bas Rodenburg[2,4]

[1] Animal Breeding and Genomics, Wageningen University & Research, 6700 AH Wageningen, the Netherlands;
[2] Animals in Science and Society, Faculty of Veterinary Medicine, Utrecht University, 3508 TD Utrecht, the Netherlands;
[3] Cobb Europe, 5831 GH Boxmeer, the Netherlands;
[4] Adaptation Physiology Group, Wageningen University & Research, 6700 AH Wageningen, the Netherlands

Submitted

Abstract

The activity level of individual broilers is an important trait to monitor in relation to health, welfare and performance. The correlation between activity and gait that has been observed in other studies suggests that activity is a key candidate as a leg health proxy. An easy-to-measure proxy for leg health would be valuable, because including leg health in broiler breeding schemes has been shown to successfully reduce the prevalence of lameness and current recording methods are time-consuming and subjective. However, before activity is implemented as a proxy in breeding programs, it is important to determine whether activity is heritable in broilers. In this study, a radio frequency identification (**RFID**) tracking system was implemented to continuously record individual activity of broilers throughout life, and to determine genetic parameters for activity. Using individual activity data on 387 pure line broilers, a heritability of activity across the full production period of 0.31 ± 0.11 was estimated. Heritability estimates for each week in the production period showed a decreasing heritability of activity as the birds aged. The genetic and phenotypic correlations between activity levels recorded in different weeks were positive and most of them were moderate to strong, and the correlations between adjacent weeks were strongest. Overall, our results suggest that genetic selection on activity is feasible. Selection for increased early-life activity could potentially improve broiler welfare through the hypothesized positive effects on leg health.

6.1 Introduction

Selection for increased growth rate and feed conversion, together with changes in the diet, has had a strong effect in broilers (e.g., Havenstein et al. (2003)). However, the increased growth rate and higher body weight have raised welfare concerns in broilers. One of the major welfare concerns in broilers is leg health (SCAHAW, 2000), and research has indicated that higher growth rates and body weights of broilers are linked to increased lameness (Kestin et al., 2001).

Leg health or strength is included in selection schemes in breeding programs of broilers (reported in Hiemstra and ten Napel (2013)) to reduce the prevalence of lameness. Indeed, this approach has been shown to be successful for reducing specific leg health problems, such as hock burn and tibial dyschondroplasia (**TD,** a spontaneously occurring lesion, characterized by an avascular cartilage mass in the proximal metaphysis of the tibiotarsus and tarsometatarsus (Leach and Monsonego-Ornan, 2007)). For example, Kapell et al. (2012) studied the development of leg health over up to 25 years of selection in three purebred commercial broiler lines. They observed that the prevalence of hock burn (i.e., lesions on broilers' hocks (Greene et al., 1985)) decreased mainly in the first ten years of recording, with a range of -1.3 to -1.5% per year, after which it stabilized. The prevalence of TD was observed to decrease by -0.4 to -1.2% per year. However, detailed information on the leg health of individual birds is required for this selective breeding. Many of the leg health traits are scored manually, which can be time-consuming and subjective, as visual observations by human assessors are required. For example, gait is commonly scored manually by an experienced assessor, observing individual birds while they walk, according to the scoring system that was developed by Kestin et al. (1992). Furthermore, some leg conditions, like femoral head necrosis (**FHN**), can only be accurately assessed *post mortem*. For practice, this means that selection cannot be performed on the individual for which the trait is assessed, but only on its siblings, which reduces the accuracy of the breeding value (Siegel et al., 2019).

For this reason, there would be added value to an easy-to-measure proxy for leg health. Studies have indicated that there is a relationship between gait and activity in broilers (Aydin et al., 2010; van der Sluis et al., 2021; Van Hertem et al., 2018a), suggesting that activity can potentially serve as a proxy for gait.

Furthermore, there are indications that activity may serve as a proxy for other health and production parameters as well. In terms of health, diseased animals generally spend more time resting (Gregory, 1998). In terms of production, differences in activity have been observed between lightweight and heavyweight birds. Bokkers et al. (2007) tested broilers in an operant runway test and observed that lightweight birds walked longer distances than heavyweight birds. Also when activity was studied in the home pen of broilers, i.e., without walking for a reward, lightweight birds were observed to walk longer distances (van der Sluis et al., 2019). These relationships between activity and several health, welfare and performance components indicate that activity may indeed be a useful proxy to record in broilers.

Before activity is implemented as a proxy for other traits in breeding programs, it is important to determine whether activity is heritable in broilers. Some work in this direction has already been done. For example, Kjaer (2017) estimated the heritability of locomotor activity at around five weeks old in laying hen lines that were divergently selected on average level of spontaneous locomotor activity, using a New Hampshire line as founder line. Heritabilities of 0.38 ± 0.08 and 0.33 ± 0.06 were reported, for the low and high activity lines, respectively. Mignon-Grasteau et al. (2017) studied the heritability of activity at 16 days old in a cross between two broiler lines divergently selected on digestive efficiency and observed a low heritability for the number of observations during which an animal was moving (0.09 ± 0.07) that was not significantly different from zero. However, these studies have looked at activity at specific ages, while traits might show changes in heritability with increasing age, as the same trait may be influenced by different genetic and environmental factors at different ages (Visscher et al., 2008). Therefore, in this study a passive radio frequency identification (**RFID**) tracking system was implemented to continuously record the individual location and activity of broilers throughout life. This RFID system was validated for recording location and distances moved in an earlier study (van der Sluis et al., 2020). Using the resulting individual activity data for 387 pure line broilers, the heritability of activity and genetic correlations between activity levels at different ages were estimated to assess the feasibility of including activity as a proxy for specific health, welfare and performance traits in broiler breeding programs.

6.2 Materials and methods

6.2.1 Ethical statement

Data were collected under control of Cobb Europe. Cobb Europe complies with the Dutch law on animal well-being. This study is not considered to be an animal experiment under the Law on Animal Experiments, as confirmed by the local Animal Welfare Body (20th of June, 2018, Lelystad, the Netherlands).

6.2.2 Animals and housing

Data were collected on a broiler farm in the Netherlands. In total, 402 purebred broilers were included in the study divided over five consecutive rounds, with approximately 80 male birds per round (**Table 6.1**). These broilers were placed in a pen with a size of 4.7 m^2 from day of hatching to slaughter, although some broilers were removed from the pen in the course of the growing phase to avoid densities higher than 33 kg/m^2. In the pen, food and water were provided *ad libitum*, wood shavings were provided as bedding, and the birds were kept under the regular commercial feed, lighting and temperature schedule (Cobb, 2018).

Table 6.1. Overview of the number of birds, start and end age of the birds and the number of days included for each of the rounds.

Round	**1**	**2**	**3**	**4**	**5**
N birds start	80	82	82	78	80
Age at start of round (days)	1	1	0	1	1
Age at end of round (days)	36	33	35	35	33
N days included[1]	33	29	33	32	30

[1] Several days of data were excluded as there were limited data available. This was the case for the first and last day of the trial, and for the day on which the leg bands were switched.

6.2.3 Pedigree

For each round, mostly the same sires and dams were used, but with different numbers of offspring representing them. In total, 31 sires and 96 dams were used. Each sire was mated with approximately 3 dams, and each dam contributed on average 3.7 male offspring (1-13 offspring/dam). Approximately six generations of pedigree were included in the calculation of the relationship matrix (A).

6.2.4 Body weight

Individual body weight was generally measured at the start (day 0 or 1), at 8, 15, 22, 29 days of age, and at slaughter age (day 33-36 depending on the tracking round; see **Table 6.1**). In round 1, there was one exception where birds were weighed at 21 days old instead of at 22 days old. All weights were determined with five-gram precision, except for the start weight, which was determined with two-gram precision in rounds 1 and 3 and with one-gram precision in rounds 2, 4 and 5. Furthermore, average daily gain was calculated as the final body weight minus the start weight divided by the number of days between these two weighing days. These body weight traits were used to correct for body weight in the heritability estimates over time.

6.2.5 Activity recordings

The broilers were tracked continuously with an RFID tracking system from Dorset Identification (Dorset Identification B.V., Aalten, The Netherlands). All broilers were fitted with an RFID tag on one of their legs and the entire home pen was fitted with a grid of 30 RFID antennas underneath a false floor (van der Sluis et al., 2020). The RFID system was recording the position of the birds, i.e., the antenna grid cell where they were located, continuously throughout the round with approximately one sample per animal per second, and stored a log file with the animal ID, location and timestamp. From the recorded antenna positions over time, approximate distances moved were calculated using data from the main light period from 07.00-23.00 each day. The recorded distance was then divided by the recording duration between 07.00 and 23.00 for that specific day, to obtain an average distance moved per hour for each day. This was done to allow for comparisons between days and rounds, even when data were missing for part of a day due to for example weighing of the birds or technical difficulties. Using this average distance moved per hour for each day, the following seven activity traits were calculated:

1) the average activity over time (**Act$_{\mathbf{total}}$**) from the first day after placing the birds in the pen until the last day before the round ended (see **Table 6.1**), presented as the average distance moved in meters per hour over this period;

2) five activity traits for the average activity per week (**Act**$_{Wi}$), presented as the average distance moved in meters per hour over this period, with *i* representing the week number and week 1 including the first day after placing the birds in the pen up to and including day 8, week 2 including days 9-15, week 3 including days 16-22, week 4 including days 23-29 and week 5 containing 30 days and older.

3) the slope of the activity over time (**slope**), calculated using linear regression models in R (R Core Team, 2019). For each animal, the average distance moved per hour was fitted as a function of time (i.e., day of tracking).

The number of days included in the calculations could differ per round, due to the differing round lengths and missing data for some days, for example caused by technical failure or by switching of the RFID leg bands, which resulted in changes in the RFID codes linked to specific individuals and therefore incomplete data for the day of switching. In **Table 6.1**, it is indicated how many days of data were included for each of the rounds. For some birds, the number of days may have been lower, for example due to loss of leg tags. Due to wet bedding in round 1, which was avoided in later rounds, the antennas underneath the drinker could not detect the presence of tags well, resulting in lower recorded activity levels in this round.

6.2.6 Data analysis

Several animals were excluded from the data analyses. Females (mis-sexed) were removed from the data (leaving n = 390 males). Furthermore, animals with only start bodyweight, i.e., that died before further recordings were made, were removed from the data, resulting in 387 animals (round 1: n = 80; round 2: n = 77; round 3: n = 82; round 4: n = 72; round 5: n = 76). For these 387 animals, extreme outliers were identified based on a threshold of four times the standard deviation. Within each round, outliers were determined and excluded for each of the seven activity traits, the body weights over time and ADG separately. All outliers were identified using the complete data available for that round, after which outliers for each of the variables were removed separately. R software version 3.6.1 (R Core Team, 2019) was used to decide which fixed effects to include in the model for estimating genetic parameters for activity and to calculate residuals for body weight, fitting body weight as a function of round and, for body weight D8, D15, D22 and D29, also start weight. The initial model included fixed effects for round and body weight residuals. For each of the weekly activity levels, the body weight residuals for the weighing moment at the start of the respective week were included (**Table S6.1 in Supplementary data**). For the overall average activity and slope in activity, the residuals for ADG were included. The software package ASReml (Gilmour et al., 2015) was used to estimate genetic parameters for activity. First, a linear animal model was used:

$$y = Xb + Za + e \quad [1]$$

where y is a vector of observed average activity across the round, weekly average activity or slope of activity, b is a vector of fixed effects, with incidence matrix X linking observations to fixed effects, a is a vector of the breeding values, with incidence matrix Z linking observations on individuals to their breeding values, and e is a vector of random residuals. Second, a multivariate animal model was used to estimate genetic and phenotypic correlations between activity at different ages, and to account for repeated measurements, again including round and the respective body weight residuals as fixed effects.

6.3 Results

6.3.1 Body weight

The average body weight over time in the different rounds is shown in **Figure 6.1**. Overall, there were no large differences in body weight between the different rounds, except for the final body weight. However, this is likely because the different rounds ended at different ages (**Table 6.1**), as the trend of body weight over time is similar for all rounds.

6.3.2 Activity

Table 6.2 shows the overall average activity and slope in activity per round. The average distance moved ranged from 9.3 through 14.0 m per hour per day (from 7:00 until 23:00). As expected, the slope was negative, meaning a decrease in activity over time, ranging from −0.6 through −1.0 m per hour for every additional day of age. Overall, the recorded activity in round 1 was lower, compared to the other rounds. **Figure 6.2** shows the average activity per week for each round. There was a clear decrease in the average activity level over time, as well as a decrease in standard deviation of the observed activity levels.

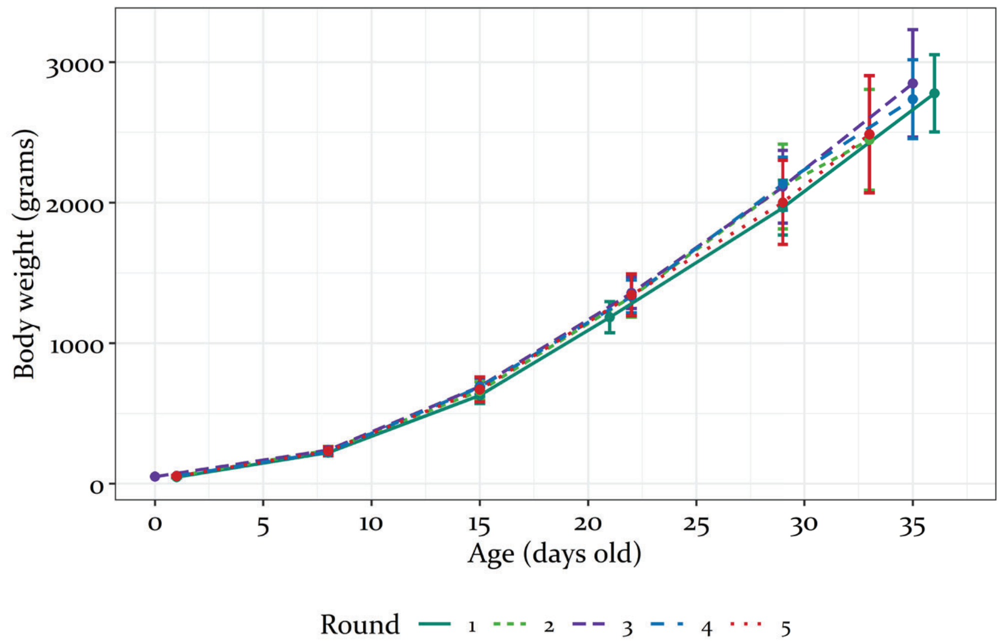

Figure 6.1. Average body weight over time for the different rounds. Error bars indicate SDs. Note that body weight recordings were made at different ages in some instances.

Table 6.2. Activity levels over time per round (in m per hour). The sample size is indicated between brackets, and can differ due to outlier removal. Act_{total} = activity measured across the whole round.

Round	1	2	3	4	5
Act_{total}	9.3 ± 1.9 (80)	13.1 ± 2.2 (77)	12.7 ± 2.3 (82)	12.7 ± 2.0 (71)	14.0 ± 3.5 (76)
Slope	-0.6 ± 0.2 (80)	-0.9 ± 0.2 (77)	-0.7 ± 0.2 (82)	-0.8 ± 0.2 (72)	-1.0 ± 0.3 (76)

6.3.2.1 Heritability estimates for activity over time

The genetic parameters for the different activity traits are shown in **Table 6.3**. Overall, the heritabilities were moderate, ranging from 0.11 ± 0.08 (Act_{W4}) to 0.45 ± 0.13 (Act_{W1}). The heritability of activity decreased over time. The heritabilities of the total average activity and the slope were also moderate (0.31 and 0.41, respectively; **Table 6.3**).

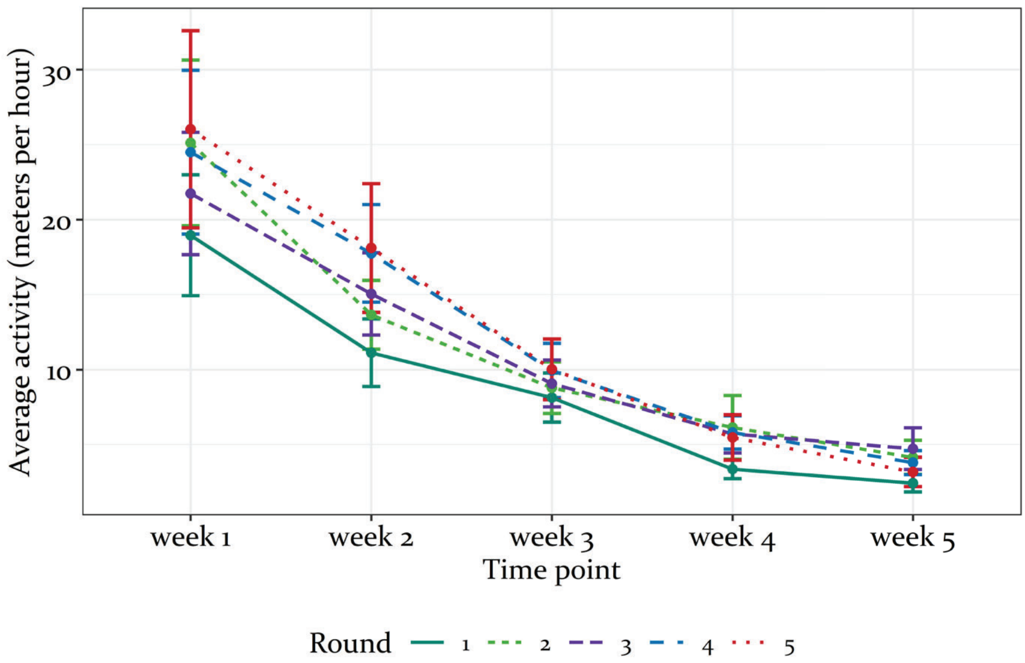

Figure 6.2. Average distance moved per hour over time for the different rounds. Error bars indicate SDs. Each time point represents approximately one week of data, with week 1 including the first day after placing the birds in the pen up to and including day 8, week 2 including days 9-15, week 3 including days 16-22, week 4 including days 23-29 and week 5 containing 30 days and older.

Table 6.3. Genetic parameters for average activity. σ_A^2 is the genetic variance; σ_P^2 is the phenotypic variance; h^2 is the heritability; Act_{total} = activity measured across the whole round; Act_{Wi} = the activity measured in a certain week (i = 1-5).

Trait	σ_A^2	σ_P^2	h^2
Act_{total}	1.86 ± 0.71	5.93 ± 0.47	0.31 ± 0.11
Act_{W1}	12.47 ± 4.21	27.66 ± 2.41	0.45 ± 0.13
Act_{W2}	3.49 ± 1.30	9.09 ± 0.77	0.38 ± 0.12
Act_{W3}	0.87 ± 0.37	2.97 ± 0.24	0.29 ± 0.11
Act_{W4}	0.22 ± 0.16	1.91 ± 0.15	0.11 ± 0.08
Act_{W5}	0.13 ± 0.10	1.00 ± 0.08	0.13 ± 0.10
Slope	0.02 ± 0.01	0.05 ± 0.01	0.41 ± 0.12

6.3.2.2 Genetic and phenotypic correlations between activity levels over time

The genetic and phenotypic correlations between activity measured at different ages are shown in **Table 6.4**. The phenotypic correlations were moderately positive and significantly different from zero, ranging from 0.18 ± 0.06 (Act_{W1}-Act_{W5}) to 0.77 ± 0.02 (Act_{W4}-Act_{W5}), and were stronger for adjacent weeks than for weeks further apart. The genetic correlations were positive, moderate to strong, and significantly different from zero, except for the genetic correlations between Act_{W1} and both Act_{W4} and Act_{W5}. The genetic correlations between adjacent weeks were stronger than for weeks that were further apart (0.73 ± 0.24 to 0.94 ± 0.11 for adjacent weeks, 0.08 ± 0.41 for Act_{W1}-Act_{W5}).

Table 6.4. Genetic (above diagonal) and phenotypic (below diagonal) correlations between weekly activity measured at different ages. Act_{Wi} = the activity measured in a certain week (*i* = 1-5).

	Act_{W1}	Act_{W2}	Act_{W3}	Act_{W4}	Act_{W5}
Act_{W1}		0.77 ± 0.13	0.61 ± 0.19	0.07 ± 0.34	0.08 ± 0.41
Act_{W2}	0.57 ± 0.04		0.94 ± 0.11	0.57 ± 0.30	0.51 ± 0.41
Act_{W3}	0.40 ± 0.05	0.59 ± 0.04		0.73 ± 0.24	0.65 ± 0.37
Act_{W4}	0.22 ± 0.05	0.33 ± 0.05	0.54 ± 0.04		0.86 ± 0.20
Act_{W5}	0.18 ± 0.06	0.22 ± 0.05	0.42 ± 0.05	0.77 ± 0.02	

6.4 Discussion

In this study, a passive RFID system was implemented to record the individual location and activity of broilers throughout life, with the aim of estimating the heritability of activity and the genetic correlations between activity at different ages, while housed in groups. The results showed that activity is heritable, but that the heritability changes over the growing period.

6.4.1 Activity trends over time

Within the different rounds, a clear decrease in activity over time was observed. This decrease in activity over time matches with reports in literature (e.g., van der Sluis et al. (2019); Weeks et al. (2000)). Overall, the recorded activity in the first round was somewhat lower than for the other rounds. Likely, this is due to difficulties that were experienced with wet bedding in this round. When analyzing this round, it was discovered that the number of antenna registrations underneath the drinker was very low at the end of the trial, very likely caused by wet bedding blocking the RFID signal. In the consequent rounds, the bedding was replaced periodically to avoid this issue. The reduced recording capability in the first round was accounted for by including a round effect in the analyses. Furthermore, analyses without data of the first round included resulted in very similar conclusions.

6.4.2 Heritability estimates for activity over time

The heritability of activity across the full recording period was estimated to be 0.31 ± 0.11. When looking at weekly activity levels, a decreasing trend in heritability of activity over time was observed. Heritability decreased from 0.45 ± 0.13 (week 1) to 0.11 ± 0.08 (week 4). Concurrently, the genetic and phenotypic variance decreased, indicating that the individual activity levels of broilers became more similar to each other as the broilers aged. Moreover, in comparison with the variance values of activity in first week of life, the genetic variance in the last days of the recording period was relatively small compared to the phenotypic variance. This suggests that as broilers grow older, their recorded activity level is influenced to a relatively larger extent by factors other than genetic variation for activity. At different ages, different factors may play a role, or to a different extent. Many factors have been suggested to affect activity levels in broilers, besides the earlier mentioned role of age and body weight (e.g., van der Sluis et al. (2019); Weeks et al. (2000)). These include, but are not limited to, leg health (e.g., Weeks et al. (2000)), light intensity (e.g., Blatchford et al. (2009)), environmental enrichment (e.g., Vasdal et al., (2019)), placement of feeders and drinkers (e.g., Reiter and Bessei (2009)), and housing density (e.g., Hall (2001)). Likely, over the growth period these factors play a role to different extents, resulting in different heritability estimates for activity and suggesting that activity is not the same trait at different ages in broilers. Other studies have also estimated the heritability of activity in poultry. For example, Kjaer (2017) reported heritabilities of 0.38 ± 0.08 and 0.33 ± 0.06 for locomotor activity at approximately five weeks old, for divergently selected low and high activity lines of laying hens, respectively. This is higher than our heritability estimate at this age, but it is important to note that five weeks old is relatively young in laying hens, while for broilers this is already near the end of the production period. While around this age the broilers in our study on average weighed more than 2600 g, the laying hens in the study by Kjaer (2017) had mean body weights in the range of around 200 to 400 g. Given the earlier-mentioned relationship between activity, age and body weight, it is not surprising that the reported heritability of activity for broilers is lower than for laying hens at this age. When we look at broilers with body weights similar to these laying hens, which in our data set is closest to 1 week old, the heritability estimate of activity was 0.45 ± 0.13 and resembles the heritability observed for laying hens (Kjaer, 2017). Others have also studied the heritability of activity in broilers. Mignon-Grasteau et al. (2017) reported a low heritability (0.09 ± 0.07) for the number of observations during which an animal was moving at 16-days-old that was not significantly different from zero for a cross of two divergently selected broiler lines, selected on digestive efficiency. This estimate is much lower than our observed estimate of 0.38 ± 0.12 around this age. However, the activity levels in the study by Mignon-Grasteau et al. (2017) were determined in a different way compared to our activity recordings, that is, they determined activity levels through the use of scan sampling of one-hour video recordings, in which the number of observations during which an animal was moving was counted. Possibly, the recordings of activity in our study, using distances moved between 07.00 and 23.00 each day, reflect a different underlying trait, resulting in a different heritability estimate.

6.4.3 Genetic and phenotypic correlations between activity levels over time

The genetic correlations that were observed between the activity recordings at different ages were moderately to strongly positive and significantly different from zero, except for the genetic correlations between activity in week 1 and activity in weeks 4 and 5. Generally, the genetic correlations between activity levels recorded closer in time were higher than those recorded further apart. Activity levels at different ages might represent different traits, as was mentioned earlier, but the closer they are in time, the more these traits might resemble each other. This trend for lower genetic correlations for recordings further apart in time has also been observed in other studies (e.g., for several weight and body composition traits in cattle (Johnston et al., 2003) or for body weight in broilers (Chu et al., 2020)). The phenotypic correlations resembled the genetic correlations in terms of the overall pattern that was shown, with observations closer in time having higher correlations than observations further apart in time. As activity levels are known to change over time in broilers, that is, showing a decreasing trend (van der Sluis et al., 2019; Weeks et al., 2000), this pattern of observations further apart in time resembling each other less was as expected.

6.4.4 Relationships between activity and body weight

The current data set was too limited to reliably estimate genetic correlations between activity and body weight. However, the estimates of the body weight residuals indicated a negative relationship between activity and body weight (**Table S6.1 in Supplementary data**). This negative relationship between activity and body weight has been observed earlier (Tickle et al., 2018; van der Sluis et al., 2019) and there are multiple possible explanations for this relationship. For example, Tickle et al. (2018) studied the energetics and behavior of broilers across the growing period and observed that the costs of resting metabolism increased over development. This was hypothesized to limit the energy available for other activities, including locomotion. Another possible explanation could be that the negative estimates between body weight and activity are related to leg health. Higher body weights have been linked to increased leg problems (Kestin et al., 2001), and these leg problems may reduce birds' activity levels (Weeks et al., 2000).

6.4.5 Implications for practice

The observations in this study suggest that genetic selection on activity is feasible. However, before activity is implemented in breeding programs, it is of great importance to determine whether there are no unintentional side effects of selection on activity. The data from the current study were too limited to assess relationships with other traits, but some indications for relationships with body weight were discussed earlier and other indications for potential side effects have been reported in literature. Krause et al. (2019) studied laying hens lines that were divergently selected on general locomotor activity (**GLA**) and studied the behavioral consequences of this selection. Compared to the low GLA line, the high GLA line showed - as expected - a higher GLA, but also a higher body weight at 30 days of age (but not at adulthood), and an increased fearfulness (measured in a tonic immobility test). In contrast, the opposite has been observed in Japanese quail chicks that were selected on locomotor activity, in which the inactive line was observed to be more fearful than the active line (R. B. Jones et al., 1982). Krause et al.

(2019) furthermore observed that the two laying hen lines did not differ in gentle feather pecking, severe feather pecking, aggressive pecking, and several social behavior traits. Contrastingly, Lutz et al. (2016) studied genetic correlations between feather pecking, feather eating (**FE**) and GLA in laying hens and estimated a positive genetic correlation between GLA and FE. In broilers, Skinner-Noble et al. (2003) studied whether improved feed conversion was associated with increased lethargy and observed, among other things, a correlation between resting and the feed conversion ratio from 40 to 47 days of age which indicated that broilers with a good feed conversion ratio were less lethargic. Overall, these studies indicate that activity is related to other traits in poultry, although the directions are sometimes unclear. Therefore, before activity is implemented in breeding programs, careful examination of unintentional side effects is of great importance. This study indicated that activity levels at different ages might not represent the same trait. This highlights the importance of the timing of activity recordings that are used as a proxy for health, welfare or performance. A major benefit of the RFID system that was implemented in this study was that activity levels could be recorded continuously throughout life, and this would be advisable for future studies into the relationship between activity and other traits in broilers as well, to determine the best age at which to record activity. Increased activity can be beneficial for leg health (e.g., Reiter and Bessei (2009)), and it is hypothesised that especially increased activity early in life might be beneficial (e.g., Bizeray et al. (2000)). Therefore, selection for increased early-life activity could potentially improve broiler welfare, as leg weakness is thought to be painful for the birds (Danbury et al., 2000), might result in difficulties in competing for resources with other broilers in more severe cases (Kestin et al., 1992), and may result in a limited ability to perform behaviors such as dustbathing (Vestergaard and Sanotra, 1999). Furthermore, a reduction in leg health problems can have economic benefits (discussed in Bradshaw et al. (2002)). Altogether, the results of this research can contribute to a sustainable breeding goal.

6.5 Conclusions

This study examined the heritability of activity, recorded using an RFID system, over time and the genetic correlations between activity levels at different ages. This was done with the aim of assessing the feasibility of including activity as a proxy for health, welfare and performance traits in broiler breeding programs. Overall, activity was observed to be heritable, with decreasing heritability estimates as the broilers aged, suggesting that activity levels at different ages might not represent the same trait and highlighting the importance of the timing of activity recordings that are to be used as a proxy for specific correlated traits. Although more research into potential unintentional side effects of selection on activity is required, the results of this study suggest that genetic selection on activity is feasible.

6.6 Acknowledgements

This work was financially supported by the Dutch Ministry of Economic Affairs (TKI Agri & Food project 16022) and the Breed4Food partners Cobb Europe, CRV, Hendrix Genetics and Topigs Norsvin. The authors thank Twan van Rijswijck for his help with the experimental work and for taking care of the broilers, as well as the employees of Cobb Europe (Boxmeer, the Netherlands) for their help with the experimental work.

6.7 Supplementary information

Supplementary Table S6.1. Estimates for the body weight residuals for the different ages of the birds. Reported numbers are rounded to three decimals. Act_{total} = activity measured across the whole round; Act_{Wi} = the activity measured in a certain week (*i* = 1-5).

Age	Body weight parameter	Estimate ± SE	*p*-value
Act_{total}	ADG	-0.006 ± 0.012	0.603
Act_{W1}	Start weight	-0.079 ± 0.058	0.177
Act_{W2}	Body weight day 8	-0.022 ± 0.005	2.34e-05
Act_{W3}	Body weight day 15	-0.006 ± 0.001	1.24e-05
Act_{W4}	Body weight day 22	-0.002 ± 0.001	2.94e-04
Act_{W5}	Body weight day 29	<-0.001 ± <0.001	0.797
Slope	ADG	0.002 ± 0.001	0.098

Chapter 7:
General discussion

7.1 Activity tracking in broilers

Broilers are nowadays kept in large groups, where individual identification and monitoring of activity levels are challenging. This is a pity, since individual data on activity are hypothesized to be informative for broiler health, welfare and performance. However, novel approaches like sensor technologies can provide solutions. In this thesis, my goal was to implement automated activity tracking and shine light on the relationships between activity, body weight and leg health in broilers, to ultimately be able to select for improved health and welfare. The first objective was to determine the suitability of different sensor technologies for individual activity tracking in broilers, and validate these technologies in an experimental setup that resembled the commercial environment, but on a smaller scale. I focused on two sensor technologies: an ultra-wideband (**UWB**) system and a radio frequency identification (**RFID**) system. Both were implemented and validated by comparing the recordings to video observations. I addressed this objective in **chapters 2** and **4**. The second objective was to examine the relationships between individual activity, body weight and leg health in broilers. For this, I recorded individual activity, body weight and leg health and studied 1) how these traits were correlated, and 2) the heritability of activity over time. I addressed this objective in **chapters 3**, **5** and **6**.

In this final chapter, I will discuss and bring together the main findings from the different chapters and will place them in the broader context of implementation. I will start with the technological context and compare the two implemented sensor technologies to each other and to computer vision (**CV**), to highlight their advantages and limitations. I will then move to the use of activity as a proxy for health and welfare, for which I will elaborate on the heritability of activity and the relationships between activity, body weight and leg health, based on the results from the different chapters in this thesis. I will end the discussion with my view towards implementation in the future, for which I will discuss how we can record activity in larger-scale environments in a simplified manner (with a lower level of detail) or in an alternative way.

7.2 Sensor technologies: advantages and limitations

In this thesis, both UWB and RFID technology were used to collect individual broiler activity data. Which of these two sensor approaches is most suitable for further implementation depends on the specific question one wants to answer, as well as the broiler's age and environment for which one wants to do so.

7.2.1 Main findings from the validation studies

The UWB system and the RFID system were successfully validated for broiler activity tracking in **chapters 2** and **4**, respectively. Both systems performed well when estimating relative distances moved, that is, distances moved by individual birds compared to other birds that are tracked at the same time in the same setup. Moreover, the two systems appeared to be in agreement when looking at relative activity levels (r_s = 0.70, 95% CI 0.41-0.86; **chapter 4**). However, the recorded distances did not reflect true distances moved (as determined from video recordings) well. As was shown in **chapters 2** and **4**, the UWB system on average overestimated the true distance moved when the true distances moved were low, but underestimated the true distance moved when the true distances moved were high. Similar observations, regarding UWB tracking not always reflecting true distances moved well, have been reported for fast-growing broiler tracking indoors under commercial conditions by Baxter and O'Connell (2020). They observed that the location accuracy level was high, but that the distances that were travelled by the broilers were consistently overestimated. They did, however, observe a positive correlation between the recorded and true distance, indicating that activity differences can be studied even though absolute distances are difficult to determine. Conversely, the RFID system generally underestimated the true distance moved, as was shown in **chapter 4**. The reasons for these observed over- and underestimations were highlighted in **chapters 2** and **4**: for the UWB system these were mainly linked to the sampling rate and noise of the system (**chapter 2**) and for the RFID system these were mainly due to the fact that the RFID system cannot detect movement within antenna grid cells (**chapter 4**). Overall, it can be concluded that both the UWB system and the RFID system are suitable for relative activity tracking in broilers. However, given that RFID can be implemented throughout life in broilers, and UWB cannot, RFID seems the most promising approach for future implementations in broilers.

7.2.2 Comparison of sensor technologies

To highlight the advantages and limitations of UWB and RFID, a comparison between UWB, RFID and CV - as a popular contrasting approach - is shown in **Table 7.1**. It is important to note here that this comparison is specifically for the two systems that were studied in this thesis, with the implemented level of detail and in the settings we tested in - with a small pen of birds inside a barn - and at the time of buying these systems (see **chapters 2** and **4** for the system specifications). For other systems using a similar underlying technology or for these systems obtained at a later point in time, this comparison might look slightly different, for example due to reductions in tag sizes and costs over time. The overarching categories in this comparison are based on a technology-comparison framework that is used at the Animal Breeding and Genomics group of Wageningen University and Research and was developed by Ellen et al. (2017).

I will highlight some differences between the three approaches. To start, both the UWB and RFID system are relatively expensive compared to CV, which is mainly due to the required antennas and tags for UWB and RFID, of which multiple are needed to allow for tracking of individual birds kept in groups. On the other hand, data storage for CV can be relatively expensive compared to UWB and RFID, although this strongly depends on which data are stored and for how long. If the raw data are stored, video files require the largest storage capacity, as UWB and RFID generally only store log files with numbers, while the raw video data contain image data. However, the raw data might only need to be stored temporarily and only the final results from the CV, UWB and RFID data processing, for example a distance moved per animal per day, may require longer-term storage (Ellen et al., 2019). Furthermore, the system setup is somewhat difficult for UWB and RFID, because of the precise calibration that is required at installation. For CV, installation of cameras is relatively straightforward, but the data analysis might be more difficult to get up and running, although this depends on the available software. When one wants to implement CV in a novel environment, additional work might be needed as CV algorithms often show poor generalization across different environments for animals (G. Li et al., 2021). Consequently, many images (generally in the range of several thousands; reviewed by G. Li et al. (2021)) will have to be annotated for the tracking to ‘learn’ to recognise animals in the

Table 7.1. Comparison between UWB, passive RFID and CV for different aspects relevant for implementation in a broiler barn.

Class	Item	UWB	RFID	CV
Costs	Costs base system and software	Relatively high	Moderate	Relatively low
	Required data storage	Moderate	Moderate	High[1]
	Additional costs per animal	Per tag, relatively high	Per tag, relatively low	No
	Additional costs with increasing area	Yes, for large additions	Yes	Yes
	Re-usable	Yes	Yes	Yes
	Expected cost reduction near future	Yes	Yes	Yes
Accuracy of recording	Detailed behaviours	No	No	Possibly[2]
	Absence human error	Yes	Yes	Yes
	Direct line of sight required	No[3]	No	Yes
	Objective	Yes	Yes	Yes
What can be recorded?	Multiple behaviours	Only location or distance[1]	Only location or distance[1]	Possibly[2]
	Individual recognition	Yes	Yes	Difficult
	Multiple animals at the same time	Yes	Yes	Yes
On-site applicability	Can it handle dust and flies?	Yes	Yes	Not in front of lens[2]
	Can it handle faeces?	Yes	No, might block signal	Can change contrast[2]
	Can it handle water?	Not on tag circuitry	Not on antennas and readers	Not on camera circuitry
	Water interference	No	Possibly[4]	No
	Metal interference	Possibly[5]	Possibly[4]	No
	Easy to adjust	Difficult calibration	Difficult calibration	Easier to adjust
Practical use	Continuous recording	Yes	Yes	Yes
	User-friendly setup	No	No	Moderate
	Specialists required for installation	Yes	Yes	No
	Time-consuming (once implemented)	No	No	No
	Weight of tag	~ 25 grams	<1 gram	N/A
	Batteries required	Yes[6]	No[7]	No

[1] Ellen et al. (2019); [2] Wurtz et al. (2019); [3] Theoretically not (Alarifi et al., 2016), but blocking the line of sight can cause some problems in terms of registration success (Stadig et al., 2018a); [4] Depending on operating frequency (Ruiz-Garcia and Lunadei, 2011); [5] It is generally mentioned that metals affect the signals less than for RFID (e.g., Nekoogar and Dowla (2011)), but still interference may occur (Liu et al., 2007); [6] Alarifi et al. (2016); [7] Brown-Brandl et al. (2017), Roberts (2006).

environment, which makes the implementation time-consuming. However, once the systems are set up, they all require only limited time investments to keep the data collection running. For both UWB and RFID, periodical checks of the tags are needed to ensure the tags are still correctly attached to the animals, and for video periodical maintenance of the cameras is needed, to remove for example dust or insects (Wurtz et al. 2019). Overall, UWB, RFID and CV differ in their advantages and limitations, and which system is most suitable for broiler tracking depends on the specific behaviour(s) one wants to track (see also **section 7.4 'Towards practice'**).

7.3 Activity as a proxy

The sensor technologies that were tested and implemented in this thesis successfully provided individual-level activity data. In **chapter 1** it was hypothesized that such activity data have potential to serve as a proxy for health, welfare and performance traits in breeding programs. However, successful implementation of activity as a proxy required two important aspects: 1) insight into the heritability of activity and 2) knowledge on how activity relates to other health, welfare and performance traits. In this section, I will discuss these two aspects in relation to the observations that were made in this thesis (**Figure 7.1**).

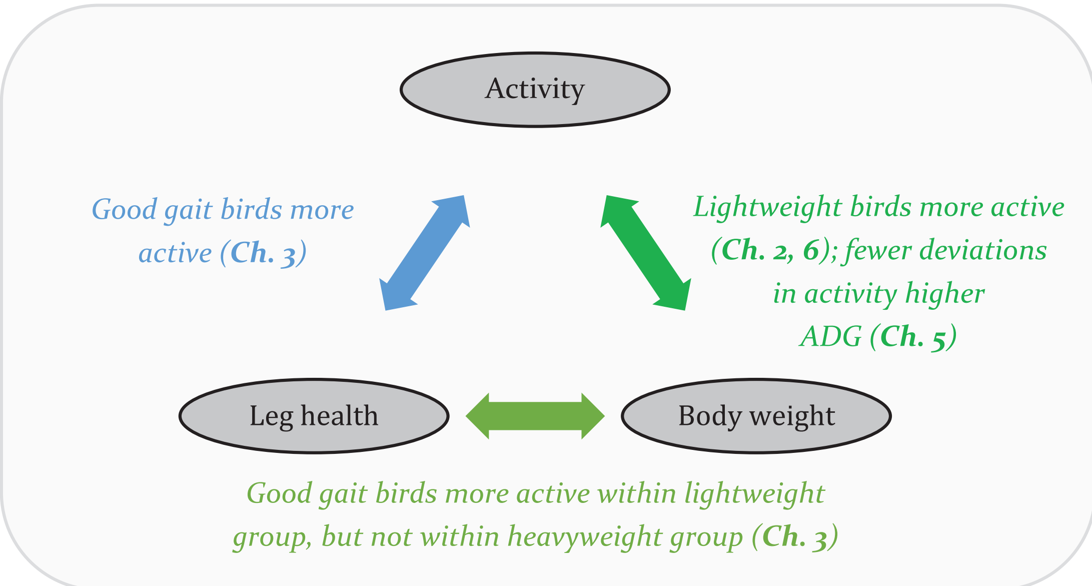

Figure 7.1. Relationships between activity, body weight and leg health that were observed in this thesis. ADG = average daily gain.

7.3.1 Heritability of activity

In **chapter 6**, it was shown that activity is heritable, with an estimated heritability of 0.31 ± 0.11 for activity across the full production period. As the broilers aged, the genetic and phenotypic variance decreased, indicating that the broilers' individual activity levels became more similar to each other over time. In accordance, the heritability of activity decreased. It is not uncommon for heritability estimates to change over time, as the same trait may be influenced by different genetic and environmental factors at different ages (Visscher et al., 2008). Several factors affecting activity in broilers were discussed in **chapter 6**, such as light intensity (Blatchford et al., 2009), distance between feeders and drinkers (Reiter and Bessei, 2009), housing density (Hall, 2001) and more. The different heritability estimates for activity at the different ages may complicate the use of activity for breeding: which time point do you pick and how does this affect what you actually select for? There are several considerations for deciding on the timing of the activity recordings.

First, if activity is the trait one directly wants to select for, a consideration would be at which time point one is most interested in changing the observed activity levels. To give an example, it is often suggested, for example by Bizeray et al. (2000), that there is a phenotypic relationship between early-life activity and leg health and that increased activity early in life can improve leg health in broilers, especially because the first weeks of age form a critical period for bone development (Sanchez-Rodriguez et al., 2019). Indeed, it has been observed that increased early-life activity can contribute to reduced locomotion disorders later in life, for example through the provisioning of bright red light from 7 to 22 days old to stimulate activity (Prayitno et al., 1997). If one wants to increase early-life activity, it would make most sense to record and select on early-life activity.

Second, how well one can record activity at different ages may play a role in deciding on the timing of the activity recordings. For example, one might be interested in activity in the second week of life, but due to constraints (for example, the UWB system has tags that are too heavy to implement at this age, see **chapter 2**) it is very difficult to record activity at this age or recordings at this age are generally more inaccurate than at other ages. In that case, it might make more sense to select another time point that has a strong genetic correlation with activity at the age of interest. When looking at the genetic correlations between activity levels recorded at different points in time, most of the correlations were moderately to highly positive and the correlations between adjacent weeks were strongest. This trend for lower genetic correlations for recordings further apart in time has also been observed in other studies, for example for several weight and body composition traits in cattle (Johnston et al., 2003) and for body weight in broilers (Chu et al., 2020). The moderate to high correlations between activity levels recorded in adjacent weeks suggest that it would indeed be possible to, for example, select on activity in the third week of life and achieve changes in the activity level in the second week (genetic correlation of 0.94 ± 0.11, **chapter 6**).

Third, if one wants to use activity as a proxy for another trait, the preferable time point might be the point at which the genetic correlation between activity and the trait of interest is strongest. Conversely, if there are also genetic correlations with traits one does not want to change, the preferable time point might be the point at which this genetic correlation is lowest. How activity phenotypically is related to body weight, leg health and several other traits in broilers, and what the consequences are for selection on activity, is elaborated on in the coming sections.

7.3.2 Relationships between activity and body weight

Selection for increased growth rates and changes in the diet have led to broilers that gain weight at a fast rate (Havenstein et al., 2003; see also **Box 1.2** in **chapter 1**). It has been suggested that this increased growth rate and the high body weights are related to reduced activity levels (Weeks et al., 2000). When comparing fast- and slow-growing broiler breeds, the fast-growing broilers have been observed to spend less time in locomotion and more time sitting (Bokkers and Koene, 2003; Dixon, 2020; Wallenbeck et al., 2016). In this thesis it was shown that also within fast-growing broilers there were differences in activity levels between lighter and heavier birds (**chapter 2**).

7.3.2.1 Differences in average activity between lightweight and heavyweight birds

Birds that were heavyweight at approximately two weeks old on average walked shorter distances than lightweight birds in the period from 16 to 32 days old (**chapter 2**). This observation of shorter distances walked in heavier birds has also been noted in other studies, for example in an operant runway test (Bokkers et al., 2007) or when walking voluntarily, that is, not necessarily for a reward, in a test using weight load reduction (Rutten et al., 2002). At the same time, decreases in walking distance or time spent walking are seen when broilers age, for example in **chapters 2** and **5** of this thesis and in Weeks et al. (2000) and Tickle et al. (2018). Tickle et al. (2018) studied energetics and behaviour in a commercial broiler breed from hatch to slaughter and suggested that broilers have a restricted total metabolic energy budget. They observed that the costs of resting metabolism increased over development and this was hypothesised to limit the energy available for other activities, including locomotion. This decrease in activity as broilers age has been suggested to be linked to the increase in body weight over time. Tickle et al. (2018) observed, among other things, rapid declines in the proportion of time spent actively moving as the broilers aged, that were correlated with increasing body mass. However, there was a breakpoint in this relationship at a value of approximately 0.9 kg, and after this point, further declines were negligible. The data presented in this thesis also include activity and body weight records over time, and therefore allow for examination of whether there is a similar breakpoint. **Box 7.1** shows this analysis.

In the breakpoint analysis from **Box 7.1**, there is a possible confounding effect of age, as the body weights were recorded at different ages. Examination of the relationship between body weight and activity within a specific age group could provide important clues about the relationship between body weight and activity without the confounding effect of age. Therefore, relationships between body weight and activity at the four different ages that were included in the breakpoint analysis were studied, i.e., at 8, 15 (excluding round 4), 22 (21 in round 1) and 29 days old. This was done for each of the rounds separately, to also exclude round effects. Linear regression models were used, modelling distance as a function of body weight, for each of the age categories and rounds separately. The results are shown in **Table 7.2**. There was still a statistically significant negative relationship between body weight and activity in most rounds when recorded at either 8 or 15 days old, when the birds on average weighed 231 (SD 28) or 662 (SD 73) grams, respectively. This matches with the observations seen in the breakpoint analysis in **Box 7.1** and the results from Tickle et al. (2018). At 22 and 29 days old, when the birds

Box 7.1. Breakpoint analysis

To study a breakpoint in the relationship between activity and body weight, the (extended) dataset from **chapter 5** was used. Solely recordings of activity and body weight at the same age were included. This resulted in activity and body weight records for birds of 8, 15 (excluding round 4 that had no activity data available on that day), 22 (21 in round 1) and 29 days old. Extreme outliers for body weight were excluded by round and age, based on a threshold of four times the standard deviation. Using these data, a breakpoint detection and estimation analysis was performed, using the segmented package (Muggeo, 2008) in R. The underlying linear regression model was distance as a function of body weight, with bird ID as a random factor to take repeated measures on the same individuals into account. A breakpoint was detected at a body weight of approximately 712 (95% CI 674-749) grams. The estimated slope up to this breakpoint was -0.029 ± 0.001, meaning that a one-gram increase in body weight resulted in a decrease in average hourly distance moved of approximately 0.03 meters. The estimated slope after this breakpoint was -0.003 ± 0.000, meaning that a one-gram increase in body weight resulted in a decrease in average hourly distance moved of approximately 0.003 meters. The breakpoint and slopes are also indicated in **Figure B7.1.1**, which visualizes the relationship between body weight and activity. Overall, these observations resemble what Tickle et al. (2018) noted in their study. There is a clear change in the relationship between activity and body weight with increasing body weight, with a smaller impact of increases in body weight on activity levels when the birds are already relatively heavy, that is, from 911 grams onwards in the study by Tickle et al. (2018) and from 712 grams onwards in the data presented here. However, the exact location of the breakpoint differs between the current observation and the estimate from Tickle et al. (2018). This might be due to their activity measurement being the proportion of time spent actively moving, and not the average hourly distance moved used here. Another possible explanation is that only a limited range of body weights was available here, as body weights were recorded once per week and no activity data were available for 15 days old in round 4. Consequently, there were few records of birds weighing approximately 300-500 and 800-1000 grams (see **Figure B7.1.1**).

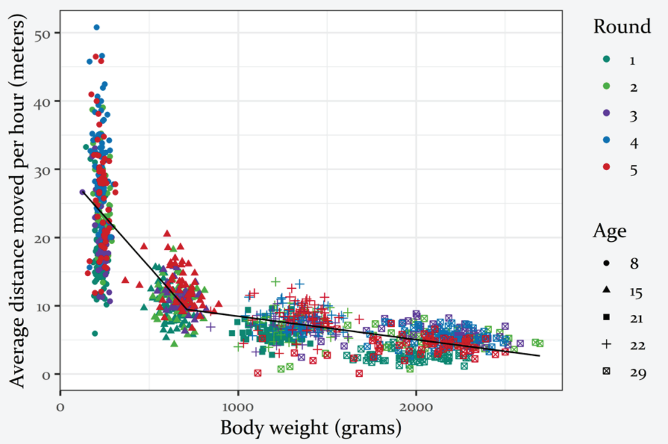

Figure B7.1.1. The relationship between body weight and activity, with breakpoint analysis. Shapes represent individual data points per age at which the body weight was recorded, with colours indicating the recording round. The black line indicates the regression.

weighed on average 1315 (SD 139) and 2076 (SD 258) grams, respectively, there was no longer a very clear and strong relationship between body weight and activity, which also matches with the observations made by Tickle et al. (2018). Overall, these results indicate that 1) there is a negative relationship between body weight and activity and 2) this relationship is most pronounced when the birds have relatively low body weights. These observations reflect what was also seen in **chapter 6** (using largely the same data), where the estimates for the body weight residuals at one to four weeks old approached zero as the birds aged.

Table 7.2. Linear regressions for the relationship between activity (outcome variable) and body weight (predictor) at different ages. R = round; BW = body weight.

Age	R	Intercept ± SE	Estimate for BW ± SE	df	t-value BW	Pr(>|t|) BW
8 days	1	34.684 ± 5.884	-0.081 ± 0.027	60	-3.045	0.003
	2	40.706 ± 6.294	-0.070 ± 0.026	60	-2.668	0.010
	3	38.403 ± 4.346	-0.081 ± 0.018	52	-4.396	<0.001
	4	38.185 ± 7.532	-0.033 ± 0.033	68	-0.997	0.322
	5	36.373 ± 8.127	-0.045 ± 0.035	56	-1.293	0.201
15 days	1	20.440 ± 2.861	-0.017 ± 0.005	62	-3.679	<0.001
	2	13.685 ± 2.964	-0.004 ± 0.004	60	-0.987	0.327
	3	19.264 ± 2.809	-0.012 ± 0.004	51	-2.972	0.005
	5	21.312 ± 2.869	-0.013 ± 0.004	52	-3.088	0.003
22 days[1]	1	9.872 ± 2.141	-0.003 ± 0.002	57	-1.830	0.073
	2	7.832 ± 2.318	-0.000 ± 0.002	59	-0.184	0.855
	3	8.798 ± 1.877	-0.001 ± 0.001	51	-0.871	0.388
	4	10.102 ± 2.336	-0.002 ± 0.002	66	-0.936	0.353
	5	9.238 ± 2.441	-0.001 ± 0.002	58	-0.341	0.734
29 days	1	3.519 ± 1.003	-0.000 ± 0.001	58	-0.955	0.343
	2	3.784 ± 1.327	0.001 ± 0.001	56	0.912	0.366
	3	6.720 ± 1.634	-0.000 ± 0.001	51	-0.545	0.588
	4	6.572 ± 1.704	-0.001 ± 0.001	68	-0.663	0.510
	5	2.116 ± 1.184	0.001 ± 0.001	58	1.691	0.096

[1]21 days in round 1.

7.3.2.2 Differences in slope of activity between lightweight and heavyweight birds

To summarize, up to this point we have seen that 1) body weight is related to activity up to a certain breakpoint and 2) after this breakpoint, body weight is related to activity to a much smaller extent. A question that might follow from this is, what happens over time with the activity levels of birds that had different body weights at an early point in time? Besides a higher average activity, **chapter 2** showed that lightweight birds, as determined at approximately two weeks old, had a steeper decline in activity in the period from 16 to 32 days old than heavyweight birds. Consequently, the difference in average activity between lightweight and heavyweight birds became less pronounced over time. What is uncertain, however, is whether this is a consequence of the birds gaining weight over time, or whether activity levels approach each other over time regardless of the body weight of birds.

Based on the breakpoint analysis from **Box 7.1**, it would be expected that the activity of the two body weight groups differs up to a weight matching that of a breakpoint, and then becomes similar for both groups. This would mean that both the lightweight and the heavyweight group would show a decrease in activity as they age, or gain weight, up to a certain weight, and from that point onwards would remain stable over time or decrease at a much lower pace. However, since lightweight birds likely take longer to reach this body weight breakpoint, a larger part of their activity trajectory over time consists of the 'fast' decrease occurring before the breakpoint, resulting in a steeper overall drop in activity over time. This hypothesis is visualised in **Figure 7.2**.

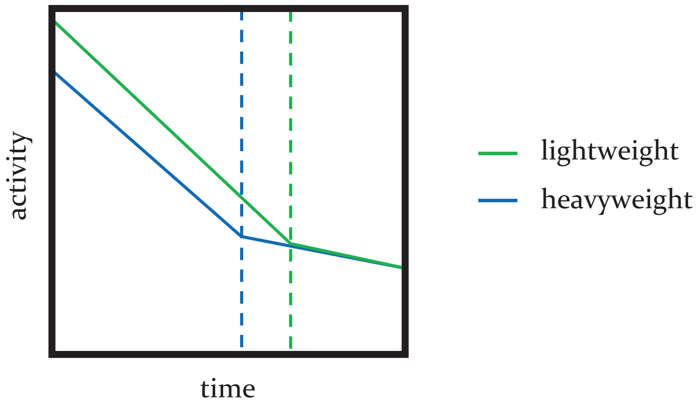

Figure 7.2. Visualisation of the breakpoint-based hypothesis. The dashed green line represents the point in time where the lightweight birds reach the 'breakpoint' body weight, while the solid green line represents the activity level over time of the lightweight group. The dashed blue line represents the point in time where the heavyweight birds reach the 'breakpoint' body weight, while the solid blue line represents the activity level over time of the heavyweight group.

Data from **chapter 5** were suitable to test this hypothesis. To replicate the analysis from **chapter 2**, the 318 individuals included in the data from

chapter 5 were ranked in ascending order based on the body weight at 15 days old, as this weighing moment was closest to the records from **chapter 2**. The weight of the 70^{th} individual from the top was taken as the upper boundary of the lightweight birds' body weight, to select approximately the top 25% of body weights when duplicate body weights were included as well. The weight of the 70^{th} individual from the bottom was taken as the lower boundary of the heavyweight groups' body weight. This selection resulted in 151 birds, due to some duplicates of the body weights that formed the boundaries. This total number is close to the number of birds included in **chapter 2** (137 individuals). To extend the earlier analysis, the period from 9 to 32 days old was used, resulting in a total of 3338 observations for the analysis. To test whether these two groups showed a different slope in activity over time, a model similar to the earlier-used model (**chapter 2**) was implemented here, but without cross, as only a single line was included in the current dataset. The results from this model for the body weight groups were similar to those reported in **chapter 2**, with a higher average activity level for lightweight birds (averaged across rounds and days, estimate = 1.69 ± 0.27, $p < 0.001$) and a faster decrease in activity compared with heavyweight birds (estimate for interaction lightweight and day in trial = -0.06 ± 0.02, $p < 0.001$). Of course, activity was modelled here as a linear function of time, round and weight category, without possible breakpoints being taken into account. To examine whether the expected pattern from **Figure 7.2** indeed occurs, a breakpoint analysis was performed for the two body weight groups in this dataset separately. For both body weight groups, activity was modelled as a function of the day in the trial and with bird ID as random effect, similar to the earlier breakpoint analysis (**Box 7.1**). The analysis revealed that there was no large difference in the location of the breakpoint for the groups: the breakpoint was estimated to lie at 13.72 days (95% CI 13.04 – 14.40) for the lightweight group and at 14.65 days (95% CI 14.26 – 15.04) for the heavyweight group (**Figure 7.3**). Moreover, the slight difference in breakpoint location was in the opposite direction of what was hypothesised. Furthermore, the observed breakpoint lay outside of the range of days that was studied in **chapter 2**, where the difference in slope of activity over time was also observed. Overall, this suggests that the location of the breakpoint does not explain the difference in slope of activity over time for the two body weight groups.

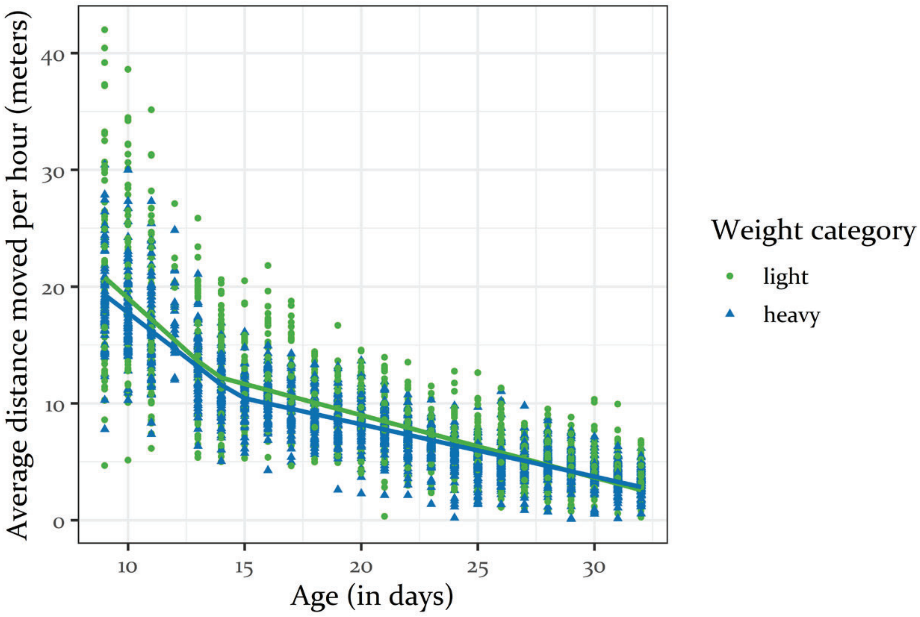

Figure 7.3. Average activity per day over time, for the lightweight (green) and heavyweight (blue) birds. Shapes represent individual data points per weight group. Lines represent the breakpoint regression for both groups.

Further examination of the pattern shown in **Figure 7.3** revealed that the slope in activity was steeper for the lightweight birds than for the heavyweight birds both before and after the breakpoint (-1.79 ± 0.12 versus -1.54 ± 0.05 before, and -0.53 ± 0.01 versus -0.45 ± 0.01 after). Since lightweight birds had a higher estimated activity at the start point of both slopes, this resulted in the two body weight groups approaching each other in activity level over time. In **chapter 2**, it was hypothesised that the decreasing difference in activity between light- and heavyweight birds could be a consequence of a decrease in the relative difference in weight of the lightweight and heavyweight birds. The weight difference was therefore also examined here. At 15 days old, the lightweight and heavyweight groups weighed 560 (SD 64) and 755 (SD 40) grams, respectively. At 21 or 22 days old, depending on the trial, they weighed 1132 (SD 121) and 1448 (SD 99) grams, and at 29 days old, they weighed 1851 (SD 211) and 2246 (SD 246) grams, respectively. In terms of relative weight, expressed as the average weight of the lightweight birds as a percentage of the average weight of the heavyweight birds, this is 74%, 78% and 82% over time. So similar to what was seen in **chapter 2**, the relative difference in body weight decreased over time. However, this still leaves us with the question whether this is indeed the underlying cause of the decrease in difference in activity level for the two body weight groups over time, or whether this decrease in difference in activity level over time occurs regardless of the body weight of the birds. To examine this, a group of birds with little variation in body weight was studied here. A total of 51 birds with body weights at 15 days old ranging from 655 to 675 grams were selected from the data of **chapter 5** and their activity from 9 to 32 days old was studied. For these birds, the slope in activity across the period from 9 to 32 days old was estimated using linear regression, with activity modelled as a function of age, for each of the birds separately. Then, the rank correlation between their activity level at 9 days old and the slope in activity was examined. A strong negative correlation was observed ($r_s = -0.84$ (95% CI -0.91 – -0.73), $p < 0.001$).

Overall, this indicates that birds with a higher activity level early in life show a steeper decline in activity over time, regardless of body weight changes. Therefore, it appears that the relatively low body weight early in life is related to the higher activity that is observed at this time point and that this difference persists over time, but becomes gradually less pronounced as all broilers approach nearly the same final activity level near slaughter age (**chapter 6**).

7.3.2.3 Causality in the relationship between activity and body weight

The causality in the relationship between activity and body weight early in life cannot be determined with certainty based on the data from this thesis and requires further examination. However, other studies have provided some clues as to what might be happening. Rutten et al. (2002) implemented a suspension device which they used to alleviate half of the weight-load on meat chickens' legs. They studied three treatments: birds that were fitted with a harness and the suspension device (**S**), birds that were fitted with the harness of the suspension device, but did not receive load reduction (**H**), and control birds that were not fitted with a harness or suspension (**C**). They implemented the treatments from an age of five days old, when all groups had similar body weights, until 19 days old. They observed two things happening that shine more light on the relationship between body weight and activity. First, they observed that for all groups, the distance travelled decreased over time, but the decrease in activity was smaller for S birds, and consequently the distance travelled in the period from 13 to 19 days old by the S birds was higher compared to both H and C birds. Second, they observed that both S and H birds had lower body weights at 19 days old compared to C birds. Rutten et al. (2002) hypothesised that this was due to the insulating effect of the harness that was used. To summarize, S birds had body weights similar to H birds, but showed a higher activity level than both H and C birds did. This suggests that the higher activity of the S birds was not the main cause for the lower body weight at 19 days, since H birds had similar body weights without increased activity levels in the preceding days. Given that only the S birds showed higher activity levels, it appears likely that the 50% weight load reduction was the main cause of the increased activity. However, one would then expect that the small reduction in body weight of the H group versus the C group would also result in a higher activity in the H group compared to the C group, which was not observed. Possibly, with only a small difference in body weight (586 g for the H birds versus 642 g for the C birds; a smaller difference than in the earlier-performed analysis in this chapter), the effect on activity is limited. Other studies have looked into the effect of activity on production parameters. Reiter and Bessei (1998) studied fast- and slow-growing broilers and subjected half of the birds to daily training on a treadmill. Within either fast- or slow-growing broilers, no difference in body weight at 7 weeks old was observed between the groups that received training and those that did not. Furthermore, at 7 weeks old no significant correlation was observed between body weight and distance walked on the treadmill in the fast-growing broilers (Reiter and Bessei, 1998). Overall, these studies provide support for the idea that body weight is the causal factor in the relationship with activity, and that this is mainly visible early in life.

7.3.2.4 Deviations in activity levels in relation to body weight gain

Besides our observation of a relationship between body weight and average activity levels, it was also observed that deviations in activity levels over time are related to average daily gain (**ADG**) in broilers (**chapter 5**). Broilers with a higher root mean square error (**RMSE**) of activity, that is, more deviations from the expected linear trend in activity, had a lower ADG. However, as discussed in **chapter 5**, with RMSE values alone it is difficult to determine the direction of the deviations, as both higher and lower activity levels (or a combination of both) than predicted can result in an increased RMSE. Therefore, the relationship with the reduced ADG is yet unclear. However, in

chapter 5, it was hypothesised that fluctuations in activity may be indicative of fluctuations in feeding or feeding motivation, possibly resulting in fluctuations in feed intake as well. Fluctuations in feed intake may be negatively related with ADG. For example, Putz et al. (2019) observed a negative genetic correlation between the RMSE of feed intake and finishing ADG in pigs. The observation of a strong correlation between total distances moved and number of feeder visits (see **section 7.4.1** 'RFID at key resources in the pen') provides support for this hypothesis, but more research is required to examine the relationship between deviations in activity levels and broiler growth in more detail.

7.3.3 Relationships between activity and leg health

In **chapter 3**, a relationship between activity and leg health was observed, with suboptimal gait (**SG**) birds showing lower average activity levels between 16 to 32 days of age than good gait (**GG**) birds. These observations are in agreement with other studies that observed lower activity levels for higher gait scores (e.g., Van Hertem et al. (2018a); Weeks et al. (2000)).

7.3.3.1 Causality in the relationship between activity and leg health

It is unclear which factor is causal in the relationship between activity and leg health. Observations have been reported for both directions. In terms of gait affecting activity levels, it has, for example, been suggested that a poor gait is painful for birds (Danbury et al., 2000; McGeown et al., 1999) and that birds may consequently reduce their activity levels (Weeks et al., 2000), as inhibition of activity can be an adaptive reaction to pain (Vierck and Cooper, 1984). Furthermore, it has been suggested that lameness can, albeit in severe cases, limit birds in performing specific behaviours (Kestin et al., 1992), such as dustbathing or preening while standing (Vestergaard and Sanotra, 1999; Weeks et al., 2000). On the other hand, it has also been suggested that increased locomotor activity may contribute to the prevention of gait problems. For example, Reiter and Bessei (2009) compared two distances between feeders and drinkers, of two and twelve meters. It was observed that the groups of broilers with the larger distance had fewer cases of leg weakness and that the locomotor activity in this treatment was higher. Kaukonen et al. (2017) studied environmental enrichment as a way to increase activity and hereby possibly improve gait in broilers. They implemented perches and elevated platforms and observed lower mean gait scores for the birds in the platform-equipped houses. It was hypothesized that the platform access increased walking to reach the platforms and enabled more versatile movement, which could have positively impacted gait (Kaukonen et al., 2017), although it must be noted that in other studies investigating environmental enrichment, increases in duration of active behaviours were observed but no effects on walking ability were found (de Jong et al., 2021). Overall, it appears that there is a two-way relationship between activity and leg health, with higher activity levels helping to prevent gait problems and, once gait problems have arisen, gait problems reducing the observed activity levels.

7.3.3.2 Distinguishing between birds with different gait scores

As was already indicated in **chapter 3**, there was considerable overlap in the activity levels of birds with a good gait and birds with a suboptimal gait. Consequently, it was very difficult to distinguish between GG and SG birds using only average distances moved. In this section, I will highlight some possible directions for future research to allow for a more complete distinction between GG and SG birds. A visual representation of this approach is shown in **Figure 7.4**. A first step would be to look at activity levels over longer periods of time and record gait scores periodically as well. By having records over time for both activity and gait, it becomes feasible to look into when the birds start to experience gait problems and how this relates to activity both before and after onset. Given the hypothesis that especially early-life increased activity can positively affect gait scores observed later in life (Bradshaw et al., 2002), it is advisable to also include the first weeks of life in these recordings. Furthermore, information on other behaviours that can be recorded in an automated manner, besides distance moved, could prove to be insightful. For example, feeder visits might be informative, as it has been observed that compared to 'gait score 0 birds' (that is, birds that walked normally with no detectable abnormality (Kestin et al., 1992)), 'gait score 3 birds' (that is, birds with an obvious gait defect which affects the ability to move about (Kestin et al., 1992)) visited the feeder less often per day, but increased the visit duration accordingly, resulting in an overall time spent feeding that was similar to that of gait score 0 birds (Weeks et al., 2000). Such feeder visits could be recorded with the RFID system described in **chapter 4**, if the placement of feeders within the pen is known and the associated antenna visits are logged. Moreover, use of the area might be interesting to include, as movement towards areas without feeders or drinkers (or not on the direct path between feeders and drinkers) is likely not essential for survival, and therefore any movement towards, or in, those areas could be indicative of locomotion not coming at a cost to the animals, for example in terms of pain. Use of the area can be recorded with the RFID system as well. Lastly, the speed of movement could be interesting to record, as this is also affected by birds' gait (Kestin et al., 1992). This would likely not be feasible to be recorded with the RFID system in its current form, as it was observed that the RFID system does not always register birds as soon as they move and no movement within antenna grid cells can be recorded (**chapter 4**). To solve this issue, one could potentially use a camera to track the movement speed of birds, while using RFID to identify the birds (see also **section 7.4.2**), but this is a challenging approach and requires further investigation. Overall, the here-described setup could potentially provide more information on birds' walking ability and allow for a more complete distinction between birds with different gaits. However, this also requires body weight recordings, as body weight is known to not only affect activity, but has also been linked to gait, and can thus be a confounding factor in the relationship between activity and gait.

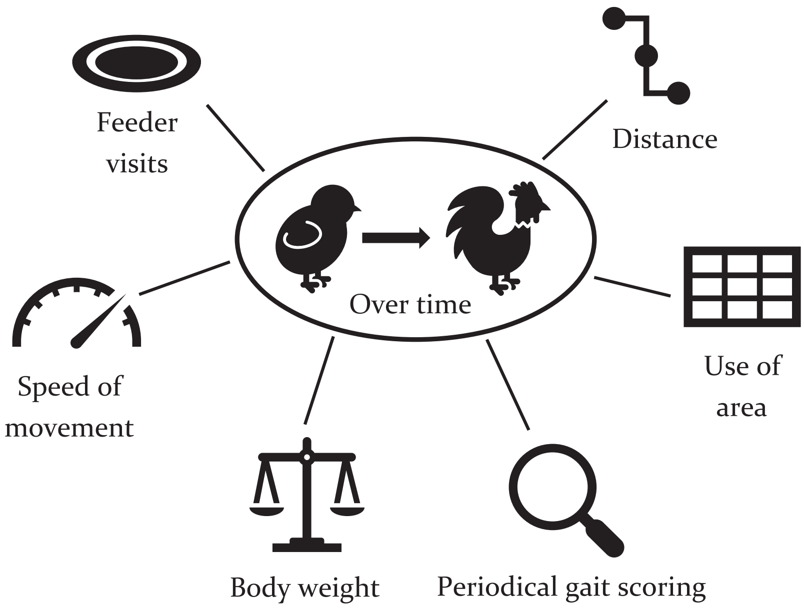

Figure 7.4. Visual representation of suggested research setup to potentially allow for a more complete distinction between birds with good and suboptimal gait.

7.3.4 Relationships between body weight and leg health

The gait problems that are observed in broilers have been hypothesised to be a result of (the selection for) high body weights and fast growth rates, and there are many studies that support this hypothesis (e.g., Kestin et al. (2001); Robinson et al. (1992); Sørensen et al. (1999)). For example, Kestin et al. (2001) evaluated thirteen different genotypes of broilers on two different feeding regimes and recorded the birds' liveweight and degree of lameness after 54 and 81 days. They observed that the modern genotypes tended to have a worse gait than the slower growing genotypes, when fed with the same diet. Furthermore, within genotypes, the birds fed with the non-limiting diet were more lame than birds fed with the more restricted diet. However, when corrections were made for differences in liveweight of the birds, it was observed that the differences were attributable to a higher liveweight. The results from Kestin et al. (2001) indicated that above a threshold of approximately 1.25 kg at 54 days, lameness increased with increasing liveweight. However, a role of growth rate was also observed, as the gait score of birds was worse at 54 days than at 81 days when corrected for liveweight differences. If birds reached a high body weight faster, their gait score was also worse compared to birds that reached this body weight slower (Kestin et al., 2001). In **chapter 3**, it was also observed that there were more SG birds in the heavyweight group than in the lightweight group, with 38 SG birds in the heavyweight group and 20 SG birds in the lightweight group (**Table 3.3**). To test if this was a statistically significant difference between the body weight groups, a chi-square test was performed here. This test revealed that the two body weight groups indeed had different gait classification distributions (χ^2 = 6.63, df = 1, p = 0.010).

Not only was there a difference in gait classification distribution between the body weight groups, there was also an interaction between body weight and gait classification in the estimated distances moved by the birds (**chapter 3**). Within lightweight birds, a difference in activity could be observed between GG and SG birds, but in heavyweight birds, this difference was no longer clearly visible. In **chapter 3**, it was hypothesised that this could be a consequence of heavier birds already limiting their activity levels to the minimally required level to obtain sufficient water and feed. In that case, having a suboptimal gait instead of a good gait might not result in a further decrease in activity. Because of this interaction, distinguishing between GG and SG birds based on distances moved is difficult in heavyweight birds. With the earlier-described research setup from **Figure 7.4** it might still be possible to distinguish between different gait scores, for example using the speed of movement, but this requires further research.

7.3.5 Potential unintentional side effects of selection on activity

In this thesis, the relationships between activity, body weight and leg health were examined, as well as the heritability of activity. However, selection for increased activity can potentially have unintentional side effects for other traits as well. For example, divergent selection on general locomotor activity in laying hens has indicated that there are behavioural consequences of selection for increased activity, namely increased fearfulness of the more active line (Krause et al., 2019). In Japanese quail chicks selected on locomotor activity, the opposite has been observed, with the inactive line being more fearful than the active line (R. B. Jones et al., 1982). Such side effects are important to study, as fear constitutes one of the negative emotions that should be avoided according to the Welfare Quality protocol for poultry (Welfare Quality®, 2009; see also **chapter 1**), although it must be noted that fear can also be functional, i.e., an adaptive state to protect the animal from injury. However, fear responses that are appropriate in natural environments can be harmful to birds in production systems, where expression of adaptive responses may be restricted (R. B. Jones, 1996). Skinner-Noble et al. (2003) studied whether improved feed conversion was associated with increased lethargy in broilers and observed, among other things, a positive correlation between resting and the feed conversion ratio from 40 to 47 days of age. Lutz et al. (2016) studied genetic correlations between feather pecking, feather eating (**FE**) and general locomotor activity (**GLA**) in laying hens and estimated a positive genetic correlation between GLA and FE (0.47 with an SEM of 0.19). Overall, these studies show that activity is (genetically) correlated with other traits or behaviours in poultry, besides body weight and gait, and therefore it is advised for future studies to carefully examine the physiological and behavioural consequences of genetic selection on activity.

7.4 Towards practice

The sensor technologies that were implemented in this thesis are in their current setups likely too expensive and detailed for practice, although it must be noted that UWB has by now been successfully tested under commercial conditions on a small subset of birds in a flock (Baxter and O'Connell, 2020). In this section, two alternative approaches for individual-level broiler activity tracking in practice are discussed. Both of these approaches involve the use of RFID, and not UWB, as passive RFID allows for tracking throughout life, due to the small and lightweight tags that do not require batteries (Finkenzeller, 2010; Roberts, 2006). The two approaches are 1) using RFID at key resources in the pen, and 2) a sensor-fusion approach, through combination of RFID and CV.

7.4.1 RFID at key resources in the pen

To simplify the RFID system for implementations in practice, RFID antennas could potentially be placed near key resources in the pen, such as the feeders, instead of throughout the full pen. This would result in a lower level of detail in activity compared to when the full pen is fitted with antennas, but I hypothesised that visits to key resources like the feeders will correlate well with overall activity levels. To test this, I used the (extended) raw RFID data from **chapter 5**, which consisted of recorded antenna positions over time. In **chapter 5**, the resulting calculated distances moved were used, but the data also allow for examination of how often birds visited specific areas, such as the feeders. To test whether distances moved and feeder visits correlated well, the antenna grid cells within the pen that contained feeders on a specific day, which could change for example due to additional feeding trays being provided during the first days after hatching, were assigned as 'feeders'. I calculated how often animals visited the feeders, coming from a non-feeder-containing antenna. If an animal moved antennas within the feeding area, this was not counted as a new visit to the feeders. Overall, when combining all ages and rounds, a strong Kendall's rank correlation was observed between the daily frequency of feeder visits and distance moved (tau = 0.74 (95% CI: 0.73-0.74), $z = 108.31$, $p < 0.001$). To assess whether this relationship was different for birds at different ages and for different rounds, Kendall's rank correlations were also calculated within days and rounds. Days on which the feeder position was changed during the day were excluded. **Figure 7.5** shows the rank correlation values over time for the different rounds. The horizontal line and shaded area represent the boundary for a strong association for Kendall's tau values, based on van den Berg (2021). However, the interpretation requires some caution, as there appears to be little consensus on the interpretation of the strength of Kendall's rank correlations. Overall, it appears that on most days, the total distance moved and the number of feeder visits, both determined by the RFID system, show a strong correlation. This suggests that monitoring visits to the feeder area using RFID antennas could provide a good indication of overall activity levels of broilers.

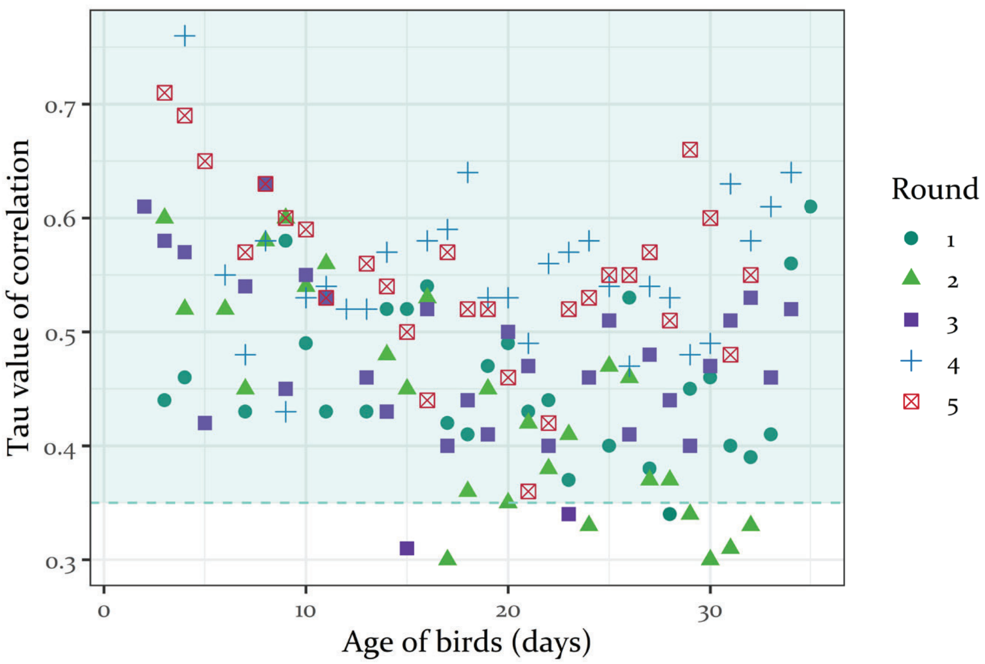

Figure 7.5. Kendall's rank correlation values between the distance moved and the number of feeder visits over time for the different rounds. All correlations are *p* < 0.01. Different shapes and colours represent different rounds. The dashed line and shaded area represent the threshold for a strong association between the two variables (tau = 0.35; van den Berg (2021)).

7.4.2 Sensor-fusion approach: combining RFID and computer vision

Based on the correlation between the distance moved and the frequency of feeder visits, it seems feasible to monitor feeder visits to obtain information on activity levels in broilers. However, what if one wants to track birds throughout the whole area available to them? A sensor-fusion approach may provide the solution. Sensor-fusion approaches are combinations of different sensors, where the different sensors provide different pieces of the required information (Ellen et al., 2019). One way to track individual broilers throughout the area available to them, besides fitting the whole area with RFID antennas, would be to combine RFID and CV. Computer vision has been suggested to have potential for providing great behavioural detail and for recording multiple behaviours (reviewed by Wurtz et al. (2019)). However, to do so at the individual level, individual identification of animals is required, which can be difficult for CV approaches (Ellen et al., 2019; van der Zande et al., 2021). Main problems for tracking multiple individuals in real time from a technical perspective include the initial object recognition, that is, separating birds from the background, which is computationally challenging, and the visual occlusion that may occur when birds for example show piling behaviour (Ellen et al., 2019). There could be great added value to combining CV and RFID, with CV for the main behaviour tracking and RFID as a reliable identification backup system. The CV could then track the broilers throughout the pen, and when birds visit the feeder, the RFID system could detect the birds and send the identification codes to the CV algorithm, to update the identities of the birds in the video. In other species, several studies have implemented CV in combination with RFID, with RFID being used for (backup) identification of individuals or tracks, for example for position tracking or locomotion scoring in dairy cows (Guzhva et al., 2018; Van Hertem et al., 2018b), position tracking in mice (Dandan and Lin, 2016) and analysis of social behaviour in mice (Peleh et al., 2019). Nakarmi et al. (2014) developed an automated behavioural quantification system for locomotion, perching, feeding, drinking and nesting of individual laying hens in small groups using CV and RFID. They fitted a small area (1.2 x 1.2 m) with a 3D camera to obtain CV data and placed twenty RFID antennas underneath the floor surface to identify individuals wearing RFID leg tags, as a recovery system for when the imaging system lost the identities of the birds. The system was shown to be capable of tracking and identifying individual laying hens (tested in groups of 5 and 10), with a 95% agreement for the movement trajectories compared to human labelling. Although quite some work needs to be done before this can be successfully implemented on a larger scale and with fewer RFID antennas, and preferably also with a regular camera instead of a 3D camera to reduce the costs, this does seem to be a very promising approach for the future.

7.5 Conclusions and future prospects

Automated tracking of individual broiler activity in small groups was shown to be feasible in this thesis using sensor technologies. Especially the passive RFID system implemented and tested in this thesis has potential for collecting activity data throughout life and potentially, albeit with a lower level of detail, on a larger scale. The systems were tested only for broilers, but with some adaptations they are likely also applicable for other species, as broilers were a challenging starting point given their small size (see **chapter 1**) and implementations for larger animals are potentially easier, although other species of course come with their own challenges. Being able to monitor individual animals is highly relevant at present, given the growing concern of citizens and consumers about the effect of animal production intensification on animal welfare (reviewed by Alonso et al. (2020)). For example, the End the Cage Age European Citizens' Initiative recently resulted in an agreement to phase out cages for farmed animals across Europe (End the Cage Age, 2021). The tracking systems discussed in this thesis have potential to, in the future, help farmers to keep track of their animals and provide detailed background information to customers on the life the animals' behind their products have had, although this remains to be tested in practice. For broilers specifically, it was recently announced that all fresh chicken meat in supermarkets in the Netherlands will, from at the latest the end of 2023 onwards, have at least one 'Beter Leven' star (de Volkskrant, 11 August 2021). Beter Leven is an animal-friendliness rating system from the Dutch Society for the Protection of Animals (Dierenbescherming, 2021) and the rating of one star means that the chickens will be slower growing than the regular fast-growing chickens and will be provided with daylight, more space and a covered run (de Volkskrant, 11 August 2021). With the earlier-mentioned higher activity levels that are often observed for slower growing broilers, uncharacteristic activity decreases might be even easier to detect and more informative or predictive of (leg) health issues, but this remains to be investigated.

In this thesis, relationships between broiler activity, leg health and body weight were observed, which indicated that a suboptimal gait is paired with reduced locomotion, at least in lightweight birds, and that higher body weights are linked to decreased activity levels. Activity data have potential for inclusion in broiler breeding programs, given the observed heritability of activity. However, more research is needed to obtain more clear distinctions between birds with different gait scores and to assess any unwanted side effects of selection on activity. Overall, the results of this thesis contribute to an improved understanding of activity levels in broilers, and the relationship with body weight and leg health. This knowledge can help us to maintain a sufficient supply of animal protein to feed the world, while ensuring the welfare of our production animals.

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Summary

One-third of the human population's protein intake is provided by livestock products and there is a rapidly increasing demand for these products. To meet this demand, animal agriculture has intensified. With the resulting large numbers of animals per farm, keeping track of individual animals is challenging. However, individual records, for example of activity, are of great relevance for assessing animal welfare and for implementation of traits in breeding programmes. The main aims of this thesis were 1) to study whether, and how, detailed activity data on individual broilers can be collected in an automated manner throughout life, while they are housed in a group, and 2) to study the relationship between individual activity, leg health and body weight, with the ultimate goal of examining whether selecting on activity to improve health and welfare of broilers is feasible.

In **chapters 2** and **3**, we implemented an ultra-wideband (**UWB**) tracking system to collect data on individual activity levels of broilers from approximately two-weeks-old onwards. In **chapter 2**, we compared the UWB recordings to video recordings and observed a moderately strong agreement between the two approaches. Furthermore, we observed that, with the UWB system, we could detect decreases in activity as the broilers grew older, as well as differences in activity levels between body weight categories. On average, lightweight broilers were observed to move longer distances than heavyweight broilers. Overall, this chapter indicated that the relative activity of broilers can be tracked well with the UWB system, from approximately two-weeks-old onwards. In **chapter 3**, we used the UWB data to assess the relationship between activity and gait in broilers. We found indications for relationships between gait classification and different measures of activity, with lower activity levels for birds with a suboptimal gait. Furthermore, we observed a tendency for an interaction between gait classification and weight category. In this interaction, a difference in level of activity was observed between gait classifications in lightweight birds, with lower activity levels for birds with a suboptimal gait, but not in heavyweight birds. It has to be further investigated if this is a consequence of higher body weight already limiting activity levels. However, in general the differences in activity levels of birds with different gait classifications were not very clear and therefore it remained difficult to distinguish gait classifications based on distances moved during the period from 16 to 32 days old.

The UWB system was not suitable for collecting activity data early in life, due to the relatively large and heavy tags that had to be fitted to the birds. In **chapters 4-6**, we therefore implemented a passive radio frequency identification (**RFID**) tracking system to collect data on individual activity levels of broilers from hatching onwards. In **chapter 4**, we validated the RFID system and observed that in 62.5% of the cases the RFID system was in full agreement with video in terms of the location of the animals. In 99.2% of the cases, near matches (allowing for a deviation of one antenna grid cell) were observed. Furthermore, we observed that there were moderately strong correlations in terms of distances moved between RFID, video and UWB. We concluded that the RFID system could accurately register among-individual variation in activity throughout life and continued collecting activity data with this system. In **chapter 5**, we used the resulting RFID data to further elaborate on the relationship between activity patterns early in life and body weight, or growth, over time. In this chapter, we not only looked at average activity levels, but also at dynamic descriptors of activity. We found a negative correlation between average daily gain (**ADG**) and the root mean square error of activity, indicating that broilers with more deviations from the expected linear trend in activity had a lower ADG. In **chapter 6**, we used RFID data to estimate the heritability of activity in broilers. We observed an estimated heritability of 0.31 ± 0.11 across the full production period and a decrease in heritability of activity as the birds aged. Furthermore, we observed positive and mostly moderate to strong genetic and phenotypic correlations between activity levels recorded in different weeks, with the correlations between adjacent weeks being strongest. Overall, our results suggested that genetic selection on activity is feasible, which could in the future potentially help to improve broiler welfare, through the hypothesized positive effects of increased activity on leg health.

In **chapter 7**, the general discussion, I brought together the results from the different chapters. I discussed the differences between, and advantages and limitations of, UWB and passive RFID tracking systems for individual activity tracking of group-housed broilers. I concluded that passive RFID systems are better suitable for activity tracking throughout life, due to their small and lightweight tags that can be fitted to broilers from hatching onwards. I furthermore addressed the relationship between activity, leg health

and body weight in more detail and showed that these are all related. Finally, I discussed potential directions for future implementation of RFID tracking systems in larger scale broiler systems, including less detailed RFID tracking and a sensor-fusion approach of RFID and computer vision.

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Curriculum vitae

About the author

Publications

Training and education

About the author

Malou van der Sluis was born on the 24th of December 1993 in Utrecht, the Netherlands. She obtained her BSc in Biology at Utrecht University in 2015, for which she also did a research internship at seal care centre Pieterburen. She obtained her MSc in Environmental Biology – Behavioural Ecology at Utrecht University in 2017. In her MSc research projects, she studied feather damaging behaviour in Grey parrots at Utrecht University and drinking behaviour of beak-trimmed and non-beak-trimmed laying hens at Wageningen University & Research. Her passion for improving animal welfare and positively affecting the lives of production animals made her want to continue working on production animal welfare related topics. In 2017, she started as a PhD candidate at Wageningen University & Research, in the Breed4Food Individual Tracking project. The results of this project are described in this thesis. Since November 2021, she works as a researcher at the Animal Breeding and Genomics group of Wageningen University & Research.

Publications

Peer reviewed scientific publications

Ellen, E.D., van der Sluis, M., Siegford, J., Guzvha, O., Toscano, M.J., Bennewitz, J., van der Zande, L.E., van der Eijk, J.A.J., de Haas, E.N., Norton, T., Piette, D., Tetens, J., de Klerk, B., Visser, B. and Rodenburg, T.B. (2019) Review of sensor technologies in animal breeding: phenotyping behaviours of laying hens to select against feather pecking. Animals, 9, 108.

van der Sluis, M., de Klerk, B., Ellen, E.D., de Haas, Y., Hijink, T. and Rodenburg, T.B. (2019) Validation of an ultra-wideband tracking system for recording individual levels of activity in broilers. Animals, 9, 580.

van der Sluis, M., de Haas, Y., de Klerk, B., Rodenburg, T.B. and Ellen, E.D. (2020) Assessing the activity of individual group-housed broilers throughout life using a passive radio frequency identification system - a validation study. Sensors, 20, 3612.

van der Sluis, M., Ellen, E.D., de Klerk, B., Rodenburg, T.B. and de Haas, Y. (2021) The relationship between gait and automated recordings of individual broiler activity levels. Poultry Science, 100, 101300.

Ellen, E.D., van der Sluis, M., de Klerk, B., Henshall, J., de Haas, Y. and Rodenburg, T.B. Genetic parameters for activity recorded using RFID throughout life in broilers. *Submitted*.

Contributions to scientific conferences

van der Sluis, M., Pieters, R.P.M., Muijres, F.T. and Rodenburg, T.B. (2017) Drinking behaviour and performance of drinking systems in poultry: beak-trimmed versus non-beak-trimmed laying hens. International Society for Applied Ethology (ISAE) Benelux conference. Hoogeloon, the Netherlands.

van der Sluis, M., Ellen, E.D., de Haas, Y. and Rodenburg, T.B. (2018) Tracking and monitoring of individual animals kept in large groups. WIAS Science Day. Wageningen, the Netherlands.

van der Sluis, M., Ellen, E.D., de Haas, Y. and Rodenburg, T.B. (2018) Tracking and monitoring of individual chickens housed in groups using passive radiofrequency identification. Precision Livestock Farming Workshop Seminar. Wageningen, the Netherlands.

van der Sluis, M., Ellen, E.D., de Haas, Y. and Rodenburg, T.B. (2018) Radiofrequency identification systems: Advantages and constraints for tracking and monitoring of individual animals. Measuring Behavior – 11th International Conference on Methods and Techniques in Behavioral Research. Manchester, United Kingdom.

Ellen, E.D., van der Sluis, M., de Haas, Y. and Rodenburg, T.B. (2018) Using sensor technologies in animal breeding: reducing damaging behaviour of animals kept in groups. Measuring Behavior – 11th International Conference on Methods and Techniques in Behavioral Research. Manchester, United Kingdom.

van der Sluis, M., de Klerk, B., Rodenburg, T.B., de Haas, Y., Hijink, T. and Ellen, E.D. (2019) Automated tracking of individual activity of broiler chickens. 53rd International Congress of the International Society for Applied Ethology (ISAE). Bergen, Norway.

Rodenburg, T.B., van der Zande, L., de Haas, E.N., Kostal, L., Pichova, K., Piette, D., Tetens, J., Visser, B., de Klerk, B., van der Sluis, M., Bennewitz, J., Siegford, J., Norton, T., Guzhva, O. and Ellen, E.D. (2019) Reduce damaging behaviour in laying hens and pigs by developing sensor technologies to inform breeding programs. 53rd International Congress of the International Society for Applied Ethology (ISAE). Bergen, Norway.

van der Sluis, M., de Klerk, B., Ellen, E.D., de Haas, Y., Hijink, T. and Rodenburg, T.B. (2019) Assessing individual activity levels in two broiler lines using an ultra-wideband tracking system. 9th European Conference on Precision Livestock Farming (ECPLF). Cork, Ireland.

Rodenburg, T.B., Bennewitz, J., De Haas, E.N., Košťál, L., Pichová, K., Piette, D., Tetens, J., Visser, B., De Klerk, B., van der Sluis, M., van der Zande, L.E., Siegford, J., Toscano, M., Norton, T., Guzhva, O. and Ellen, E.D. (2019). Developing sensor technologies to inform breeding approaches to reduce damaging behaviour in laying hens and pigs: The GroupHouseNet approach.

9th European Conference on Precision Livestock Farming (ECPLF). Cork, Ireland.

Ellen, E.D., van der Sluis, M., de Klerk, B., de Haas, Y., Hijink, T. and Rodenburg, T.B. (2019) On-farm automated tracking of group-housed poultry. 70th Annual Meeting of the European Federation of Animal Science (EAAP). Ghent, Belgium.

van der Sluis, M., de Klerk, B., Ellen, E.D., de Haas, Y., Hijink, T. and Rodenburg, T.B. (2020) Assessing individual activity levels in broilers using an ultra-wideband tracking system. WIAS Annual Conference. Lunteren, the Netherlands.

van der Sluis, M., de Haas, Y., de Klerk, B., Rodenburg, T.B. and Ellen, E.D. (2020) Validation of a passive radio frequency identification tracking system to monitor individual broiler activity throughout life. International Society for Applied Ethology (ISAE) Benelux meeting. Online.

van der Sluis, M., de Klerk, B., de Haas, Y., Rodenburg, T.B. and Ellen, E.D. (2020) Tracking individual broiler activity using a passive radio frequency identification system. 71st Annual meeting of the European Federation of Animal Science (EAAP). Online.

van der Sluis, M., Ellen, E.D., de Haas, Y., de Klerk, B. and Rodenburg, T.B. (2021) Automated activity recordings throughout life in broilers: heritability of activity and the relationship with body weight. 54th Congress of the International Society for Applied Ethology (ISAE). Online.

van der Sluis, M., Ellen, E.D., de Haas, Y., de Klerk, B. and Rodenburg, T.B. (2021) Using automated recordings of broiler activity levels to assess individual gait. 8th International Conference on the Assessment of Animal Welfare at Farm and Group level (WAFL). Online.

Ellen, E.D., van der Sluis, M., de Haas, Y., de Klerk, B. and Rodenburg, T.B. (2021) Estimating the heritability of broiler activity throughout life as recorded by using an RFID system. 72th Annual meeting of the European Federation of Animal Science (EAAP). Davos, Switzerland.

Other publications related to this thesis

van der Sluis, M. (2021) Bob the Bird. FameLab Wageningen University and Research.

van der Sluis, M. (2021) Activity tracking in chickens. FameLab Netherlands Final.

Training and education

The Basic Package (3 ETCS[1])	
WIAS course Introduction Day, Wageningen	2017
WGS course Scientific Integrity & Animal Ethics, Wageningen	2018
WIAS course Essential Skills, Wageningen	2018

Disciplinary Competences (17 ECTS)	
Writing a research proposal for WIAS	2017-2018
Laboratory Animal Science course - basics	2018
Laboratory Animal Science course - species-specific (poultry and other birds)	2018
Falconer study group (book on quantitative genetics)	2018-2020
Organising and attending Quantitative genetics Discussion Group	2019-2020
GroupHouseNet course on Genetics, epigenetics and neurobiology of damaging behaviour	2018
Introduction to R for statistical analyses	2018

Professional Competences (7 ECTS)	
Project and time management	2018
Scientific Writing 2	2019
Survival guide to peer review	2019
PhD Workshop carousel	2018
Organising B4F workshop (ROS)	2019
Last stretch of the PhD programme	2021
Writing propositions	2021
The final touch: writing the general introduction and discussion	2021
Career Orientation	2021

Societal Relevance (2 ECTS)	
Societal impact of your research	2020

Presentation Skills (maximum of 4 ECTS)	
WIAS Science Day, Wageningen (NL), 5 February, poster	2018
Precision Livestock Researchers Workshop Seminar, Wageningen (NL), 3-4 May, oral presentation	2018
Measuring behavior, Manchester (UK), 6-8 June, oral presentation	2018
ISAE, Bergen (Norway), 5-9 August, oral presentation	2019
ECPLF, Cork (Ireland), 26-29 August, poster	2019
WIAS Annual Conference, Lunteren (NL), 13-14 February, poster	2020
ISAE Benelux, online, 3 November, oral presentation	2020
EAAP, Porto (online), 1-4 December, oral presentation	2020
FameLab WUR, online, 30 April, oral presentation	2021
FameLab Netherlands Final, online, 1 June, oral presentation	2021
ISAE, online, 2-6 August, oral presentation	2021
WAFL, online, 16-19 August, oral presentation	2021

Teaching Competences (4 ECTS)	
Supervising a master student (minor)	2018
Supervising a bachelor student (thesis)	2019
Supervising a bachelor student (thesis)	2020
Supervising a master student (writing assignment; Utrecht University)	2020

Education and Training Total (36 ECTS)

[1] One ECTS credit equals a study load of approximately 28 hours.

Colophon

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