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Never wake a sleeping broiler

An undisturbed natural resting pattern in broilers

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Abstract

The importance of sleep and rest for animals is well known, but rarely mentioned and considered in the handbooks for broiler production, nor when welfare issues are discussed in relation to the production settings. Resting behaviours make up a large part of the daily time budget of broilers, making the quality of rest highly important for the welfare of the birds. Behaviour data on rest can give a lot of valuable information on the quality of resting and thereby, indirectly, on the quality of sleep. Poor quality resting can negatively affect the welfare of the birds but also disturb important functions of sleep related for instance to restoration, growth and cognitive functioning. Therefore, this thesis investigated three possible treatments to improve the quality of resting behaviour; elevated platforms, artificial brooders and intermittent lighting. The results show that all three treatments reduce physical disturbances between birds and increase resting bout duration. Intermittent lighting also increase the synchronisation of resting behaviour. No negative effects were found on production or clinical welfare parameters. A reduction of fear was seen for broilers reared with elevated platforms, artificial brooders or intermittent lighting, indicating increased welfare. Altering the broilers' environment to promote more natural resting patterns have positive effects on the resting behaviour of the birds and thus likely also on their sleep.

Keywords: Broiler chickens, animal behaviour, animal welfare, sleep, rest, elevated platforms, artificial brooders, intermittent lighting scheme

Låt sovande kycklingar sova - ostörd och synkroniserad vila hos slaktkycklingar

Sammanfattning

Vikten av sömn och vila för djur är välkänd, men nämns och beaktas sällan i handböckerna för slaktkycklingproduktion, inte heller när välfärdsfrågor diskuteras i relation till produktionsmiljöerna. Vilobeteenden utgör en stor del av slaktkycklingarnas dagliga tidsbudget, vilket gör vilokvaliteten mycket viktig för fåglarnas välfärd. Beteendedata om vila kan ge mycket värdefull information om kvaliteten på vilan och därmed, indirekt, om kvaliteten på sömnen. Vila av dålig kvalitet kan negativt påverka fåglarnas välbefinnande, men också störa viktiga sömnfunktioner relaterade till exempelvis återhämtning, tillväxt och kognitiv funktion. Därför undersökte denna avhandling tre möjliga behandlingar för att förbättra kvaliteten på vilobeteendet; upphöjda plattformar, mörka ruvare och intermittent ljus. Resultaten visar att alla tre behandlingarna minskar fysiska störningar mellan fåglar och ökar vilotiden. Intermittent ljus ökar också synkroniseringen av vilobeteenden. Inga negativa effekter hittades på produktions- eller kliniska välfärdsparametrar. En minskning av rädsla sågs för slaktkycklingar som fötts upp med upphöjda plattformar, mörka ruvare eller intermittent ljus, vilket tyder på ökad välfärd. Att förändra slaktkycklingarnas miljö för att främja mer naturliga vilomönster har positiva effekter på fåglarnas vilobeteende och därmed sannolikt även på deras sömn.

Keywords: Slaktkyckling, djurs beteende, djurvälfärd, sömn, vila, upphöjda plattformar, mörka ruvare, intermittent ljusschema

Dedication

I dedicate this thesis to all the broiler chickens of the world.

“So we can take the world back from the heart-attacked. One maniac at a time we will take it back.”

Fall Out Boy, The Phoenix

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List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I. Forslind, S., Blokhuis, H. J., & Riber, A. B. (2021). Disturbance of resting behaviour of broilers under different environmental conditions. *Applied Animal Behaviour Science*, 242, 105425.
- II. Forslind, S., Hernandez, C. E., Riber, A. B., Wall, H., & Blokhuis, H. J. (2022). Resting behavior of broilers reared with or without artificial brooders. *Frontiers in Veterinary Science*, 9.
- III. Forslind, S., Hernandez, C. E., Riber, A. B., Wall, H., Wattrang, E., & Blokhuis, H. J. Effect of artificial brooders on some production, immune and welfare parameters in broilers. (Manuscript)
- IV. Forslind, S., Riber, A. B., Wall, H., & Blokhuis, H. J. Improvement of resting behaviour of broilers through an intermittent lighting scheme. (Submitted)

Papers I-II are reproduced with the permission of the publishers.

The contribution of Sara Forslind to the papers included in this thesis was as follows:

- I. Collected the data from video recordings and carried out the analyses and summarised the results together with the co-authors. Wrote the manuscript with input from the co-authors. Responsible for writing and completing the manuscript including correspondence with the journal.
- II. Contributed to designing the study. Was responsible for planning and performing the experiment. Carried out the analyses and summarised the results with input from the co-authors. Responsible for writing and completing the manuscript, with input from the co-authors, including correspondence with the journal.
- III. Contributed to designing the study. Was responsible for planning and performing the experiment. Carried out the analyses and summarised the results with input from the co-authors. Responsible for writing and completing the manuscript, with input from the co-authors.
- IV. Contributed to designing the study. Was responsible for planning and performing the experiment. Carried out the analyses and summarised the results with input from the co-authors. Responsible for writing and completing the manuscript with input from the co-authors, including correspondence with the journal.

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Abbreviations

| | |
|-----|-----------------------------|
| EEG | Encephalography |
| EU | European Union |
| FCR | Feed Conversion Ratio |
| IBV | Infectious Bronchitis Virus |
| REM | Rapid Eye Movement |
| TI | Tonic Immobility |

1. Introduction

1.1 Chickens (*Gallus gallus*)

The ancestor of the domestic chicken (*Gallus gallus domesticus*) is the red junglefowl (*Gallus gallus*) that lives in natural conditions in the jungle of Southeast Asia. The red junglefowl is a dimorphic species where the female has plumage in brown that camouflages her well in the environment and grows to around 1kg whereas males have a more colourful plumage with red ornaments on the head and they reach an adult weight of around 1,5kg. The jungle provides a habitat where most large predators (e.g. civets and jackals) live on the ground. Therefore, the red jungelfowl generally rest and sleep perched in the trees as an anti-predator behaviour (Collias and Collias, 1967; Newberry et al., 2001). The day length does not vary much and is around 12 hours during the whole year whereas the light under the trees varies both in intensity and spectrum.

1.1.1 Natural behaviour

The wild red junglefowl live in small mixed-sex groups, often with only one male and several females (Collias and Collias, 1996), with sex-dependent social hierarchies (Banks, 1956). The male(s) defend the territory from intruders whereas females brood and care for their offspring (Collias and Collias, 1967). In natural conditions, a mother hen builds a nest in dense grass or bushes on the ground in which she lays her eggs. After 21 days of brooding, the chicks hatch and stay with the mother hen for 5 - 6 weeks (Guhl, 1968), get sexually mature around 18 weeks and reach adulthood at around 6 months of age. To the young chicks, the mother hen is an important role model from which they learn important skills (Edgar et al., 2016).

However, chickens are a precocial species and the chicks can fend for themselves after hatching, apart from the thermoregulation. The mother hen broods the chicks regularly during the day and thus synchronises the behaviour of the group in resting and active periods (Edgar et al., 2016). The typical brooding cycle of the mother hen with chicks in a warm environment is described as consisting of periods, where the hen induces resting for 40 minutes followed by activity for 40 minutes in cycles during the day (Wood-Gush et al., 1978; Malleau et al., 2007). In 1-16 days old layer chicks, resting periods of chicks, brooded by a hen, have been found to be 15 minutes (Riber et al., 2007). At first, brooding and resting takes place on the ground, but due to the threat of predators, resting is moved up in the trees when the chicks are old enough to fly up there. Perching may start as early as one week of age (Workman and Andrew, 1989). Adult red junglefowl spend the majority (around 60 %) of the day foraging, i.e. exploratory pecking and scratching along with the feeding (Dawkins, 1989).

1.1.2 Domestication

Domestication of the red junglefowl occurred around 8000 years ago (Wang et al., 2020) and the primary reason was for cock fighting although chickens had importance aesthetically and religiously (Wood-Gush, 1959). Humans have since then been selecting chickens to several different breeds with different purposes, such as aesthetics, egg production or meat production.

1.1.3 Broiler chickens

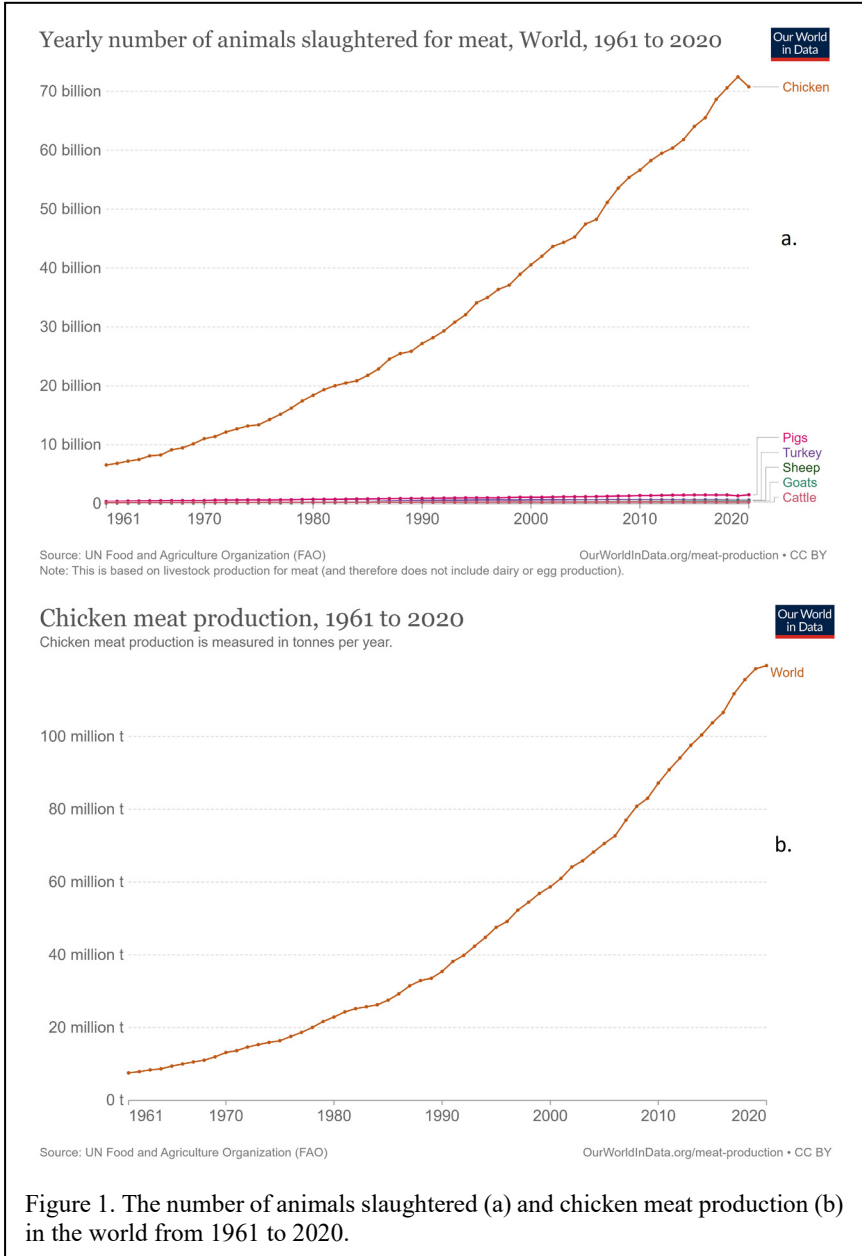
Chickens specifically selected for meat production are called broilers. The purpose of broiler selection is to achieve faster growth rate with higher meat yield using less feed. Broilers reach a slaughter weight of 1,6-4 kg, with a feed conversion ratio of 1,3-1,5, at 4-6 weeks for most commercial strains (e.g. Ross 308, Aviagen, 2022) but there are slower growing strains reaching slaughter weight at around 14 weeks. Thus, broiler chickens do not reach sexual maturity before slaughter, fast-growing birds do not even reach the age when they should have left their mother in natural circumstances. The fast growth rate of broiler chickens was already identified as a welfare issue for more than 20 years ago as it is associated with poor leg health, which could result in lameness (Kestin et al., 2001; Sanotra et al., 2001), Pulmonary arterial hypertension (ascites syndrome) (reviewed in Wideman et al., 2013) and Sudden death syndrome (reviewed in Siddiqui et al., 2009).

The behaviour of domestic chickens is very similar to that of the junglefowl (Kruijt, 1964). However, as the broiler chickens have a short life span, the similarities are mainly to the young junglefowl chicks. Like junglefowl chicks, broiler chickens show motivation to perform natural behaviours, such as perching (Ventura et al., 2012; Bailie and O'Connell, 2015). Though the heavy body weight impairs the broiler chicken's ability to use perches and to reach them by flight (Yngvesson et al., 2018). Broiler chickens also show differences from red junglefowl where the most prominent difference is the level of activity or inactivity. Broiler chickens spend between 60 and 80 % of the day time resting, depending on age and the conditions of rearing (Weeks et al., 2000; Cornetto and Estevez, 2001).

1.2 Intensive broiler production

Poultry production for meat consumption purposes has been increasing a lot in the last couple of decades, both in the number of animals slaughtered as well as meat production (Our World in Data, 2022, Figure 1). Broilers are grown for human consumption, giving humans a great responsibility for their care and resulting health and welfare. The huge number of chickens slaughtered yearly indicate the incomprehensible amount of individuals that are affected by our way of keeping and caring for them.

There are several factors that influence the welfare of broiler chickens, starting with the care of the egg, chick handling at the hatchery, housing and management at the rearing farm, transport between the various facilities and finally stunning and slaughter conditions. The focus here is on the on-farm period, when the chicks are growing.



1.2.1 Housing

Modern broiler production is very intensive. Generally, large flocks of thousands of birds are kept in a facility with stocking densities up to 42kg/m² live weight, which is the maximum allowed stocking density in the EU (European Commission, 2007). The facilities often have high standards with regard to biosecurity. This is important for the health of the birds staying safe from diseases outside of the facility. This also allows for less use of antibiotics or no use of antibiotics at all (e.g. in Sweden antibiotics are only used if there is an outbreak of disease in a flock (Svensk Fågel, 2022)). Avoiding use of antibiotics in broiler production is important to reduce the risk of antibiotic resistance, which is seen as a global concern for animal and human health (Roth et al., 2019). Broiler houses generally include control systems for ventilation, ambient temperature, humidity and lighting. The broiler chickens are raised with *ad libitum* access to feed and water and in the EU a layer of litter on the floor must be provided (European Commission, 2007). Otherwise, the chickens experience a barren environment unless enrichment is provided (see 1.3.3).

1.2.2 Lighting

An important factor for broiler welfare is light, where the intensity, source, spectrum and schedule all play a role (reviewed in Olanrewaju et al., 2006; Pal et al., 2019). Light is important for sight, help establish rhythmicity (and synchronisation) of essential functions such as body temperature and the metabolism as well as stimulating hormonal secretions that for example control growth and reproduction (Olanrewaju et al., 2006). The intensive systems often use low intensity and due to the available light sources also a limited spectrum. The lighting schedules are often kept at continuous or near continuous light, but great variation occurs. In the EU, the maximum allowed light period is 18 hours a day, but for up to the first seven days of age and the last three days of life 24 hours of light per day is allowed (European Commission, 2007). Thus, in the EU, there has to be 6 hours of darkness per day where at least 4 hours are continuous and the intensity has to be above 20 lux at animal level (European Commission, 2007).

1.2.3 Environmental enrichment

Environmental enrichment is defined as a modification of the environment that improves the biological functioning of captive animals (Newberry,

1995). The barren environment commonly provided in intensive broiler production can be enriched by for example structures or objects that enhance natural behaviours. The main focus on enrichment in broiler facilities has been to increase the activity of the birds, as a higher activity can improve leg health (reviewed in Riber et al., 2018). Vasdal et al. (2019) compared broilers reared with enrichment (peat, bales of lucerne hay, and elevated platforms) with broilers reared without enrichment and found an increase in several active behaviours such as wing flapping, body shaking and ground scratching. Environmental enrichment for broilers has been seen to broaden the variety of behaviours performed and to increase activity (reviewed in Riber et al., 2018). As broilers tend to avoid open areas and gather along walls (Buijs et al., 2010) dividers or barriers, objects that block the view, can more evenly distribute the birds resulting in less crowded areas giving opportunities to perform a wider variety of behaviours (Riber et al., 2018). Straw bales, dividers and elevated platforms have been suggested to be the most promising enrichments for broilers as they can be used for perching as well as giving areas for undisturbed resting (Riber et al., 2018).

1.3 Animal Welfare

There are several definitions or descriptions of animal welfare as it is considered a complex subject. One of the first descriptions of how to realise good welfare that got widely accepted were the five freedoms proposed by the Farm Animal Welfare Council in the UK (FAWC, 1993). These five freedoms guarantee animal welfare when the animal: 1. is free from hunger and thirst, 2. is free from physical and thermal discomfort, 3. is free from pain, injury and disease, 4. is free to express normal and natural behaviour, 5. is free from fear and distress. Since then, animal welfare definitions has been an ever developing subject, with increasing complexity towards 'a life worth living' (Mellor, 2016).

Fraser (2008) aims to summarise the complexity by describing three overlapping concepts of animal welfare. The first concept consists of the animal's basic health and functioning, describing for instance the importance of avoiding sickness and injuries, to have good welfare. The second concept considers affective states, where positive feelings increase the welfare whereas negative feelings are detrimental. The third concept focuses on 'natural living' and describes good welfare as fulfilling the animal's needs

which are developed during its evolutionary history. Fraser (2008) highlights the importance of addressing all the three concepts of animal welfare for the assessment of an animal's welfare state.

A more recent description of animal welfare is presented by Mellor et al., (2020), after several updates from the original formulation made in 1994 (Mellor and Reid, 1994) who describes a five domains model of animal welfare. The model does not define the state of the welfare, if it is good or bad, but rather provides a tool for a systematic and coherent assessment of animal welfare. Three domains focus on the internal and external aspects that need to be adapted to the specific animal(s): nutrition, environment and health. If these three domains are well adapted, the animal will experience positive affects. If anything in any of these domains is not well adapted, for example water is not satisfactory provided, the animal will experience negative affects, such as thirst. The fourth domain focuses on the possibilities of behavioural interactions, such as interactions with the environment (including challenges of confinement and space availability) and interactions with other animals (and/or humans). A motivation to perform a certain behavioural interaction will either enhance welfare if the animal is able to perform the motivated behaviour and otherwise diminish welfare. The fifth domain connects the previous domains and focuses on the animals' emotions and mental health based on the first four domains. Combining all these five domains, the welfare state of the animals can be assessed in a way that subjectively shows the experiences perceived by the animals (Mellor et al., 2020).

1.4 Sleep and rest

Sleep is a complex subject and it is undoubtedly important for most animal species, most likely serving multiple functions but not yet fully understood (Krueger and Obal, 2003; Krueger et al., 2016). Suggested functions include: tissue restoration and growth, energy conservation, neurobehavioral and neurocognitive performance, memory processing and learning as well as increased waste clearance in the brain (Carskadon and Dement, 2005; Siegel, 2005; Assefa et al., 2015). Sleep can be defined as a specific state of rest with altered consciousness, reduced responsiveness to external stimuli and homeostatic regulation (Carskadon and Dement, 2005). Sleep consists of several different psychophysiological states such as REM sleep and different

stages of nonREM sleep, that can be measured by encephalography (EEG) (Šušmáková, 2004). EEG consists of electrodes attached to the head, measuring brain activity through electrical impulses. Most sleep research is done in humans (reviewed in Šušmáková, 2004), but can likely be implemented in other mammals and birds as the sleep functions are probably similar (Blokhuys, 1983; Siegel, 2005). One large area of sleep research in humans relates to sleep disorders and the consequences of sleep disturbances as it affects human welfare negatively (reviewed in Šušmáková, 2004). For example cognitive and physical performance deteriorate with sleep loss and progressively gets worse (Doran et al., 2001). Thus, this is also likely in animals.

Measuring sleep in animals is more difficult, as using EEG measurements can be complicated. To measure sleep, one can instead observe the behaviour when resting. Rest may be defined as a prolonged period of inactivity that can clearly be distinguished from other maintenance behaviours (Blokhuys, 1984). However, it is sometimes impossible to tell whether animals are sleeping or not based on the behaviour, that is why the term resting is more appropriate when using behavioural observations. Resting and sleeping positions for adult hens were described previously by Blokhuys (1984). Most commonly a hen is dozing with the head withdrawn (Figure 2) and sleeping with the head tucked under the feathers on the wing base (Figure 3). However, to my knowledge, resting or sleeping positions for young chicks are not described.



Figure 2. Dozing hen. From Blokhuis (1984) with permission from the publisher¹.



Figure 3. Sleeping hen. From Blokhuis (1984) with permission from the publisher¹.

¹This article was published in Applied Animal Behaviour Science, 12(3), Blokhuis, H. J., Rest in poultry, 289-303, Copyright Elsevier (1984).

1.4.1 Sleep and rest in broiler production settings

Broiler production raises various welfare issues, e.g. health problems, impaired locomotion and behavioural restrictions as described by EFSA (2012). Often neglected behaviours in this context are rest and sleep. It has been shown that resting behaviour commonly gets disrupted by physical disturbances by other individuals (Yngvesson et al., 2017). Very limited research on sleep and rest in broilers can be found even though sleep most likely has an extensive impact on the welfare of the birds. There are however several aspects in broiler production that can affect rest and sleep due to the unnatural circumstances for the birds:

- High stocking density, more birds occupy the space giving less room to rest undisturbed.
- Large flock sizes, a lot of individuals that can disturb each other.
- No mother hen, no individual present to induce rest and provide conditions for undisturbed rest for the young chicks.
- Light schedule, if not adapted to the natural resting patterns of young chicks.
- Barren area, no specific resting places or change to perform motivated resting behaviours such as perching.

1.5 Disturbed sleep and animal welfare

There are two important links between disturbed sleep and animal welfare: animal behaviour and the function of sleep (Figure 4). Firstly, sleep is a highly motivated behaviour and disturbances of such behaviours could lead to frustration and stress (Fraser, 2008). Frustration is defined as an aversive state arising when animals are prevented from performing behaviour that they are strongly motivated to perform (Fraser, 2008) but is not always visible in the behaviour of the animal. Secondly, sleep serves vital functions and disturbances of sleep may impair these functions, possibly resulting in impaired health, reduced growth and/or loss of cognitive functioning (Rial et al., 2007; Assefa et al., 2015).

Thus, disturbances of sleep and possible loss of sleep may have detrimental effect on animal welfare, it is relevant to highlight the significance of rest and sleep for the welfare of broilers.

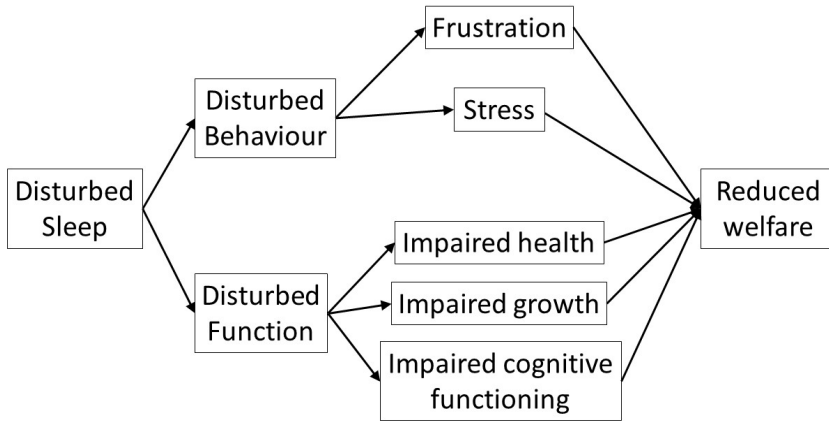


Figure 4. The effect of disturbed sleep on animal welfare.

2. Aims of the thesis

The general aim of this thesis was to investigate and extend the limited knowledge of resting behaviour of broilers. Also, aiming to investigate the effects of disturbed sleep or rest on the behaviour of the broilers and the function of sleep.

The specific aims in the different studies were:

- Study I: to investigate the effects of adding a separate resting place in the form of an elevated platform on the resting behaviour of broilers. Also, investigating the effect of a reduction in stocking density on the resting behaviour (Paper I).
- Study II: to investigate the effects of adding a separate resting place in the form of artificial brooders on the resting behaviour and welfare of broilers (Paper II and III)
- Study III: to investigate the effects of an intermittent light programme on the resting behaviour and welfare of broilers.

3. Materials and Methods

Information on the full materials and methods is found in the printed papers provided at the end of this thesis (Paper I-IV).

In Study I birds were kept under intensive production settings and resting behaviour was studied in two treatments: 1) using elevated platforms and 2) applying reduced stocking density, and compared to a control (Paper I). In Study II the effect of artificial brooders on resting behaviour (Paper II), health and production parameters (Paper III) was studied. Study III consisted of two treatments with different light programmes, and effects on resting behaviour, health and production parameters were observed (Paper IV).

3.1 Ethical statement

All studies conducted for this thesis included work with live animals. Study I was conducted in Denmark and was carried out according to the guidelines of the Danish Animal Experiments Inspectorate with respect to animal experimentation and care of animals under study. Study II and III were conducted in Sweden and were approved by the Animal Research Ethics Committee in Uppsala (Dnr 5.8.18-17765 2018).

3.2 Study I (Paper I)

3.2.1 Animals and housing

This experiment was conducted at AU Foulum, Aarhus University, Denmark. Mixed-sex Ross 308 broilers were kept for a full production period under commercial conditions with a stocking density at an expected 40 kg/m² at slaughter age. In the facility, ten pens of 3.1m x 9.6m (29.8m²) were used.

Water and feed were provided ad libitum, light intensity was 27 lux at animal level and the light schedule was programmed for 18L: 6D from day 6 of age (and 24L before that).

3.2.2 Experimental design

The treatments were arranged in a randomized block design, consisting of six blocks, where the first started up in September 2016 and the last was completed in July 2017. The three treatments were, treatment EP consisting of an elevated platform (L×W×H: 5.40m × 0.60m × 0.30m, stocking density at 40 kg/m², Figure 5), treatment SD consisting of one type of manipulation of the environment (low stocking density at 34 kg/m²) and control C (no platform, stocking density at 40 kg/m²). In the treatment EP, two access ramps at an incline of 14.5° were provided to ease the access to the platforms. Both platforms and access ramps consisted of perforated plastic slats. The area underneath the platforms and ramps was fenced off and not accessible to the birds.



Figure 5. Pen with elevated platforms.

3.2.3 Behavioural observations

Behavioural observations were made from video recordings of the pens from day 20 and 34 of age using focal animal sampling. The focal animals were followed during a complete resting bout where the length of each resting bout as well as the occurrence of disturbances were registered. In addition, the position in the pen while resting was registered.

3.3 Study II (Paper II and III)

3.3.1 Animals and housing

This experiment was conducted at Lövsta Research Center, Swedish University of Agricultural Sciences, Uppsala, Sweden. Mixed-sex Ross 308 broilers were kept for a full production period with a stocking density at an expected 20 kg/m² at slaughter age. In the facility, twelve pens of 2m x 3,5m (7m²) were used. Water and feed were provided ad libitum, light intensity at 27 lux at animal level and the light schedule was programmed for 18L: 6D from day 6 of age.

3.3.2 Experimental design

Two treatments were used in this study, artificial brooders and control without brooders. In the treatment with brooders, each pen had three artificial brooders (40cm × 60cm) with the sides of the brooders covered with flaps of tarp to make the area under the brooders dark (Figure 6). The brooders were removed at 21 days of age, when all chicks no longer could fit under them and the heat provided no longer was necessary.



Figure 6. Pen with artificial brooders.

3.3.3 Behavioural observations (Paper II)

Behavioural observations were made from video recordings of the pens from day 20 and 34 of age using focal animal sampling. The focal animals were followed during a complete resting bout where the length of each resting bout as well as the occurrence of disturbances were registered. In addition, the position in the pen while resting was registered. Data on the use of the brooders were collected using scan sampling four times a day on days 6, 13 and 20.

3.3.4 Production and health parameters (Paper III)

Growth, feed consumption and mortality were monitored throughout the experiment. Walking abilities, litter quality and other clinical health parameters were measured at the end of the experiment. All chicks received a commercial live vaccine against Infectious Bronchitis Virus (IBV) (Nobilis® IB Ma5 vet, MSD Animal Health) at 7 days of age. Ten individually identified chicks per pen were monitored for antibodies to IBV in serum during the experiment. Blood samples were collected from the jugular vein at 7 days of age, prior to vaccination, and subsequently at 22, 29 and 36 days of age. The levels of antibodies to IBV in sera were quantified by a commercial ELISA-test (IDEXX IBV Ab Test) performed according to the manufacturer's instructions.

3.3.5 Fear (Paper III)

Three different fear tests were performed during the experiment. A Tonic Immobility test was done at 24 days of age. Five individuals per pen were tested individually, placed carefully on their back in a cradle measuring latency to movement after induction of TI. A longer duration spent in TI indicates increased fearfulness. A Novel Object test was done at 10 and 32 days of age. A novel object was placed in the pen and the full pen was recorded for 10 minutes, and the number of individuals within three bird lengths from the object were noted every minute. Less birds near the object indicated a more fearful group of birds. An Avoidance Distance test was done at 35 days of age. An observer walked slowly through the pen stopping at four different locations. At every location, the observer counted the number of birds within an arm's length after 10 seconds. Less birds near the observer indicated a more fearful group of birds.

3.4 Study III (Paper IV)

3.4.1 Animals and housing

This experiment was conducted at Lövsta Research Center, Swedish University of Agricultural Sciences, Uppsala, Sweden. Mixed-sex Ross 308 broilers were kept for a full production period with a stocking density at an expected 20 kg/m² at slaughter age. In the facility, twelve pens of 2m x 3,5m (7m²) were used (Figure 7). Water and feed were provided ad libitum, light intensity at 27 lux at animal level and the light schedule was programmed for 18L: 6D from day 6 of age.



Figure 7. Pen layout during Study III.

3.4.2 Experimental design

Two treatments were included in this study, intermittent lighting and a control setting with a continuous light scheme. In the treatment with intermittent lighting, there was a longer night period of 4h darkness (22:00-02:00) and six shorter dark periods of 20min evenly spread over the day. The control treatment had one dark period of 6h (22:00-04:00) followed by 18h light. For all transitions from light to dark or dark to light, five minutes of dimming were added.

3.4.3 Behavioural observations

Data on synchronisation of resting behaviour were collected from video recordings at day 6, 20 and 34 of age using scan sampling. Each pen was divided in two parts, one with feeders and drinkers and the other one an empty area. Only the empty area was observed as the chickens were expected to rest there, away from active chicks foraging. The proportion of resting individuals was observed every ten minutes for two hours twice each day. Behavioural observations were made from video recordings of the pens from day 6, 20 and 34 of age using focal animal sampling. The focal animals were followed during a complete resting bout where the length of each resting bout as well as the occurrence of disturbances were registered. In addition, the position in the pen while resting was registered.

3.4.4 Production and health parameters

Growth, feed consumption and mortality were monitored throughout the experiment. Walking abilities, litter quality and other clinical health parameters were measured at the end of the experiment.

3.4.5 Fear

Three different fear tests were performed during the experiment. A Tonic Immobility test was done at 24 days of age. Five individuals per pen were tested individually, placed carefully on their back in a cradle measuring latency to movement after induction of TI. A longer duration spent in TI indicates increased fearfulness. A Novel Object test was done at 10 and 32 days of age. A novel object was placed in the pen and the full pen was recorded for 10 minutes, and the number of individuals within three bird lengths from the object were noted every minute. Less birds near the object indicated a more fearful group of birds. An Avoidance Distance test was done at 35 days of age. An observer walked slowly through the pen stopping at four different locations. At every location, the observer counted the number of birds within an arm's length after 10 seconds. Less birds near the observer indicated a more fearful group of birds.

3.5 Statistics

Statistical analyses were performed using R with a significance level of 0.05.

All data collected of durations was controlled for normal distribution and homogeneity of variances and if necessary and possible transformed logarithmically. If normally distributed a parametric test, t-test or ANOVA test, was used to compare differences between for example treatment groups, ages, periods of the day, resting positions in the pen and interactions between factors were included. Pen was used as a random factor. Post hoc comparisons of significant factors were performed using Tukey's test (Tukey's HSD test).

For data collected where data was not normally distributed a non-parametric test was done, as for proportions a Chi-squared test was performed. The explanatory factors were for example treatment, age, position in the pen and interactions between factors were included. Pen was used as a random factor. Post hoc comparisons of significant factors were performed using Pairwise Nominal Independence. For all other comparisons, a Kruskal Wallis test was done as the data were not normally distributed.

The experimental unit was the pen since individuals within the pen interact and thus influence each other.

4. Summary of results

In this chapter, a summary of the results from each study is presented. For full details, see the respective paper.

4.1 Study I (Paper I)

4.1.1 The effect of disturbances on resting bout duration

Physical disturbances between birds shortened the duration of resting bouts during the night at 20 days of age (Disturbance 279.68 ± 41.02 s; No disturbance 345.10 ± 40.12 s, $p = 0.005$) and tended to do the same at 34 days of age (Disturbance 194.60 ± 21.86 s; No disturbance 225.24 ± 20.23 s, $p = 0.058$). This effect was not found during the day.

4.1.2 The effect of disturbances on the activity duration

At both 20 and 34 days of age, the duration of activity between resting bouts during the day decreased if the preceding resting bout had been ended due to disturbance (20 days of age, Disturbance 12.26 ± 2.10 s; No disturbance 27.45 ± 6.21 s, $p = 0.046$; 34 days of age, Disturbance 12.97 ± 2.56 s; No disturbance 21.12 ± 3.52 s, $p = 0.009$) but no effect was found during the night.

4.1.3 Proportion of disturbances

Disturbances occurred in all treatments and situations (Table 1). The proportion of disturbances was lower when the resting was performed on top of a platform compared to open areas in the control setting.

Table 1. Proportion of resting bouts where the resting bird is disturbed by companions shown separately for day and night and divided into occurrences in different treatments and positions in the pen. The statistical values indicated are for the interactions ‘treatment×position in the pens’.

| | Treatment | | | | | | | p |
|---|--------------------|--------|--------|---------|--------|-------------|--------|--------|
| | Elevated platforms | | | Control | | Low density | | |
| Proportion | Plat- form | Open | Wall | Open | Wall | Open | Wall | |
| ion disturbe d during day | 0.40a | 0.46ab | 0.64ab | 0.77b | 0.58ab | 0.54ab | 0.56ab | 0.0018 |
| ion disturbe d during night | 0.30a | 0.43ab | 0.48ab | 0.55b | 0.52ab | 0.32ab | 0.56b | 0.0225 |

Different letters within period of the day indicate significant differences

4.1.4 Duration of resting bouts and activity between resting bouts

Resting bout duration was different in different locations within and between treatments (Table 2). The duration was the longest for birds resting on top of platforms during night. The activity between resting bouts was generally short and did not differ between treatments and locations.

Table 2. Duration (s) of resting bouts during day and night, respectively, and duration of activity between resting bouts during the day (mean \pm SE). The statistical values listed are for the interaction treatment x positions in pen.

| | Treatment | | | | | | | p |
|---------------------------|---------------------------|------------------------|------------------------|---------------------------|-------------------------|------------------------|---------------------------|--------|
| | Elevated platforms | | | Control | | Low density | | |
| | Plat- form | Open | Wall | Open | Wall | Open | Wall | |
| Resting bout, day | 116.13 \pm 17.30a | 66.29 \pm 11.15ab | 99.29 \pm 17.30ab | 60.85 \pm 5.95b | 107.63 \pm 10.83ab | 98.10 \pm 11.15ab | 127.88 \pm 15.88a | <0.01 |
| Resting bout, night | 500.64 \pm 66.96a | 207.04 \pm 44.24b | 240.36 \pm 63.58b | 247.90 \pm 34.91b | 167.77 \pm 23.54b | 261.58 \pm 38.84b | 257.48 \pm 32.43b | <0.001 |
| Activity | 11.07 \pm 1.92a | 18.54 \pm 6.25a | 15.34 \pm 4.44a | 12.38 \pm 2.25a | 11.03 \pm 1.93a | 13.99 \pm 3.15a | 10.11 \pm 1.47a | 0.429 |

Different letters within parameter indicate significant differences

4.2 Study II (Paper II and III)

4.2.1 The effect of disturbances on resting bout and activity duration (Paper II)

Physical disturbances between birds shortened the duration of resting bouts (Disturbance 98.4 ± 3.4 s; No disturbance 257.9 ± 4.7 s, $p < 0.001$).

Physical disturbances between birds increased the duration of activity between resting bouts (Disturbance 9.98 ± 1.0 s; No disturbance 61.0 ± 2.4 s, $p < 0.001$).

4.2.2 Proportion of disturbances (Paper II)

Disturbances occurred in both treatments and all situations. There was a difference in the proportion of disturbances between the treatments both at 20 days of age ($p < 0.001$) and at 34 days of age ($p < 0.001$) with a lower proportion of resting bouts being disturbed in the treatment with brooders (0.15 and 0.25 disturbed) than in the control treatment (0.48 and 0.42 disturbed).

4.2.3 Duration of resting bouts and activity between resting bouts (Paper II)

The treatment with brooders had longer resting bouts than the control treatment (260.7 ± 5.2 s vs. 132.8 ± 5.3 s, $p < 0.001$). Resting bouts taking place under the artificial brooder (329.4 ± 8.4 s) were longer than in open areas (176.5 ± 6.3 s, $p < 0.001$) and near walls (182.7 ± 6.0 s, $p < 0.001$). Duration of resting bouts taking place in open areas did not differ from resting bouts taking place near walls ($p = 0.254$). The resting bouts were longer in the evening ($p < 0.001$) than in the morning (Evening vs. Morning: 229.1 ± 6.4 s vs. 190.0 ± 6.4 s). The resting bouts were longer for older birds ($p = 0.02$) than younger birds (20 vs. 34 days of age: 202.3 ± 6.4 s vs. 216.8 ± 6.5 s).

The treatment with brooders had longer activity between resting bouts than the control treatment (49.2 ± 2.4 s vs. 40.0 ± 3.3 s, $p < 0.001$). The position in the pen while resting prior to becoming active also affected the duration of activity between resting bouts ($p < 0.01$) where birds resting in open areas (37.7 ± 2.7 s) were active for a shorter duration than birds resting under the artificial brooders (52.2 ± 4.2 s, $p = 0.013$) and near walls (50.0 ± 3.5 s, $p = 0.006$). Duration of activity after resting near walls did not differ from resting under brooders ($p = 0.935$).

4.2.4 Production parameters (Paper III)

There were no differences in mean body weight at different ages between the treatments (Table 3), neither in feed intake ($p = 0.15$) or FCR ($p = 0.75$) at 35 days of age.

Table 3. Body weight (g) of the chickens at different ages in the two treatments (mean \pm SE).

| Age (days) | Body weight (g) | | p |
|------------|-------------------|-------------------|------|
| | Brooders | Control | |
| 1 | 39.9 ± 0.04 | 40.0 ± 0.03 | 0.34 |
| 7 | 212.9 ± 2.2 | 212.2 ± 2.1 | 0.68 |
| 21 | 1279.0 ± 16.2 | 1260.9 ± 14.9 | 0.41 |
| 28 | 2049.7 ± 28.1 | 2005.7 ± 25.1 | 0.24 |
| 35 | 2857.3 ± 40.8 | 2831.9 ± 39.6 | 0.66 |

There was no difference in mortality and culls between treatments ($p = 0.89$). The total mortality was 7.3% in the brooder treatment and 7.9% in the control.

4.2.5 Antibodies to IBV in serum (Paper III)

Serum levels of IBV specific antibodies, measured prior to vaccination at seven days of age and at 15, 22 and 29 days after vaccination, are shown in Figure 8. Serum levels of IBV specific antibodies prior to vaccination show that the majority of chickens in both groups had maternally derived antibodies to IBV (98 % of birds in both groups had a titer ≥ 100 ; 81 % and 84 % of brooder and control birds, respectively, had a titer ≥ 300). At 15 days after vaccination, i.e. 22 days of age, serum titers to IBV were significantly decreased compared to those measured before vaccination for both groups (Figure 8). At 21 and 29 days after vaccination serum titers to IBV gradually increased for both treatments and were 52.8 ± 19.1 % and 72.8 ± 36 % of pre-vaccination titers for brooder and control, respectively, on day 29 after vaccination, i.e. 36 days of age. There were no statistically significant differences in serum titers to IBV between treatments at any of the sampling occasions.

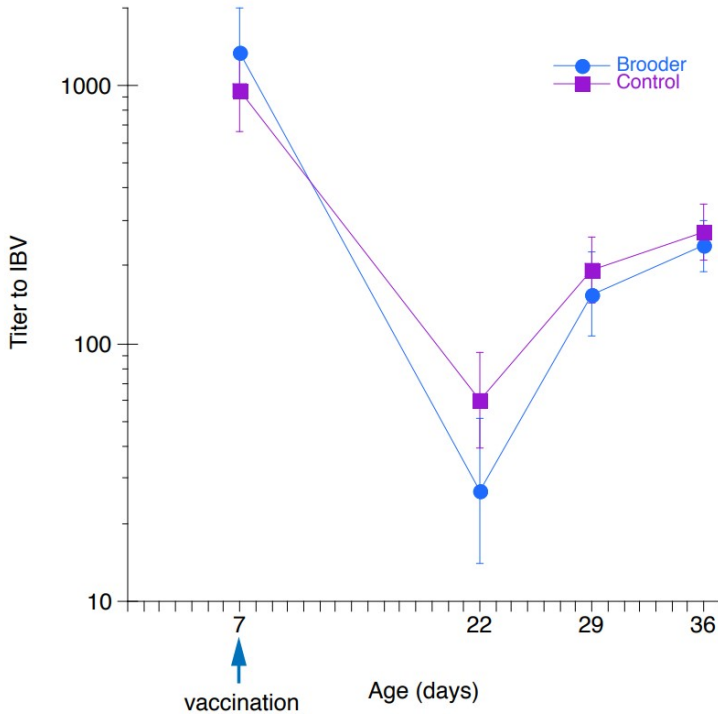


Figure 8. Titers to IBV in sera collected on the indicated days of age from chickens with access to artificial brooders (Brooders, blue circles) and control chickens without access to brooders (Control, purple squares). All chickens were vaccinated with a live IBV vaccine at 7 days of age (arrow). Values are geometric group mean values \pm 95% confidence intervals.

4.2.6 Clinical welfare measurements (Paper III)

The assessment of walking ability at 35 days of age showed a tendency of a lower gait score in the brooder treatment (brooders: 0.42 ± 0.05 , control: 0.59 ± 0.06 ; $p = 0.0504$). No hock burns were found in any of the treatment. Occurrences of footpad dermatitis were found in only three birds (two in the control treatment and one in the treatment with brooders). No difference was found in litter quality between different treatments (brooders: 0.2 ± 0.07 ,

control: 0.23 ± 0.08 ; $p = 1$). Litter quality was good throughout the experiment.

4.2.7 Fear (Paper III)

The Tonic Immobility test, performed at 24 days of age, showed a longer duration of tonic immobility for chickens reared without brooders, both for first head movement (brooders: 197.2 ± 15.9 s, control: 307.6 ± 17.9 s, $p < 0.001$) and for the chicken to turn itself around (brooders: 255.0 ± 21.7 s, control: 372.0 ± 25.8 s, $p < 0.001$). The number of inductions did not differ between the treatments (brooders: 1.1 ± 0.06 , control: 1.2 ± 0.08 , $p = 1$).

The Novel Object tests, performed at 10 and 34 days of age, showed a difference between treatments in the number of birds being within 3 bird lengths from the object; more chickens reared with brooders were in the vicinity of the novel object compared to the control chickens (brooders: 2.79 ± 0.13 birds, control: 1.76 ± 0.11 birds, $p < 0.001$). No difference was found between ages ($p = 0.93$) nor between objects ($p = 0.53$).

The Avoidance Distance test, performed at 35 days of age, showed a difference between treatments where more chickens reared with brooders were within an arm's length from the observer compared to the control chickens (brooders: 9.75 ± 0.24 birds, control: 4.79 ± 0.21 birds, $p < 0.001$).

4.3 Study III (Paper IV)

4.3.1 Synchronisation of resting behaviour

The proportion of birds resting simultaneously was higher in the treatment with intermittent light than in the control treatment (0.72 ± 0.003 vs. 0.60 ± 0.003 , $p < 0.001$). There was also a higher proportion of birds resting simultaneously in the morning than in the evening (0.71 ± 0.003 vs. 0.61 ± 0.003 , $p < 0.001$). Age did not affect the proportion of birds resting simultaneously ($p = 0.94$).

4.3.2 Resting behaviour and disturbances

The percentage of broilers being physically disturbed by other birds during resting was ranging from about 11% to about 55%, depending on treatment, position in the pen and age (Table 4). No interactions of factors were

significant, wherefore age and period of the day were analysed separately for the two treatments.

Table 4. Proportion of resting bouts where the resting bird was disturbed by companions shown separately for different factors (mean \pm SD).

| Factor | | Proportion disturbed by other birds | p |
|---|-----------------------|--|---------|
| Treatment | Intermittent lighting | 0.29 \pm 0.04 | < 0.001 |
| | Control | 0.48 \pm 0.08 | |
| Age (Intermittent lighting) | 6 days of age | 0.18 \pm 0.01 | < 0.001 |
| | 20 days of age | 0.29 \pm 0.03 | |
| | 34 days of age | 0.40 \pm 0.06 | |
| Age (Control) | 6 days of age | 0.40 \pm 0.07 | < 0.001 |
| | 20 days of age | 0.48 \pm 0.07 | |
| | 34 days of age | 0.55 \pm 0.09 | |
| Period of day (Intermittent lighting) | Morning | 0.11 \pm 0.07 | < 0.001 |
| | Evening | 0.27 \pm 0.09 | |
| Period of day (Control) | Morning | 0.17 \pm 0.08 | < 0.001 |
| | Evening | 0.48 \pm 0.11 | |

The resting bouts were longer in the treatment with intermittent lighting than in the control treatment (223.4 s \pm 4.3 vs. 129.1 s \pm 4.3, $p < 0.001$), longer in the evening than in the morning (199.9 s \pm 5.2 vs. 152.5 s \pm 4.4, $p < 0.001$) and longer near walls than in open areas (180.6 s \pm 4.9 vs. 171.9 s \pm 5.0, $p = 0.045$). No differences between the other factors or interactions of factors were found. The duration of activity between resting bouts was longer in the

treatment with intermittent lighting than in the control treatment (41.2 ± 1.8 vs. 37.3 ± 2.2 , $p < 0.001$), longer near walls than in open areas (43.1 ± 2.1 vs. 35.5 ± 1.9 , $p = 0.001$) and longer for younger birds than older (6 days: 47.0 ± 3.2 , 20 days: 39.3 ± 2.3 , 34 days: 31.7 ± 1.7 , $p = 0.015$). Specifically, birds of 6 days of age had longer activity between resting bouts than birds of 34 days of age ($p = 0.016$). No differences between the other factors or interactions of factors were found.

4.3.3 Fear tests

The Tonic Immobility test, performed at 32 days of age, showed a shorter duration of tonic immobility for chickens reared with intermittent lighting, both for first head movement (intermittent lighting: 206.3 ± 15.4 s, control: 296.3 ± 17.8 s, $p < 0.01$) and for the chicken to turn itself around (intermittent lighting: 271.1 ± 24.1 s, control: 361.6 ± 267.0 s, $p < 0.01$). The number of inductions did not differ between the treatments (intermittent lighting: 1.2 ± 0.07 , control: 1.3 ± 0.11 , $p = 0.29$).

The Novel Object tests, performed at 3 and 30 days of age, showed no differences in the number of birds being within 3 bird lengths from the object between treatments (intermittent lighting: 2.63 ± 0.16 birds, control: 2.44 ± 0.11 birds, $p = 0.38$), between ages ($p = 0.23$) or between objects ($p = 0.75$).

The Avoidance Distance test, performed at 35 days of age, showed a difference between treatments where a higher number of chickens reared with intermittent lighting were within an arm's length from the observer compared to the control chickens (intermittent lighting: 6.96 ± 0.21 birds, control: 4.33 ± 0.29 birds, $p < 0.001$).

4.3.4 Health and production parameters

Body weight and FCR were accounted for on a weekly basis (Table 5).

Table 5. Body weight (g) of the chickens and FCR at different ages (days) in the two treatments (mean \pm SD).

| Age | Body weight | | | FCR | | |
|-----|--------------------|-------------------|--------------|--------------------|-----------------|-------------|
| | Intermittent light | Control | p | Intermittent light | Control | p |
| 1 | 39.9 \pm 0.03 | 39.9 \pm 0.02 | 0.57 | - | - | - |
| 7 | 214.8 \pm 2.0 | 217.4 \pm 1.6 | 0.33 | 0.94 \pm 0.02 | 0.93 \pm 0.02 | 0.87 |
| 14 | 599.8 \pm 6.2 | 575.2 \pm 5.2 | 0.003 | 0.97 \pm 0.02 | 1.01 \pm 0.01 | <i>0.08</i> |
| 21 | 1152.1 \pm 13.2 | 1132.7 \pm 13.8 | 0.31 | 1.07 \pm 0.02 | 1.09 \pm 0.01 | 0.26 |
| 28 | 1859.6 \pm 23.2 | 1820.1 \pm 25.3 | 0.25 | 1.20 \pm 0.02 | 1.21 \pm 0.01 | 0.52 |
| 35 | 2645.3 \pm 33.8 | 2553.1 \pm 37.0 | <i>0.068</i> | 1.51 \pm 0.01 | 1.52 \pm 0.01 | <i>0.08</i> |

Values considered significant are in bold ($P \leq 0.05$). Trends ($0.100 \geq P > 0.05$) are italicised.

Mortality and culls did not differ between treatments ($p = 0.28$). The total mortality was 3.9% in the intermittent lighting treatment and 2.7% in the control. Culls accounted for 9 of the total 26 in the intermittent lighting treatment and 3 of the total 18 in the control that died during the experiment. The assessment of walking ability at 35 days of age showed no differences in average gait score between the treatments (intermittent light: 0.36 ± 0.05 , control: 0.49 ± 0.06 ; $p = 0.10$). No hock burns were found in any of the treatments. Six instances of footpad dermatitis were found (two in the intermittent lighting treatment and four in the control treatment). No difference was found in litter quality between different treatments (intermittent lighting: 0.3 ± 0.09 , control: 0.37 ± 0.09 ; $p = 0.59$).

5. Discussion

This thesis investigated resting behaviour of broilers and related welfare parameters in response to specific environmental changes. Similar to many studies on broiler behaviour and welfare, the experiments in this thesis were performed in a controlled environment with smaller group sizes for the purpose of replication. This is a great way to control for consistency between treatments and reduces the risk of unnoticed differences occurring. However, it also opens questions on how to implement the results on-farm. Below, some general methodological aspects of the studies as well as observed indicators are discussed. Then, resting behaviour and animal welfare aspects are discussed and practical implementation of the results and its implications and limitations are addressed. Finally some conclusions are presented.

5.1 Methodological aspects and indicators observed

5.1.1 Fast-growing broilers

In this thesis, all data was collected using fast-growing broiler chickens. It has been shown that fast-growing broilers have lower activity levels than slower growing broilers (Lewis et al., 1997; Siegel et al., 1997) which in itself may pose a welfare problem. For example, higher activity levels have been seen to improve leg health by strengthening bone structures (Reiter and Bessei, 1998). Dawson et al. (2021) saw that fast-growing broiler strains are more inactive and use enrichments less than slower-growing strains, especially at young ages. They concluded that this has to do with the specific growth rate of the strain, where the higher growth rates show more inactivity. The inactivity is defined as rest in this thesis and broilers show high resting times (between 60 and 80 %) during the day (Weeks et al., 2000; Cornetto

and Estevez, 2001). Therefore, high quality resting periods of broiler chickens may be important for restoration of energy, making the birds able and/or motivated to engage in more energy costly activities, thus increasing their welfare. Furthermore, Sherlock et al. (2010) show that heavier birds of the same age have a worse gait score and that the activity of the birds decreases with age also indicating that fast-growing broilers are exposed to more welfare challenges than slower growing strains of broilers. The studies conducted in this thesis focused on fast-growing broiler chickens (Ross 308) with the expectation that the results could also be implemented for slower growing broilers as these show similarities but less extreme welfare implications.

5.1.2 Behavioural observations

Behavioural observations were mainly made during the latter part of the experiments based on the expectancy that disturbances of resting behaviour would occur when the space availability decreases. It was sometimes difficult to identify the behaviour of the chickens on the video recordings, especially when their yellow down matched the substrate of wood shavings. However, human presence could influence the behaviour of the animals during behavioural observations (Hemsworth et al., 1993). Therefore, video recording is more likely to show the true behaviours of the broilers as there are no people present in the facility to influence the birds. During Study II and III, the resting or sleeping positions of the broilers were photographed during the experiments (Figure 9). Compared to the descriptions of adult laying hens by Blokhuis (1984), there seems to be a wider variety of resting positions in young broiler chicks making it difficult to define specific resting or sleeping positions. However, the older and larger the broilers got, more birds tended to rest laying on one side with one leg stretched (Figure 9).



Figure 9. The most common resting or sleeping positions of young broiler chickens seen during Study II and III.

5.1.3 Stocking density

Stocking density has been shown to have an effect on several welfare indicators for broilers. Decreased stocking density has been seen to promote locomotion and foraging (Blokhuys and van der Haar, 1990; Martrenchar et al., 1997; Hall, 2001; Ventura et al., 2012), reduce leg problems (Hall, 2001), reduce lameness (Dawkins, 2004) and reduce contact dermatitis (Hall, 2001; Kyvsgaard et al., 2013). In the three studies of this thesis, different stocking densities were used: Study I, 34kg/m² or 40kg/m²; Study II, 20kg/m²; Study III 36kg/m². The stocking density affect the space availability for the chickens, especially towards the end of the rearing period when the birds take up more space. In another study of Study I, a lower stocking density positively affected locomotion during day 6 and 27 (Bach et al., 2019). Zuowei et al. (2011) found that chicks reared with a lower stocking density (26kg/m²) showed better gait scores than higher stocking density (42kg/m²). Martrenchar et al. (1997) showed that disturbances of lying bouts are more frequent for broilers reared with a stocking density of 43kg/m² compared to 27kg/m² and that 35kg/m² are more similar to the lower than the higher stocking density. Study I could not confirm that a lower stocking density reduces disturbances. And although Study II used an even lower stocking density (20kg/m²) than the other studies, disturbances still occurred. Therefore, stocking density in itself does not seem to provide opportunities for undisturbed rest in flocks of broilers, but has other welfare benefits.

5.1.4 Environmental enrichment

In this thesis, the two environmental enrichments chosen focused on fulfilling motivated resting behaviour (perching) or simulate a more natural resting situation for young chicks (brooding).

Elevated platforms

Perching is a highly motivated behaviour for resting in chickens (e.g. layers, Olsson and Keeling, 2000; Olsson and Keeling, 2002), but broiler chickens tend to not use perches due to their heavy body weight (e.g. Yngvesson et al., 2018). Therefore, the elevated platforms with access ramps were thought to be a good alternative as they ease the balance and access problems for the broilers. Indeed, in a study by Norring et al. (2016) it was seen that platforms were used by broilers to a higher extent than perches. In another study of Study I, the broilers used the platforms from an early age during both day

and night (at least from the first day of observation at 6 days of age) (Bach et al., 2019). However, layer chicks start perching during day-time at a much younger age than during night-time, where they instead chose to rest under heating lamps for the first six weeks of life (Heikkilä et al., 2006). Therefore, for the very young chicks, elevated platforms may be of little relevance. But for the older broiler chicks (of 20 and 34 days of age in Study I), the platforms show positive effects on resting behaviour, though disturbances still occur. Additionally, elevated platforms had a positive effect on foot pad health (Tahamtani et al., 2020), but do not seem to increase the general activity of the broilers (Norrington et al., 2016). This, indicates that the elevated platforms have relevance for the resting behaviour and welfare of broiler chickens, especially for the later weeks of the rearing period.

Artificial brooders

The purpose of the artificial brooders in Study II was to simulate the dark, heated area a broody hen provides for the resting bouts of her chicks. Thus, an area specifically for resting that is structurally separated from areas of high activity, such as near feeders and drinkers. Study II showed improvement of resting behaviour, a lower proportion of disturbances and increased duration of resting bouts, for broilers reared with artificial brooders regardless of where in the pen the resting took place. Also, after the removal of the brooders, the effect still remained. This indicates a long term effect of using brooders in the early rearing, which has also been seen in other behavioural aspects in layers (e.g. reduced feather pecking and fear, Riber and Guzman, 2016, 2017). Thus, the brooders could positively affect welfare of the birds in regards to resting quality. There is a very limited amount of previous research of similar brooders, making it difficult to put in a wider perspective. Stadig et al. (2018) investigated the effect of dark brooders on fear and free-range use in slower growing broiler chickens but found no effect when compared to rearing without brooders. However, slower growing broilers have been found to be less fearful in general (Abdourhamane and Petek, 2022) giving a greater need of reducing fearfulness in fast-growing broilers. In contrast to Stadig et al. (2018), Study II found that rearing broilers with brooders reduced the fearfulness in three different fear tests. This indicates that the brooders could affect fearfulness in fast-growing broilers. Furthermore, Study II found a tendency for a better gait score for broilers reared with brooders. In conclusion, the limited research on artificial brooders in fast-growing broilers makes reliable comparisons difficult and

therefore more studies need to be made to confirm the positive effects found in this study.

5.1.5 Intermittent lighting

The lighting programs used for broiler chickens in production settings vary greatly. In Study III, the focus was to compare the mandatory standards in the EU (18L: 6D where 4 hours of darkness has to be continuous (European Commission, 2007)) in two different settings. When using an intermittent lighting scheme, Study III found an increase in synchronisation and improved resting behaviour as well as decreased fearfulness. This confirms previous suggestions by Malleau et al. (2007) and Schwean-Lardner et al. (2014) that synchronisation of resting behaviour may increase welfare of the broilers. In Study III, the weight of the broilers at slaughter age tended to be higher for birds reared with intermittent lighting, without affecting the FCR. Duve et al. (2011) showed that intermittent lighting of two dark periods of 4h per day resulted in an increase in weight gain compared to using one dark period of 8h daily. Similarly, Renden et al. (1996) also found an increase in weight for birds reared with intermittent lighting (16L:2D:1L:2D:1L:2D), whereas Rahimi et al. (2005) found no increase in body weight but an improved FCR for broilers reared with intermittent lighting (1L:3D). The previous studies of intermittent lighting differ a lot in the used lighting schemes, making it difficult to do a direct comparison, but it may be concluded that changing the lighting scheme can improve resting behaviour and may have the potential to improve the growth and/or feed conversion of broiler chickens.

5.1.6 Clinical welfare indicators

Most of the clinical welfare parameters were collected at the end of each study, following the Welfare Quality[®] protocol (Welfare Quality, 2009). This was done to make the data more comparable to other studies and potential on-farm results in the future. Leg problems and contact dermatitis are two major welfare concerns for broiler chickens (EFSA, 2012). Leg problems and walking difficulties occur as a result of the fast growth rate, the legs are simply not strong enough to hold up the heavy body (EFSA, 2012). Contact dermatitis occurs as a result of poor litter quality and the extremely long time of sitting. Sitting and lying in fast growing broilers

increase with age during day-time from 75% in the first week to 90% at 5 weeks of age (Bessei, 2006). In another study of Study I, broilers reared with elevated platforms showed positive effects on foot pad health but similar walking abilities as the control group, whereas the lower stocking density showed an improvement in walking abilities (Tahamtani et al., 2020). Contact dermatitis often occur when the broilers have extended contact with wet or dirty substrates (Bessei, 2006). The platforms allow the broilers to leave the substrate, which could explain the improvement of foot pad health. The improvement in walking ability when applying a reduced stocking density confirms previous studies (Sørensen et al., 2000; Dawkins, 2004). In Study II, there was a tendency for a better gait score for birds reared with artificial brooders. As gait scores often are explained by activity, it is possible that the brooders increase activity similar to barriers (Bizeray et al., 2002) as they block the view in the pen in a similar manor and thus improve walking abilities. Barriers may increase activity as the chickens have to walk around them (Bizeray et al., 2002). The use of an intermittent lighting scheme in Study III found no differences in walking abilities or contact dermatitis. This is in contrast with previous research that found improvements in walking abilities when using intermittent lighting schemes (reviewed in Buyse et al., 1996). However, those lighting schemes differ a lot from the scheme used in Study III, as many of them use 1-2 hours of light followed by 3-4 hours of darkness throughout the day and compare the results to a continuous lighting scheme with only 1 hour of darkness during the night. This gives an extensive amount of darkness during 24h which could force activity during the light hours for feeding, which might be the reason for the increased walking abilities.

5.1.7 Production parameters

Interesting parameters for producers relate of course to production. In the studies mainly growth and FCR are measured. A fast growth with a good FCR increase the income per animal but are not the only parameters influencing economic results. Most economical losses of animal production relate to diseases as a result of unfavourable conditions (e.g. related to breeding, rearing, hygiene, slaughter or other external factors) (Vetter et al., 2014). The economics of the production system will influence the decision to implement changes that might increase work load, energy consumption, cost of equipment et cetera. Increased costs of implemented changes need to

be compensated by improved economic returns (e.g. by producing more meat because of healthier chickens or less mortality or by being able to sell for a higher retail price). This implies that the increase in animal welfare, but lack of increased body weight of the chickens, identified when using elevated platforms or artificial brooders may still affect economics in the broiler production. However, this needs to be further studied and specifically on farm to conclude an impact on production economics.

5.1.8 Fearfulness

Fear is an aversive emotion, which could have damaging effects and should be avoided to ensure good animal welfare (Jones and Boissy, 2011). Fear may result in adverse reactions including panicked fleeing which could lead to piling up, leading to injuries, pain, and sometimes even suffocation (Jones, 1989; Mills and Faure, 1990). In Study I, broilers reared with elevated platforms showed reduced fearfulness in a Tonic Immobility test (Tahamtani et al., 2018). Study II showed decreased fearfulness in all three fear tests for broilers reared with artificial brooders. Study III showed decreased fearfulness in two out of three fear tests for broilers reared with intermittent lighting. The Novel Object test done in Study III may have been affected by the placement of the object close to the feeders as hunger could have been of such a high motivation that it did override the fear of the object. The Avoidance distance test was done to investigate fear of humans as this has been found to be a potential limiting factor for productivity and welfare (reviewed in Zulkifli, 2013). The broilers reared in the different treatments had the same amount of human contact to avoid confounding effects of human influence. Broiler chickens that had a human present twice daily were less fearful than chickens reared without human contact and also showed a better FCR (Hemsworth et al., 1994). The reduced fearfulness in the studies of this thesis implies that the elevated platforms, artificial brooders and intermittent lighting could improve welfare of broiler chickens.

5.2 Resting behaviour

Resting behaviour of broilers occurs when the individual does not engage in any active behaviour, such as foraging, walking or preening. Resting behaviour makes up a large part of the daily time budget of broilers (Weeks et al., 2000; Cornetto and Estevez, 2001) and increases with age (e.g.

Cornetto and Estevez, 2001; Malleau et al., 2007). Possibly, the heavy body weight of older broiler chickens reduce the motivation to be active, maybe due to discomfort or pain during locomotion, causing a preference for resting during the day (Dawson et al., 2021). Malleau et al. (2007) studied resting behaviour of both layer and broiler chicks reared with different light schemes. For the first 14 days of life, both layer and broiler chicks reared with one dark period of 4 hours and 40 minutes showed similar amounts of resting behaviour, around 50 % of the time during the hours of light. Young animals tend to need more rest and sleep than older animals, possibly due to their growth and development (e.g. rats, Mendelson et al., 1999). This shows that resting is an important part of the behavioural repertoire of young chicks. Brokaw et al. (2016) saw that quiet rest periods can enhance memory, which is especially important for the young chicks during development learning about their environment and how to interact with other individuals. However, apart from sufficient good quality rest, it is also important that the birds are sufficiently active to prevent welfare issues. For instance, broilers' lack of activity has been linked to the prevalence of leg deformities and abnormal bone development (Reiter and Bessei, 1998). Higher activity levels could lead to stronger muscles and thus reduce the risk of getting leg problems due to their heavy body weight (Reiter and Bessei, 1998). In two of the experiments, the chicks had separate resting places away from feed and water. The chicks that preferred to use the resting places for resting needed to move around in the pen to reach feed and water before returning to the resting place. Chicks staying near the feeders/drinkers when resting could avoid moving around. However, staying near the feeders also means resting in the area of most activity which could reduce the quality of the rest and sleep finally leading to reduced welfare.

5.2.1 Motivation to rest

In all the three studies, the duration of activity until the next resting bout started, was taken as a measurement of motivation to continue resting after a resting bout ended. This inter-bout activity was on average less than one minute in all studies, which suggests a high motivation to rest and could explain the general high inactivity in broiler chickens (Weeks et al., 2000; Cornetto and Estevez, 2001). In Study I and II, the duration of activity between resting bouts was less if the preceding resting bout was ended by a disturbance, indicating that there might be a higher motivation to continue

resting when disturbances occur. Thus, the disturbances affect also the activity of the chickens, which in turn are important for several welfare aspects as discussed previously.

5.2.2 Sleep

Sleep and rest are rarely mentioned and considered in the handbooks for broiler production. Neither when welfare issues are discussed in relation to the production settings (EFSA, 2012). Yet, it is known that sleep is indeed important for many functions related to welfare (Krueger and Obal, 2003; Assefa et al., 2015). The broiler chickens could be in especially great need of good quality sleep as younger individuals tend to have a need for more sleep than older individuals (e.g. domesticated species, Arnold 1985; Nicolau et al., 2000; rats, Mendelson & Bergmann, 1999; humans, Feinberg, 1974; Kurth et al., 2010). This thesis shows that broiler chickens experience disturbances during resting which obviously decreases their resting quality. Because of the technical and practical difficulties of studying sleep, conclusions on sleep are approached through the study of resting behaviour of the chickens. It is very likely that disrupted rest, as seen in the studies in this thesis, also lead to disrupted sleep (or sleep fragmentation, Bonnet, 2005) and related welfare issues (e.g. Malleau et al., 2007). Future studies should identify the sleeping patterns, stages and needs of broilers to further improve their welfare.

5.2.3 Disturbances

All studies showed that physical disturbances between individuals are common regardless of which situation was observed. Study I shows that a lot of disturbances occur both during day and night, whereas study II and III only observed behaviour during the day. The birds are expected to be inactive during the darkness as the species are diurnal and only when the dark period gets very long the birds get active to feed (Nielsen et al., 2003). Study I had a higher stocking density than both the other studies and stocking density could affect the proportion of disturbances occurring during the night. As the observations were made at 20 and 34 days of age, the birds had a large body size which reduces the available open space in the pen. Therefore, the birds were resting near other individuals, increasing the risk of physical disturbances as other individuals move. Giving more space such that the birds could rest without physical contact with other individuals could reduce

the risk of disturbances. However, this was not confirmed by Study I, where the lower stocking density (34kg/m²) did not show less disturbances than the higher stocking density (40kg/m²). This is in contrast to other studies that show an increase of disturbances with an increase in stocking density (e.g. Hall, 2001; Cornetto et al., 2002; Dawkins et al., 2004; Ventura et al., 2012), but all had a larger difference in stocking density than Study I. In natural flocks, chickens would gather together for comfort and security provided by the group. This natural tendency may cause chickens, under production circumstances, to avoid large open areas and instead gather near walls for security while resting (Newberry and Hall, 1990; Arnould and Faure, 2003). This results in especially crowded areas near walls regardless of the size of the open areas. The introduction of more of such preferred areas would more evenly distribute the birds. This is shown to be the case when panels are introduced to the central part of the area (Cornetto and Estevez, 2001). The elevated platforms and artificial brooders could be assumed to be such preferred areas for resting as both simulate more natural areas for resting by perching or brooding respectively. Thus, broilers using such areas are expected to do so for the purpose of resting and those areas could be expected to show low activity and therefore less disturbances. As chickens resting on top of the platforms or under the brooders had a reduction in the proportion of disturbances, the separate resting place seem to fulfil its purpose to some extent.

5.2.4 Synchronisation of behaviours

Study III aimed to increase synchronisation of resting behaviour by using an intermittent light scheme. Most species of birds that live in flocks sleep synchronised due to the positive effect of keeping the flock together both during night and day. Chickens are no exception and wild adult birds sleep together perched in the trees (Collias and Collias, 1967) and adult captive chickens sleep together perched at night (Blokhuys, 1984). Additionally, young chicks do tend to rest together as a group at dark periods (Malleau et al., 2007). Blokhuys (1984) observed that adult captive chickens of both junglefowl and layers often rest on the ground during day-time. Young chicks do rest on the ground but can start perching as early as one week of age (Workman and Andrew, 1989). Broiler chickens in intensive production settings do rest on the ground their whole life as elevated structures for resting are seldom provided. Resting in groups on the ground, the broilers

need to be synchronised to avoid disturbances. If not synchronised, active broilers are continuously entering and leaving resting groups and areas, disturbing broilers still resting. The mother would naturally synchronise the behaviour of the chicks (e.g. activity in layers, Riber et al., 2007). However, introducing mother hens for the chicks in broiler production settings would be difficult, options that can simulate some of her influence are available. The use of artificial brooders in Study II showed positive effects on resting behaviour, but disturbances still occurred indicating that there was still a lack of synchronisation in the group. Therefore, the intermittent lighting scheme, simulating the darkness of the brooding of the hen, was tested in Study III and showed positive effects on the synchronisation of resting behaviour but also showed that disturbances still occur. Thus, adapting the lighting scheme seems like an effective solution to synchronise the behaviours, but further studies are needed to perfect the lighting scheme to avoid disturbances.

5.3 Implementation

On farm implementation of the results obtained in the studies vary in complexity. Making structural changes such as introducing elevated platforms or artificial brooders have positive effects on the welfare of the birds but may require more work by the staff. To maintain high standards of biosecurity, the structures need to be easily cleaned and disinfected. Platforms consisting of perforated plastic slats that tolerate the cleaning and that can be moved while cleaning the facility (for instance by being lifted by strings in the ceiling) are one possibility, but also comes with investment by the producer. The artificial brooders could similarly to the platforms, be lifted to the ceiling to ease the routines of cleaning. Depending on how the facilities are heated, the use of brooders could have an energy saving effect since the ambient temperature can be kept low. However, the very small brooders used in Study II are not applicable under practical conditions and need to be replaced by a larger brooder type adapted for use on farm. A practical example of the implementation of structural changes in a broiler facility is the Windstreek broiler house (Windstreek, 2023) in the Netherlands, which originated from a project at Wageningen University (Janssen et al., 2011). The Windstreek broiler facility provides natural ventilation which improves air quality, natural light which provides variation

in light intensity, artificial brooders for heat and comfort, elevated platforms for resting and other environmental enrichment.

Changing the lighting schemes not costly in comparison to structural changes and could easily be implemented. Better adapting the lighting schemes to natural conditions where the mother hen would brood the chicks regularly during the day, can be an easy and cheap alteration that may have a great effect on resting behaviour. Further studies are needed to more precisely define the lighting scheme and its adaptation over the life time of the birds.

The results from the studies in this thesis do indicate that welfare improvements in relation to resting behaviour are feasible. Further studies should focus on the implementation on farm.

5.4 Limitations and future studies

All studies in this thesis were performed in research settings, using small groups of animals for replication purposes. To confirm the results obtained by the experiments in this thesis it would be valuable to do studies on farm with larger flocks.

All studies in this thesis investigated one change of the environment compared to the control setting, limiting the risk of several factors affecting the results. However, none of the investigated environmental changes could fully eliminate disturbances or synchronise the flock. Study I on elevated platforms showed positive effects on the resting behaviour for birds resting on the platforms. Study II with the artificial brooders showed positive effects on resting behaviour for chickens reared with the brooders. Study III with the intermittent light showed positive effects on both resting behaviour and synchronisation when reared with intermittent light. Thus, all three changes to the environment show positive effects for the resting quality. What is missing though is a study where these three factors are combined in innovative ways. The roof of the brooder could for instance be used as an elevated platform, giving the opportunity to use one structure for multiple use. Chicks probably have individual preferences for resting places and providing several choices could possibly increase the use of these resting places and reduce the number of individuals resting in areas of high activity, such as near feeders and drinkers. A simultaneous introduction of an

intermittent light scheme with dimming could further synchronise the chicks, reducing the risk of disturbances during resting even further.

It is suggested here that a combined application of several environmental factors that improve the quality of rest is a promising option to use in broiler production to improve the quality of rest and sleep.

The difficulty to study sleep in large groups of animals caused this thesis to observe resting behaviour. Resting behaviour is important and can give a clue to the quality of sleep as well. However, when using only the behaviour of the broiler chickens it cannot be decided whether the animal is sleeping or just resting. The sleeping positions described by Blokhuis (1984) refer to hens. In the current experiments, many different resting positions of young chicks were observed and the most common position was lying on the side with one leg stretched (Figure 5). However, observing the resting or sleeping behaviour using video recordings from above the pen makes it even more difficult to tell if the chickens are resting or sleeping. The two dozing stages and sleeping position described by Blokhuis (1984) cannot be distinguished from the video recordings obtained in the current experiments. Therefore, for future studies it could be of interest to define actual sleep positions of young chicks reared without a mother hen. Also, because sleep measurement could be a potentially valuable tool in studies to assess animal welfare (Langford and Cockram, 2010), it would be of great interest to further extend knowledge on sleep and disturbance of sleep in broiler production.

6. Conclusions

- The barren environment commonly used in intensive broiler production exposes the birds to great challenges with regard to resting behaviour. Adding structures such as elevated platforms or artificial brooders decrease disturbances and thus enhance the quality of resting.
- Altering the lighting scheme to a more natural like pattern, improves the synchronisation of resting behaviour and reduces physical disturbances which improves the quality of resting.
- Regarding the environmental changes, no negative effects on clinical welfare measurements or production were found in any of the studies, indicating that the improvement of resting behaviour may increase the welfare of the birds while not negatively affecting other welfare indicators or productivity.
- Elevated platforms, artificial brooders and the intermittent lighting scheme all reduced fearfulness of the birds, an important indicator of improved welfare compared to the barren environment.

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Popular science summary

That sleep and rest is important for the welfare of humans is well known. Insufficient or disrupted sleep make us feel tired, losing energy to perform active tasks, reduces our memory and in the long run it reduces our overall health. The same is true for most animals, who need sufficient sleep of good quality to experience good welfare. For captive animals, we are responsible for their welfare and thus also to provide for instance environments that suite the specific species. To provide an environment that fulfil the need of sleep for the animals, one can study the resting behaviour of wild ancestors. For captive chickens, the wild ancestor still lives in the Southeast Asian jungle, the red junglefowl. The red junglefowl live in small mix-sexed groups where the male defend the territory and the hen care for her chicks. Young chicks are brooded by the hen for comfort and heat and she synchronises the behaviour of her chicks. Older chicks and adult chickens rest elevated, perched in trees to avoid the predators on the ground.

In broiler production, the chickens are often reared in an environment with high biosecurity and controllability. However, the environment and conditions are far from similar to natural conditions for the young chicks. Differences include a barren environment, large flocks, high stocking densities, fast growth and a short life span. It has previously been seen that broiler chickens experience a great amount of disturbances while resting. Active individuals physically disturb resting individuals as they share the same area for their behavioural needs. This implies that there are challenges that needs to be addressed in regards to broiler sleep and rest. However, sleep and rest is rarely mentioned and considered in the handbooks for broiler production, nor when welfare issues are discussed in relation to the production settings. This thesis aimed to investigate and extend the limited

knowledge of resting behaviour of broilers as well as the effects of disturbed rest on behaviour and other welfare indicators.

Three different changes to the environment were studied; (Study I) elevated platforms to allow perching, (Study II) artificial brooders to mimic a mother hen and (Study III) an intermittent lighting schedule as a more natural lighting (brooding) pattern. During Study I a lower stocking density was observed in addition to the elevated platforms. All studies had also a control setting where the chickens were reared in the same manors but without the above mentioned change to the environment. Observations of resting behaviour showed a decrease of physical disturbances between chickens and an increase of resting bout duration for chickens resting on top of elevated platforms, reared with artificial brooders or reared with an intermittent lighting scheme. The intermittent lighting also synchronised resting behaviour better. A reduced stocking density had no effect on resting behaviour. Physical disturbances between the chickens were though common in all studies, suggesting that implementing one change to the environment is not enough to achieve undisturbed rest. However, changing the environment to a more natural like situation in terms of resting have positive effects of the chickens' resting quality.

Additionally, the use of elevated platforms, artificial brooders and intermittent lighting also showed a decrease in fearfulness. Fear is an aversive emotion that can cause stress and has been used as a welfare indicator for captive animals. A reduction of fearfulness in broiler chickens is thus positive for their welfare. For the more commonly used welfare measurements in animal production, no negative effects were found on the measures taken on health, production parameters, mortality or litter quality. This implies that adding a structure or changing the light scheme is more important for the behaviour and welfare states of the broilers than their physical attributes.

This thesis show several opportunities to improve the resting quality of broiler chickens. However, since the chickens do still experience disturbances, more is needed to be done to ensure undisturbed rest for broiler chickens in production. Disturbances indicate poor quality resting, and with the vital needs of rest and sleep, makes it a welfare concern. However, altering the broilers' environment, to promote more natural resting patterns, have positive effects on the resting behaviour of the chickens and thus likely also on their sleep, making it a step for the better. A combination of several

alterations of the environment could possibly improve the resting quality and welfare of broilers even further.

Populärvetenskaplig sammanfattning

Att sömn och vila är viktigt för människors välfärd är välkänt. Otillräcklig eller störd sömn gör att vi känner oss trötta, tappar energi för att utföra aktiva uppgifter, reducerar vårt minne och i förlängningen minskar det vår allmänna hälsa. Detsamma gäller för de flesta djur, som behöver tillräckligt med sömn av god kvalitet för att uppleva ett gott välbefinnande. För djur i fångenskap är vi ansvariga för deras välbefinnande och därmed också för att tillhandahålla exempelvis miljöer som passar den specifika arten. För att tillhandahålla en miljö som uppfyller djurens behov av sömn kan man studera vilda förfäders vilobeteende. För höns i fångenskap lever den vilda förfadern fortfarande i den sydostasiatiska djungeln, den röda djungelhönan. De röda djungelhönsen lever i små blandkönade grupper där hanen försvarar reviret och hönan tar hand om sina ungar. Unga kycklingar ruvas av hönan för komfort och värme och hon synkroniserar beteendet hos sina kycklingar. Äldre kycklingar och vuxna kycklingar vilar upphöjt, uppflugna i träd för att undvika rovdjur på marken.

I slaktkycklingproduktion föds kycklingarna ofta upp i en miljö med hög biosäkerhet och kontrollerbarhet. Miljön och förhållandena är dock långt ifrån de naturliga förhållandena för de unga kycklingarna. Skillnaderna inkluderar en karg miljö, stora flockar, hög belägningsgrad, snabb tillväxt och kort livslängd. Man har tidigare sett att slaktkycklingar upplever en stor mängd störningar när de vilar. Aktiva individer stör fysiskt individer som vilar eftersom de delar samma område för deras beteendebestånd. Detta innebär att det finns utmaningar som måste åtgärdas när det gäller slaktkycklingars sömn och vila. Sömn och vila nämns dock sällan och beaktas inte i handböckerna för slaktkycklingproduktion och inte heller när välfärdfrågor diskuteras i relation till produktionsmiljöerna. Denna avhandling syftade till att undersöka och utöka den begränsade kunskapen

om vilobeteende hos slaktkycklingar samt effekterna av störd vila på beteende och andra välfärdsindikatorer.

Tre olika förändringar av miljön studerades; (Studie I) förhöjda plattformar för att tillåta upphöjt sittande, (Studie II) mörka ruvare för att efterlikna en höna och (Studie III) ett intermittent ljusschema som ett mer naturligt belysningsmönster (ruvning). Under studie I observerades också en lägre beläggningsgrad förutom de förhöjda plattformarna. Alla studier hade också en kontroll där kycklingarna föddes upp på samma premisser men utan ovannämnda förändring av miljön. Observationer av vilobeteende visade en minskning av fysiska störningar mellan kycklingarna och en ökning av viloperiodens varaktighet för kycklingar som vilade ovanpå förhöjda plattformar, uppfödda med mörka ruvare eller uppfödda med ett intermittent ljusschema. Intermittent ljus synkroniserade också vilobeteendet bättre. En minskad beläggningsgrad hade ingen effekt på vilobeteendet. Fysiska störningar mellan kycklingarna var dock vanliga i alla studier, vilket tyder på att det inte räcker att genomföra en förändring av miljön för att uppnå ostörd vila. Men att ändra miljön till en mer naturlig liknande situation när det gäller vila har positiva effekter på kycklingarnas vilokvalitet.

Dessutom visade användningen av förhöjda plattformar, mörka ruvare och intermittent ljus också en minskning av rädsla. Rädsla är en aversiv känsla som kan orsaka stress och har använts som en välfärdsindikator för djur i fångenskap. En minskning av rädsla hos slaktkycklingar är således positivt för deras välfärd. För de vanligare använda välfärdsmätningarna inom djurproduktionen konstaterades inga negativa effekter på observationerna av hälsa, produktionsparametrar, dödlighet eller strökvalitet. Detta innebär att det är viktigare att lägga till en struktur eller ändra ljusschemat för slaktkycklingarnas beteende och välfärd än deras fysiska egenskaper.

Denna avhandling visar flera möjligheter att förbättra vilokvaliteten hos slaktkycklingar. Men eftersom kycklingarna fortfarande upplever störningar måste mer göras för att säkerställa ostörd vila för slaktkycklingar i produktionen. Störningar indikerar vila av dålig kvalitet, och med de vitala behoven av vila och sömn gör störningarna det till ett välfärdsproblem. Att förändra slaktkycklingarnas miljö, för att främja mer naturliga vilomönster, har dock positiva effekter på kycklingarnas vilobeteende och därmed sannolikt även på deras sömn, vilket gör det till ett steg i rätt riktning. En

kombination av flera förändringar av miljön skulle möjligen kunna förbättra vilokvaliteten och välfärden för slaktkycklingar ytterligare.

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Disturbance of resting behaviour of broilers under different environmental conditions

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ABSTRACT

Resting behaviours make up a large part of the daily time budget of broilers. However, in intensive broiler production systems disruptions of resting behaviours occur, where resting individuals get disturbed by active individuals. Such interruptions of resting behaviour may negatively affect the welfare of the birds but also disturb important functions of sleep related for instance to restoration and growth. This study investigated if a lower stocking density or the provision of separate resting places in the form of elevated platforms would result in less disruption of resting patterns of fast-growing broilers. Three different treatments were used, one with an elevated platform, one with lowered stocking density and a control setting. A randomized block design was used, consisting of six blocks, giving six to eight replicates of each treatment. Depending on treatment, 422–497 broilers (Ross 308) were kept per pen. Data on duration of resting bouts, occurrence of disturbances and position in pen during resting were collected on days 20 and 34 of age. An overall effect of treatment \times position was found on duration of resting bouts both during day ($P < 0.01$) and night ($P < 0.001$). When resting on platforms the duration of resting bouts during daytime was longer compared to when birds were resting in open areas in the control groups ($P = 0.04$). During night the duration of resting bouts on platforms was longer compared to duration of resting bouts at all other locations in all treatments. In addition, resting position also had an overall effect on proportion of disturbances during day ($P = 0.0018$) and night ($P = 0.0225$). Resting on platforms reduced the number of physical disturbances of resting chickens compared to open areas in the control group both during day ($P_{\text{adj}} < 0.001$) and night ($P_{\text{adj}} = 0.01$).

Generally, the level of disturbances was high in all treatments, suggesting that birds experience disrupted rest. As rest and sleep are vital needs, it is a welfare concern that chickens negatively affect other individuals' resting behaviour. A separate resting place appears to reduce disturbances to some extent and thus potentially increases the welfare of broiler chickens. However, provision of platforms is not enough to prevent frequent disturbances of resting and it is concluded that additional changes in housing conditions of broiler chickens are needed to improve their rest and sleep.

1. Introduction

Broiler production raises various welfare issues, e.g. health problems, impaired locomotion and behavioural restrictions (EFSA Panel on Animal Health and Welfare (AHAW), 2012). Often neglected behaviours in this context are rest and sleep. Rest may be defined as a prolonged period of inactivity that can clearly be distinguished from other maintenance behaviours (Blokhuis, 1984). Sleep is a specific state of rest with altered consciousness, reduced responsiveness to external stimuli and homeostatic regulation (Carskadon & Dement, 2005). The conservation

of rest and sleep across all mammals and birds suggests that it serves a vital function. Suggested functions include: tissue restoration and growth, energy conservation, neurobehavioral and neurocognitive performance, memory processing and learning and increased waste clearance in the brain (Carskadon & Dement 2005; Siegel 2005; Assefa et al., 2015). These functions, and the notion that sleep deprivation leads to a strong need for sleeping (with associated feelings of distress in humans, likely also in animals) underline the importance of sufficient rest and sleep. Both the quantity, as in duration, and quality, as without disturbances, of rest and sleep are important. A certain duration of

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undisturbed sleep is needed to acquire both deep sleep and Rapid Eye Movement (REM) sleep, which together serve the vital function of sleep (Assefa et al., 2015). In addition to being a welfare problem, disturbance of sleep may also affect productivity in farm animals (e.g. less growth, increased sickness and possibly death) and thus profitability (Rial et al., 2007; Assefa et al., 2015).

Under natural conditions where a mother hen is present, chicks have regular rest periods throughout the day. The rest periods are induced through the periodic brooding of the hen (Shimmura et al., 2010). This behaviour results in a highly synchronised pattern of undisturbed resting behaviour (Roden & Wechsler, 1998). One of the most important factors affecting sleep and its quality in practical poultry husbandry is the duration and pattern of dark periods (Schwean-Lardner et al., 2012). This may be specifically relevant for young chicks since the light/dark schedules applied in practice differ substantially from the pattern induced by natural darkness as well as the brooding pattern of the mother hen. Modern broiler chickens are generally kept under continuous lighting with one dark period (1-6 hours). In the EU the maximum allowed light period is 18 hours a day (European Commission, 2007). For up to the first seven days of age and the last three days of life 24 hours of light a day is allowed (European Commission, 2007). These lighting conditions may result in loss of synchronisation of activity causing active and resting birds to share the same areas, leading to a high risk of birds physically disturbing resting conspecifics. A high stocking density may contribute to such disturbances as more birds share the same area. Indeed, under such circumstances frequent disturbances of resting behaviour are seen (Yngvesson et al., 2017), which, especially in young birds, may lead to sleep disturbance and sleep deprivation. Such disturbed sleeping patterns have been termed sleep fragmentation (Bonnet, 2005) and such disrupted rest has a negative effect on welfare in several ways and species (Malleau et al., 2007; Abou-Ismaïl et al., 2008; Opp & Krueger, 2015). In broilers, disrupted rest can negatively affect behavioural expressions (Schwean-Lardner et al., 2012) and cause welfare problems such as frustration (defined as an aversive state arising when animals are prevented from performing behaviour that they are strongly motivated to perform (Fraser, 2008)). Moreover, vital functions of sleep may be disturbed resulting in other (as yet unknown) welfare issues as in humans (Medic et al., 2017).

Apart from stocking density and lighting schedule, resting may also be affected by the structure of the housing. Chickens have previously shown to avoid open areas and instead gather along walls to rest when kept in a barren environment (Buijs et al., 2010). The provision of functional areas for active behaviours such as eating, drinking and dustbathing that are structurally separated from areas for resting may support undisturbed resting. A possibility to achieve this is the provision of elevated resting places where the risk of active birds disturbing resting birds is thought to be reduced. For example, Yngvesson et al. (2018) showed that broilers resting on perches were less physically disturbed by other individuals. Similarly, laying hens often rest on elevated structures such as perches as they have a natural motivation to rest above ground level (Olsson & Keeling, 2000; Olsson & Keeling, 2002). However, fast-growing broilers tend to not use perches due to their heavy weight (e.g. Yngvesson et al. (2018)), and would thus instead need something sturdier, like a platform.

In the present study, the aim was to investigate how resting behaviour and disturbances of fast-growing broilers were affected by providing platforms or by reducing the stocking density. We hypothesised that broilers kept in an environment with elevated platforms will get better rest qualitatively than broilers kept in a barren environment and that reducing the stocking density will reduce disturbances. We expected that the frequency of disturbances would increase with the bird's age as they take up more space.

2. Materials and Methods

2.1. Animals and Housing

This experiment was conducted at AU Foulum, Aarhus University, Denmark. In the building, two identical rooms (10.7 m x 16.6 m) were equipped with five pens (10 pens in total) of 3.1 m x 9.6 m (29.8m²). The pens were separated by 60 cm high dark brown panels and the floor was covered with a four-centimetre layer of wood shavings.

Mixed-sex Ross 308 broilers were delivered as day-old from a commercial hatchery (DanHatch A/S, Sønderborg, DK) to the research facilities. At delivery, the chicks were randomly divided over the pens and raised under commercial-like management practices. Commercial conditions were simulated by keeping the stocking density at an expected 40 kg/m² at slaughter age. Water was provided *ad libitum* by nipple drinkers (11.7 broilers/nipple) and feed was provided in round feeders (1.61 cm feeder space per bird). Birds were fed a recommended commercial diet *ad libitum* (feed company DLG, Tjele, DK). At one day of age, the light schedule was programmed for 23L: 1D. Subsequently, the dark period was gradually increased to 6 h on day 6 of age (18L: 6D) and maintained until the end of the experiment (the light was on 04:30-22:30). The light intensity was 27 lux at animal level. A standard temperature programme was followed, starting at 34 °C on day 0 and gradually decreased to reach 20 °C at 28 days and to the end of the growing period.

All flocks were slaughtered at 35 days of age. Further description of the animals and housing can be found in Tahamtani et al. (2018).

2.2. Experimental design

Three treatments were used in the present investigation, i.e. treatment EP consisting of an elevated platform (L x W x H: 5.40 m x 0.60 m x 0.30 m, stocking density at 40 kg/m²), treatment SD consisting of one type of manipulation of the environment (low stocking density at 34 kg/m²) and control C (no platform, stocking density at 40 kg/m²). In the treatment EP, two access ramps at an incline of 14.5 ° were provided to ease the access. Both platforms and access ramps consisted of perforated plastic slats. The area underneath the platforms and ramps was fenced off and not accessible to the birds.

Depending on treatment, a flock of 422-497 broilers was housed in each pen. The number of drinking nipples and feeding space per bird was controlled to account for differences in flock size per experimental group to preclude any confounding effects due to differences in competition for resources. The treatments were arranged in a randomized block design, consisting of six blocks, where the first started up in September 2016 and the last was completed in July 2017. The treatments were balanced between the two rooms, in order to account for any confounding effects of rooms. Each block consisted of one replicate of each treatment (also including treatments not used in this study and an additional treatment replicate). For further details see Tahamtani et al. (2018). In total, the study contained six replicates of treatment EP, six replicates of treatment SD and eight replicates of treatment C. A minor flooding during block 1 resulted in the exclusion of observations in a control group and a platform group.

2.3. Data collection

Four cameras (CCTV Camera, D1325) were placed above each pen, facing directly downwards, for an overview of the whole pen. Data were collected on days 20 and 34 of age from the video recordings using focal animal sampling. Each pen was observed two times per observation day (days 20 and 34), at night from 00:30-02:30 h and at noon from 11:30-13:30 h. On the videos, each part of the pen (covered by one camera) was divided into nine imaginary squares of equal size. Two to three individuals per part of the pen was followed during each observation period, giving a total of ten individuals per pen and observation period.

Focal animals were chosen as individuals starting to rest (defined as lying with a leg to the side or sitting with the legs under the body while not engaging in any other activities), chosen in a randomised square of the pen (randomisation through a given list of numbers between one and nine). The focal animals were followed during a complete resting bout where the length of each resting bout as well as the occurrence of disturbances (defined as physical disturbances by other individuals, causing the focal animal to change position or become active) were registered. In addition, it was registered whether the focal animal was 1) "on the platform", defined as being situated on the platform or ramp (only treatment EP), 2) close to a wall, defined as being within one bird length from a wall or 3) elsewhere in the pen.

2.4. Statistical analysis

Statistical analyses were performed using R (R version 3.6.1, R Core Team, 2019). All data were analysed separately for the period of the day (day/night). The effect of disturbances on duration of resting bouts and duration of activity between resting bouts were examined using a t-test. The data were analysed separately for the age (20/34 days of age). After a logarithmic transformation of the duration data, the data adhered to normal distribution and homogeneity of variances.

The effect of treatment, position in the pen and their interaction on the proportion of disturbances was examined using a Chi-squared test. A second Chi-squared test was used to test the change of proportion of disturbances between ages (20/34 days). The explanatory factors used in this model was the treatments, position in the pen and age, and the interactions between the explanatory factors were included.

An ANOVA test was used to compare the duration of resting bouts between the treatment groups. The explanatory factors used in this model was the treatments, position in the pen and age and the random factor used was the pen. The interactions between the explanatory factors were also included. A similar model was used in another ANOVA test to compare the duration of activity between resting bouts during the day between treatment groups. After a logarithmic transformation of the duration data, the data adhered to normal distribution and homogeneity of variances. Both in the Chi-squared test and ANOVA tests, *post hoc* comparisons of significant factors were performed using Pairwise Nominal Independence or Tukey's test (Tukey's HSD test).

2.5. Ethical statement

The experiment was carried out according to the guidelines of the Danish Animal Experiments Inspectorate with respect to animal experimentation and care of animals under study.

3. Results

3.1. Duration of resting bouts and activity between resting bouts on disturbances

At 20 days of age, the duration of resting bouts during the night decreased if the resting bout ended due to disturbance (Disturbance 279.68 ± 41.02 s; No disturbance 345.10 ± 40.12 s, $df = 175.28$, $t = 2.831$, $p = 0.005$), but no effect was found during the day (Disturbance 80.99 ± 8.78 s; No disturbance 112.53 ± 9.69 s, $df = 171.27$, $t = 0.051$, $p = 0.96$). At 34 days of age, the duration of resting bouts was not affected by whether the resting bouts were ended due to disturbance during the day (Disturbance 89.01 ± 8.47 s; No disturbance 112.87 ± 12.80 s, $df = 136.27$, $t = 0.457$, $p = 0.65$), but it tended to decrease if the birds were disturbed during the night (Disturbance 194.60 ± 21.86 s; No disturbance 225.24 ± 20.23 s, $df = 177.99$, $t = 1.905$, $p = 0.058$).

At both 20 and 34 days of age, the duration of activity between resting bouts during the day decreased if the preceding resting bout had been ended due to disturbance (20 days of age, Disturbance

12.26 ± 2.10 s; No disturbance 27.45 ± 6.21 s, $df = 167.98$, $t = 2.014$, $p = 0.046$; 34 days of age, Disturbance 12.97 ± 2.56 s; No disturbance 21.12 ± 3.52 s, $df = 152.98$, $t = 2.664$, $p = 0.009$) but no effect was found during the night (20 days of age, Disturbance 5.83 ± 0.69 s; No disturbance 7.29 ± 1.19 s, $df = 172.92$, $t = 0.358$, $p = 0.72$; 34 days of age Disturbance 5.02 ± 0.75 s; No disturbance 7.38 ± 0.97 s, $df = 177.98$, $t = 0.813$, $p = 0.42$).

3.2. Proportion of resting bouts disturbed

The percentage of broilers being physically disturbed during resting by other birds during the day and night, respectively, was ranging from about 30% to about 77%, depending on treatment and the position in the pen (Table 1). The proportion of disturbances was lower on platforms in treatment EP than in open areas in treatment C both during day ($p_{adj} < 0.001$) and night ($p_{adj} = 0.010$). During night, resting on platforms in treatment EP showed lower proportions of disturbances than resting near walls in treatment LD ($p_{adj} = 0.016$). None of the other positions in the pen differed in any treatments.

Age affected the proportion of disturbances; in some positions in the pen it increased with age whereas in others it decreased (Table 2). Comparing the proportion of disturbances during the day between ages, there is a difference between age 20 and 34 (Table 2), where the increase with age in disturbances on platforms in treatment EP differ from the decrease with age in open areas in treatment EP ($p_{adj} = 0.03$) and along walls in treatment LD ($p_{adj} = 0.03$). Also, the increase with age in open areas in treatment LD differ from the decrease with age in open areas in treatment EP ($p_{adj} = 0.02$) and along walls in treatment C ($p_{adj} = 0.04$) and treatment LD ($p_{adj} = 0.02$). Comparing the proportion of disturbances during the night between ages, there is a difference between age 20 and 34 (Table 2), where the decrease with age in disturbances in open areas in treatment EP differ from the increase with age on the platform in treatment EP ($p = 0.002$) and open areas in treatment C ($p = 0.002$) and treatment LD ($p = 0.001$).

3.3. Duration of resting bouts

During the day, there were differences in the duration of resting bouts between positions within and between treatments (Table 3). Specifically, the resting bouts were longer on platforms in the treatment EP than in open areas in treatment C (Tukey's test $p = 0.04$) and longer near walls in treatment LD than in open areas in treatment C (Tukey's test $p < 0.001$) (Table 3). No differences between the other treatments and resting positions were found during the day. There was no effect of age on the duration of resting bouts during the day ($df = 1$, $F = 0.071$, $p = 0.789$).

During the night, there were differences in the duration of resting bouts between positions within and between treatments (Table 3). Specifically, the resting bouts were longer on platforms in the treatment EP than in the other resting positions in all treatments. No differences were found between the other resting positions in any of the treatments. In the analysis of duration of resting bouts, none of the other interactions were significant. There was an effect of age on the duration of resting bouts during the night ($df = 1$, $F = 5.472$, $p = 0.0199$) where the resting bouts were on average longer for younger birds (20 vs. 34 days: 314.57 ± 28.74 s vs. 211.11 ± 14.85 s).

3.4. Duration of activity between resting bouts

No effect of treatment was found in the duration of activity between resting bouts during the day (Table 3), nor in any other factors or interactions of factors.

4. Discussion

This study as well as earlier research (e.g. Yngvevsson et al., 2018)

Table 1

Proportion of resting bouts where the resting bird is disturbed by companions shown separately for day and night and divided into occurrences in different treatments and positions in the pen. The statistical values indicated are for the interactions 'treatment × position in the pens'.

| | Treatment | | | | | | | Df | X-square | p-value |
|--|--------------------|--------|--------|---------|--------|-------------|--------|----|----------|---------|
| | Elevated platforms | | | Control | | Low density | | | | |
| | Platform | Open | Wall | Open | Wall | Open | Wall | | | |
| Proportion disturbed by other birds during day | 0.40a | 0.46ab | 0.64ab | 0.77b | 0.58ab | 0.54ab | 0.56ab | 6 | 21.0 | 0.0018 |
| Proportion disturbed by other birds during night | 0.30a | 0.43ab | 0.48ab | 0.55b | 0.52ab | 0.32ab | 0.56b | 6 | 14.7 | 0.0225 |

Different letters within period of the day indicate significant differences

showed that resting in broiler chickens is regularly disturbed by active conspecifics resulting in a fragmentation of this behaviour. As described earlier, this may affect the quality of sleep and can cause sleep deprivation (Bonnet, 2005), which may deteriorate important functions of sleep related to for instance neurobehavioral and neurocognitive performance, memory processing and learning and increased waste clearance in the brain tissue. This in turn can negatively affect behavioural expressions and cause stress and welfare problems (Schwean-Lardner et al. 2012). Moreover, a disturbed quality of sleep may affect tissue restoration and growth and energy conservation with impact on production efficiency and thereby on profitability. To be able to draw conclusions on sleep quality, further physiological characterisation of rest and sleep under optimal conditions would be needed but observation of disturbances of resting behaviour like in the present study allows the identification of causal factors and remediate measures.

In the present study there were in general a lot of physical disturbances causing individual birds to end resting bouts. Disturbances were common in all treatments and situations. Thus, both during day and night, at least 30% of the observed birds resting on top of a platform were disturbed by other birds, while up to 77% in an open area in the control group were disturbed. Similarly, in a study by Yngvesson et al. (2018), 53% of the focal birds were disturbed during resting at day 45, during daytime, again suggesting that physical disturbances are common. Platforms were suggested to be a separate resting place where birds could go to rest, away from active birds. Platforms have been shown to be a better solution than perches to the heavy body weight of broilers (Norrington et al., 2016) and broiler breeders (Gebhardt-Henrich et al., 2017, 2018). As platforms at least partially can be used to satisfy the perching motivation and as the number of disturbances was lower on the platforms, they seem to some extent to fulfil the intended purpose of a separate resting place.

In this study, the duration of resting bouts was also used to indicate the quality of rest. From other species it is known that a certain period of undisturbed sleep is necessary to reach specific sleep stages like Rapid Eye Movement (REM) sleep (e.g. rats, Trachsel et al., 1991; Frank & Heller 1997; humans, Carskadon & Dement 2005; zebra finches, Low et al., 2008). Poultry also shows REM-like sleeping patterns (Van Luijckelaar et al., 1987) and a longer period of undisturbed sleep is therefore likely to be important for chickens as well. We observed resting bouts of up to ten minutes, but most bouts only lasted a couple of minutes. The bouts ended when the bird became active, changed position or were disturbed by another individual. The duration of the resting bouts showed the longest duration in the EP treatment, specifically resting performed on a platform. However, the duration of resting bouts did not depend on whether they were ended by a disturbance or not, except during the night at 20 days of age. We had expected to find that disturbances cause shorter resting bouts but the reasons for our results are unclear. Further studies on duration of resting bouts combined with physiological measures of sleep quality are needed to determine the duration necessary to achieve a good quality of sleep.

In this study, a lower stocking density (34 kg/m²) was applied to examine if available space as such would affect disturbances. The expectation was that with additional space birds can move about with less physical contact resulting in less disturbances. However, no effect

on the number of disturbances was found although during daytime the resting bouts were longer near walls compared to the open area in the control treatment. Dawkins et al. (2004) showed that the disturbances increased with stocking densities where differences were found between a stocking density of 30 kg/m² and 42 kg/m² or higher. Also, earlier studies have shown an increase in disturbances with an increase in stocking density (Hall 2001; Cornetto et al., 2002; Ventura et al., 2012). Possibly, the difference in stocking density in this study was insufficient for an effect to be found.

If restricted space is a main factor in causing disturbances, one would also expect disturbances to increase with age as the birds take up more space. However, we did not find such an effect of age. The proportion of disturbances increased with age in some resting positions (e.g. on the platform in EP, in open areas in C and LD) within treatments but decreased in others (e.g. open areas in EP, near walls in C and LD). As the walls are a preferred resting place for broilers (Arnould et al., 2001; Buijs et al., 2010) but are a limited resource, the number of birds that fit near walls decrease with size and thus age. In our study, the first observations were made at 20 days of age and the birds might have reached such a high level of disturbances that it will not increase further with age. On the other hand, open areas are less preferred, probably due to lack of cover (Newberry & Shackleton, 1997), but with the increase in size of the birds they fill up more of the open space and therefore there is an increase in disturbances with age. The platforms seemed to be a preferred resting place due to the motivation to rest at an elevated area and with an increase in the size of the birds the competition for this space increases, which might explain why we see an increase in disturbances with age.

We also found an effect of age in the duration of resting bouts, specifically during the night, where younger birds had longer resting bouts than older birds. This has not yet been studied in broilers, but in general, younger individuals tend to have a need for more sleep than older individuals (e.g. domesticated species, Arnold 1985; Nicolau et al., 2000; rats, Mendelson & Bergmann 1999; humans, Feinberg 1974; Kurth et al., 2010).

To get an impression of the motivation to rest, and thereby of the impact of disturbances, the duration of activity between two resting bouts was observed. It was expected that when a resting bout was followed by a short phase of activity, the bird had high motivation to continue resting. In human infants the resting cycles seem to be mostly regulated by hunger and disrupted rest often results in a continuation of resting (Goodlin-Jones et al., 2000). In the current study, the average time the birds spent active was a few seconds, in all treatments. However, during the day the activity following a resting bout was shorter if the bird was disturbed. This might show a motivation to continue resting and that the resting is indeed disrupted. We could not find this link between disturbances and activity during night which might depend on the general low level of activity of broilers in darkness (Norrington et al., 2016).

A main reason for the high frequency of disturbances in all treatments is likely the lack of behavioural synchronisation. When resting, chickens seek each other's company and since they are not synchronised birds are continuously entering and leaving resting groups and areas, disturbing birds still resting. During dark periods chicks tend to rest

Table 2
Proportion of resting bouts where the resting bird is disturbed by companions shown separately for age 20 and age 34 and divided into occurrences in different treatments and positions in the pen. The statistical values indicated are for the interactions Treatment × position in the pens × age.

| | Treatment | | | | | | | | | | | | df | X-square | p-value | | |
|---------------------|--------------------|--------|--------|----------|--------|----------|-------------|----------|--------|---------|--------|--------|------|----------|---------|------|--------|
| | Elevated platforms | | | Control | | | Low density | | | Wall | | | | | | | |
| | Platform | | Open | Wall | | Open | Wall | | Open | | Wall | | | | | | |
| | Age 20 | Age 34 | Age 20 | Age 34 | Age 20 | Age 34 | Age 20 | Age 34 | Age 20 | Age 34 | Age 20 | Age 34 | | | | | |
| Day | 0.29 | 0.50 | 0.50 | 0.42 | 0.57 | 0.71 | 0.75 | 0.79 | 0.60 | 0.56 | 0.40 | 0.68 | 0.60 | 0.52 | 6 | 13.1 | 0.041 |
| Change during day | +0.21ac | | | -0.08bcd | | +0.14ace | | -0.04ade | | -0.04ac | | +0.28e | | -0.08bd | | | |
| Night | 0.23 | 0.36 | 0.60 | 0.23 | 0.54 | 0.42 | 0.50 | 0.60 | 0.55 | 0.49 | 0.24 | 0.40 | 0.60 | 0.52 | 6 | 25.8 | <0.001 |
| Change during night | +0.13a | | | -0.37b | | -0.12ab | | +0.10a | | -0.06ab | | +0.16a | | -0.08ab | | | |

Different letters within the change during period of the day indicate significant differences

Table 3
Duration (s) of resting bouts during day and night, respectively, and duration of activity between resting bouts during the day (mean ± SE). The statistical values listed are for the interaction treatment × positions in pen.

| | Treatment | | | | | | | | | | | | df | F | p-value |
|---|--------------------|-----------------|-----------------|-----------------|------------------|-----------------|-----------------|---|-------|--------|------|--|----|---|---------|
| | Elevated platforms | | | Control | | | Low density | | | Wall | | | | | |
| | Platform | | Open | Wall | | Open | Wall | | Open | | Wall | | | | |
| Resting bout during day | 116.13 ± 17.30a | 66.29 ± 11.15ab | 99.29 ± 17.30ab | 60.85 ± 5.95b | 107.63 ± 10.83ab | 98.10 ± 11.15ab | 127.88 ± 15.88a | 4 | 4.144 | <0.01 | | | | | |
| Resting bout during night | 500.64 ± 66.96a | 207.04 ± 44.24b | 240.36 ± 63.58b | 247.90 ± 34.91b | 167.77 ± 23.54b | 261.58 ± 38.84b | 257.48 ± 32.43b | 4 | 7.443 | <0.001 | | | | | |
| Activity between resting bouts during day | 11.07 ± 1.92a | 18.54 ± 6.25a | 15.34 ± 4.44a | 12.38 ± 2.25a | 11.03 ± 1.93a | 13.99 ± 3.15a | 10.11 ± 1.47a | 4 | 0.959 | 0.429 | | | | | |

Different letters within parameter indicate significant differences

together as a group (Malleau et al., 2007). Also, commercial rearing of chicks does not include broody hens. Without a broody hen, layer chicks are less synchronised in activity (Riber et al., 2007). Improved synchronisation of behavioural patterns may further reduce disturbances, possibly in combination with platforms and lower stocking densities.

4.1. Conclusion

The results of this study showed that broiler chickens experience difficulties in achieving undisturbed rest. The introduction of elevated platforms provided an opportunity for somewhat better rest, if the rest took place on a platform. Lowering the stocking density to 34 kg/m² did not affect the frequency of disturbances, but it did increase the duration of resting bouts during daytime. Disrupted rest was common in all situations suggesting that more measures than adding an elevated platform or a reduction in density from 40 to 34 kg/m² are needed to further reduce disturbances and thus increase welfare of the birds. Increased synchronisation of behavioural patterns could possibly be such a measure that further reduces disturbance, but more research is needed to determine how to induce it in broiler flocks and to evaluate its potential effects on quality of sleep.

Declarations of interest: none.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Resting behavior of broilers reared with or without artificial brooders

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Rest and sleep are important for the welfare of mammals and birds. A large part of the daily time budget of broiler chickens is taken up by resting behavior and the quality of resting is important. However, in intensive broiler production systems, disruptions of resting behaviors are common. These disruptions of resting behavior could be negative for the health and growth of the birds. This study investigated if artificial brooders that provide a delimited and darker resting place, away from active birds, reduce disruptions of resting behavior compared to a control situation without artificial brooders. Six pens of each treatment were used in the same building, keeping 60 chickens (Ross 308) per pen. The artificial brooders were removed at 21 days of age. Data on disturbances and duration of resting bouts and activity between resting bouts were collected on 20 and 34 days of age. Also, as an indicator of the quality of rest, the animals' cognitive performance was evaluated in a spatial learning test that was performed at 11 days of age. The results showed that birds housed in pens with access to brooders have longer resting bouts (260.7 ± 5.2 vs. 132.8 ± 5.3 s, $p < 0.001$) and are less likely to be disturbed during resting by other individuals (0.15 vs. 0.48 , $p < 0.001$). The effect of the artificial brooders on both the duration of resting bouts and the proportion of disturbances remained after the removal of the brooders at 21 days of age. The duration of activity between resting bouts was shorter if the resting bout was ended by a disturbance (9.98 ± 1.0 vs. 61.0 ± 2.4 s, $p < 0.001$). Birds reared with brooders were more likely to solve the spatial learning task (0.5 vs. 0.27 , $p < 0.01$), but those succeeding were not faster at solving it. Broilers may be exposed to disrupted rest due to the lack of a dedicated resting place separated from areas with high activity. Using artificial brooders reduces disturbances but does not eliminate them. Therefore, additional changes to the housing conditions or management will be needed to prevent disturbances.

KEYWORDS

broiler, resting behavior, artificial brooder, disturbance, sleep

Introduction

Rest and sleep are vital for the welfare of mammals and birds but are rarely considered in broiler production systems. Rest can be defined as a period of inactivity without any maintenance behaviors occurring, whereas sleep is a specific state of inactivity with altered consciousness and reduced responsiveness (1). Sleep cannot be distinguished from rest if only using behavioral measurements. Instead, an electroencephalogram (EEG) measuring brain activity is needed. Suggested functions of sleep include tissue restoration and growth, energy conservation, neurobehavioral and neurocognitive performance, memory processing, learning, and waste clearance of the brain (1–3). A certain duration of undisturbed sleep is needed to acquire both deep sleep and Rapid Eye Movement (REM) sleep, which together serves the vital function of sleep (3). In addition to being a welfare problem, disturbance of sleep may also affect productivity in farm animals (e.g., less growth, increased sickness, and possibly death) and thus profitability (3, 4).

Under natural conditions, a mother hen would influence the chicks' behavior to have regular resting periods throughout the day. Periodic brooding of the hen induces regular resting periods (5), but it also provides a heated dark area to rest under. The darkness provided by the mother hen, as well as the natural daily rhythm of brooding (6), differ a lot from the light/dark schedules used in broiler production systems which often consist of a long continuous light period with one dark period (1–6 h) each day. Schwan-Lardner et al. (7) showed that the duration of dark periods in poultry production systems is an important factor affecting rest. Specifically, longer periods of darkness decrease the duration of resting periods during light hours. Disturbances occur to a higher extent during the light phase than during the dark period (8) for which reason it may be expected that having dark periods during the day could possibly reduce the prevalence of disturbances. Using artificial brooders, that provide a dark and warm shelter to rest under, could attract chickens and motivate them to rest even during the day.

Apart from the lighting schedule, rest and sleep may also be affected by the design of the housing. Open areas have previously been shown to be avoided by chickens and instead, chickens gather along walls to rest when kept in a barren environment (9). The provision of functional areas for active behaviors such as eating, drinking, and dustbathing that are structurally separated from areas for resting may support undisturbed resting. A possibility to achieve this is the provision of elevated resting places. Chickens have an innate motivation to rest in elevated places, such as branches, but broiler chickens rarely use perches (10) probably due to their heavy body weight. Another elevated structure used for broilers is platforms, which are seen to be used from an early age, at least from 6 days of age (11). However, although elevated platforms reduce disturbances of rest in broilers to some extent

they are not the ultimate solution to prevent disturbances as disturbances still occur (8). Here, we focus on artificial brooders as a measure to reduce the risk of active birds disturbing resting birds.

Artificial brooders have previously been used to separate active chicks from resting chicks, with the aim of reducing feather pecking in layer pullets and hens (12, 13). Riber (14) argued that artificial brooders may improve behavioral synchronization, specifically for inactive behaviors. Sleep in birds is associated with species-specific behaviors and may be triggered by specific environmental releasers or innate behaviors (15). For broiler chickens, a broody hen can be such a trigger as the chicks seek shelter under the hen for resting. This does not differ from layer chicks, but older domestic fowl rest in elevated places. Thus, an artificial brooder could possibly work as a replacement for a broody hen, allowing sleep patterns that are more like natural sleep patterns for chickens.

A barren environment, high stocking densities, and large flocks are commonly used in broiler production and can result in disturbances of rest and sleep. Disrupted sleep could lead to sleep fragmentation, which may lead to changes in cognitive function, including poor memory and difficulties in concentration (16, 17). Tartar et al. (18) show that rats learned the location of a platform in a water maze, but for rats having fragmented sleep the distance of swimming was longer indicating poorer memory indicating that sleep fragmentation negatively impacts spatial learning. Therefore, a spatial learning task may be a good indicator of sleep fragmentation, although never previously investigated in chickens.

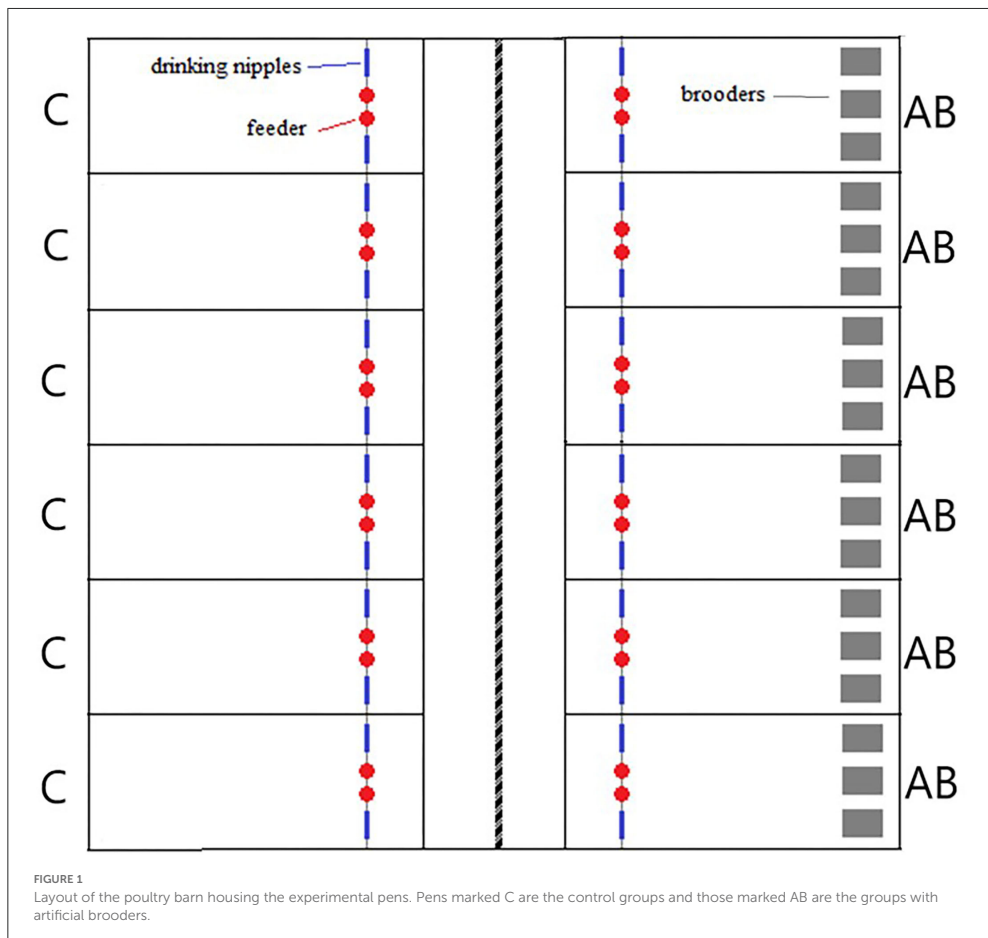
In the present study, the aim was to investigate how resting behavior, including disturbances of rest, in fast-growing broilers, is affected by providing artificial brooders. We hypothesized that artificial brooders will improve broilers' quality of rest and that this would result in better performance in a spatial learning task. We expected that the frequency of disturbances would increase with the bird's age as they take up more space, resulting in conditions that are more crowded.

Materials and methods

Animals and housing

This experiment was conducted at Lövsta Research Center, Swedish University of Agricultural Sciences, Uppsala, Sweden. In the building, one room was divided by a movable wall into two identical sections (6 m × 30 m) which were each equipped with six pens (12 pens in total) of 2 m × 3.5 m (7 m²; Figure 1). The pens were separated by 60 cm high wired fences and the floor was covered with a 4 cm layer of wood shavings.

A total of 720 Mixed-sex Ross 308 broilers were picked up as day-old from a commercial hatchery (Swehatch AB,



Väderstad, SWE) and transported by car to the research facilities. Upon arrival, the chicks were randomly allocated into the pens, resulting in 60 chicks per pen. In one section, the ambient temperature was kept according to commercial practices with a starting temperature of 34°C and gradually decreased to reach 20°C at 21 days and to the end of the growing period. The other section kept an ambient temperature of 20°C throughout the entire growing period and in each of these pens, three artificial brooders (40 cm × 60 cm, vidaXL) were provided with a starting temperature of 34°C measured on the floor. The temperature of the artificial brooders was gradually decreased to reach 28°C at 21 days of age and the artificial brooders were then removed from the pens. The stocking density was

kept at an expected 20 kg/m² at slaughter age to give room for different resting place opportunities. Water was provided *ad libitum* by nipple drinkers (10 broilers/nipple) and feed was provided in round feeders (2 cm feeder space per bird). Birds were fed a recommended commercial grower diet *ad libitum* (feed company Lantmännen, SWE). At 1 day of age, the light schedule was programmed for 24L: 0D. Subsequently, the dark period was gradually increased to 6h on day 6 of age (18L: 6D) and maintained until the end of the experiment (the light was on 04:30–22:30). No daylight was provided. The light intensity was 27 lux at the animal level and 0–2 lux under the brooders. The study ended at 35 days of age, when the birds were slaughtered.

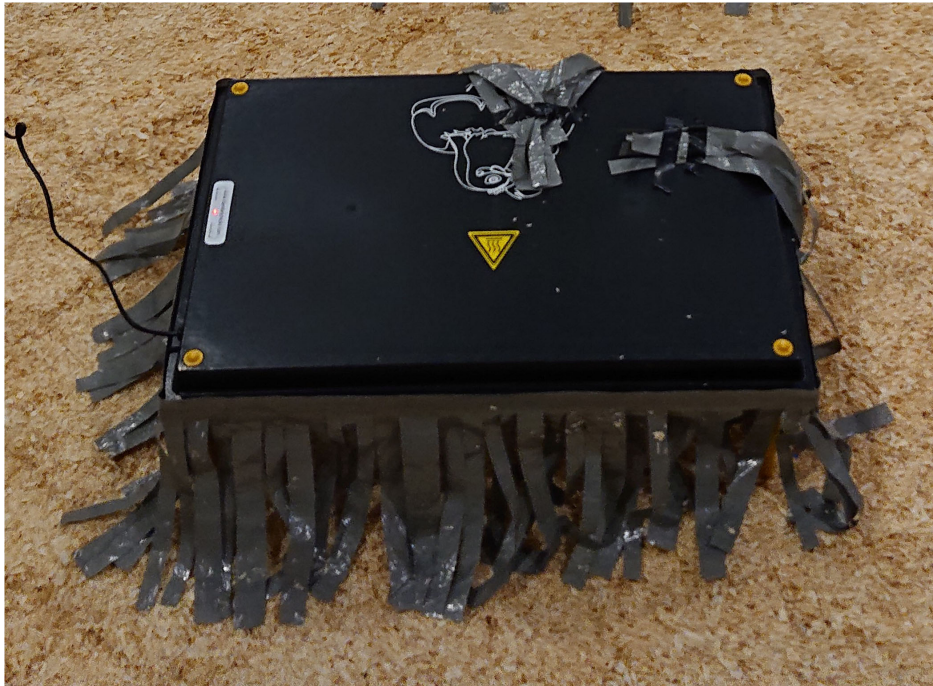


FIGURE 2
Artificial brooder with flaps of tarp. The height of the brooders was adjusted during the experiment.

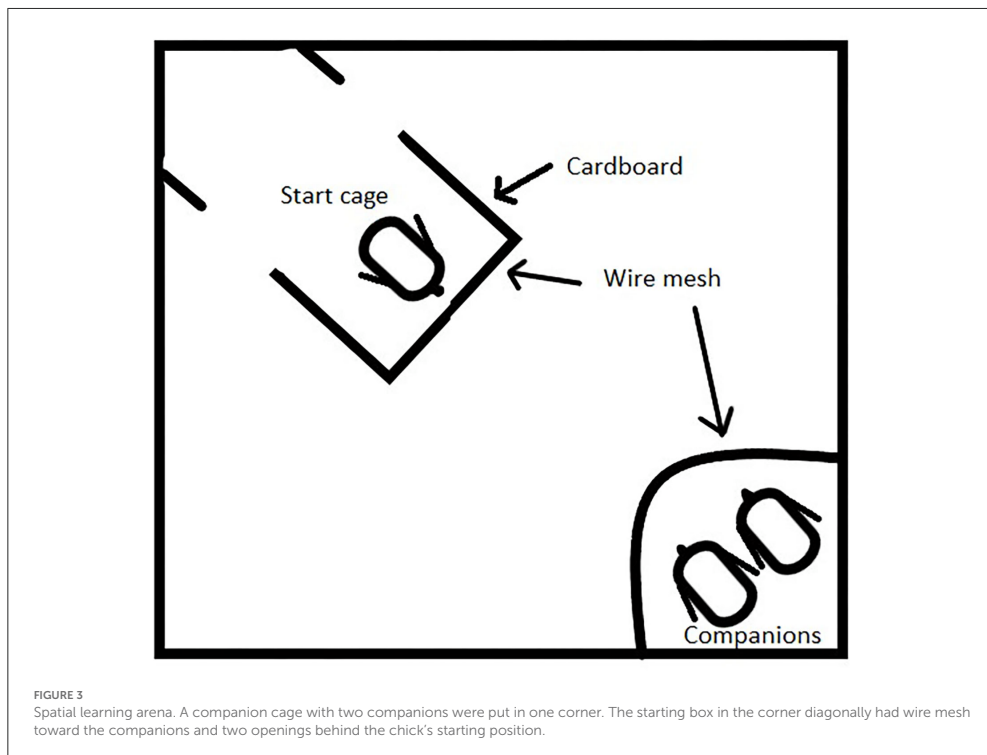
Treatments

Two treatments were used in this study, artificial brooders and control without brooders. In the treatment with brooders, each pen had three artificial brooders (40 cm × 60 cm) with the sides of the brooders covered with flaps of the tarp to make the area under the brooders dark (Figure 2). The brooders were removed when they were 21 days when all chicks no longer could fit under them and the heat provided no longer was necessary.

Data collection

Cameras (Sony SNC-CH120) were mounted on the ceiling, facing directly downwards, each camera covering two whole pens but with “dead spots” under the brooders. Small cameras (GoPro Hero 7 White) were used to record under the brooders and were only present during recording (a wire mesh cage for the camera was always present under each brooder). Data on the use of the brooders were collected using scan sampling

four times a day on days 6, 13, and 20. Data on resting behavior were collected on days 20 and 34 of age from the video recordings using focal animal sampling. Each pen was observed two times per observation day (days 20 and 34), at morning from 06:00 to 08:00h and in the evening from 20:00 to 22:00h. The videos showed that the pen was divided into nine imaginary squares of equal size. A random number was given for each new observation and an individual in that square was followed. A total of 10 individuals per pen and observation period (morning and evening of days 20 and 34) were observed. In addition, using videos recorded under the brooders, 10 individuals per pen were also followed. Focal animals were chosen once individual birds started to rest (defined as lying with a leg to the side or sitting with the legs under the body while not engaging in any other activities), chosen in a randomized square of the pen (randomization through a given list of numbers between one and nine). The focal animals were followed during a complete resting bout as well as the following activity, defined as all behaviors that do not fit in our definition of rest, until the start of the next



resting bout. Chickens that rested under brooders, but left the brooder when becoming active, were followed using the cameras above the pen. The length (in s) of each resting bout and the duration of activity between resting bouts as well as the occurrence of disturbances (defined as physical disturbances by other individuals, causing the focal animal to change position or become active) were registered. In addition, it was registered whether the position of the focal animal in the pen was (1) under the brooder (only in the treatment with brooders), (2) close to a wall, defined as being within one bird length from a wall, or (3) elsewhere in the pen (open areas). One observer collected all data from the videos to avoid the confounding effects of several observers.

A cognitive test was performed to evaluate spatial learning capacity, adapted from the study by Freire et al. (19). At 11 days of age, five birds from each pen (30 per treatment) were randomly chosen. Chicks were carried, in a box with companions, to a separate room and given 10 min to acclimatize, with the companions, to the environment. An 80 cm × 80 cm white box with 60 cm high white panels was used as an arena (Figure 3). A wire mesh cage of 15 cm × 15 cm was placed in one

corner, where two companion birds were placed and provided with feed and dried mealworms. The companions came from the same pen as the bird to be tested and were not used for testing themselves. In the corner diagonally from the cage with companion birds, a three-sided cage was placed 10 cm from the wall. The cage had two sides of cardboard and one wire mesh side facing the other cage, the back was open to allow the chick to leave the cage and get closer to the companions. The distance between the cages was 70 cm. The test started when a chick was placed in the starting cage and ended when the chick was one bird length from the companion cage or after a maximum of 10 min if the chick was unsuccessful to reach the companions. The test was recorded using GoPro Hero 7 cameras to avoid human interference and videos were later analyzed. The latency for the chick to leave the starting cage (passing one of the cardboard edges) and the latency to reach one bird length of the cage of the companion birds were noted. A shorter latency implies a better understanding of the spatial environment. One observer conducted the experiment and collected all data from the videos to avoid the confounding effects of several observers.

TABLE 1 Proportion of chicks being under the brooders during observation four times a day at ages 6, 13 and 20.

| Age (days) | Time of day | Proportion of chicks under the brooders |
|------------|-------------|---|
| 6 | 06:00 | 0.27 |
| 6 | 08:00 | 0.28 |
| 6 | 20:00 | 0.26 |
| 6 | 22:00 | 0.28 |
| 13 | 06:00 | 0.25 |
| 13 | 08:00 | 0.24 |
| 13 | 20:00 | 0.24 |
| 13 | 22:00 | 0.22 |
| 20 | 06:00 | 0.16 |
| 20 | 08:00 | 0.14 |
| 20 | 20:00 | 0.18 |
| 20 | 22:00 | 0.19 |

Statistical analysis

Statistical analyses were performed using R (R version 4.1.3, 20). The significance level used in the study was 0.05.

An ANOVA test was used to compare the durations of resting bouts and activity between resting bouts between the treatment groups. The explanatory factors used in this model were treatment, position in the pen, age, and period of the day and the random factor used was the pen. The interactions between the explanatory factors were also included in the initial model, but they were removed when not statistically significant. After a logarithmic transformation of the duration data, the data adhered to normal distribution and homogeneity of variances. *Post hoc* comparisons of significant factors were performed using Tukey's HSD test. Results are reported as means \pm SE.

A Chi-squared test was used to test the occurrence of disturbances between treatments and positions. As the brooders were removed between the two observation periods, the test was done separately for each age. The explanatory factors used in this model were treatment and position in the pen. Results are reported as means.

A *t*-test was used to investigate the effect of disturbances on the durations of resting bouts and activity between resting bouts. All original data from both treatments, age, and period of day were used. After a logarithmic transformation of the duration data, the data adhered to normal distribution and homogeneity of variances. Results are reported as means \pm SE.

A Chi-squared test was used to test the proportion of birds solving the spatial learning task between treatments. Thereafter, a Kruskal–Wallis test was used to test for latencies between treatments. Data did not adhere to normal distribution. Results are reported as means.

Ethical statement

The study was approved by the Animal Research Ethics Committee in Uppsala (Dnr 5.8.18-17765 2018).

Results

Usage of brooders

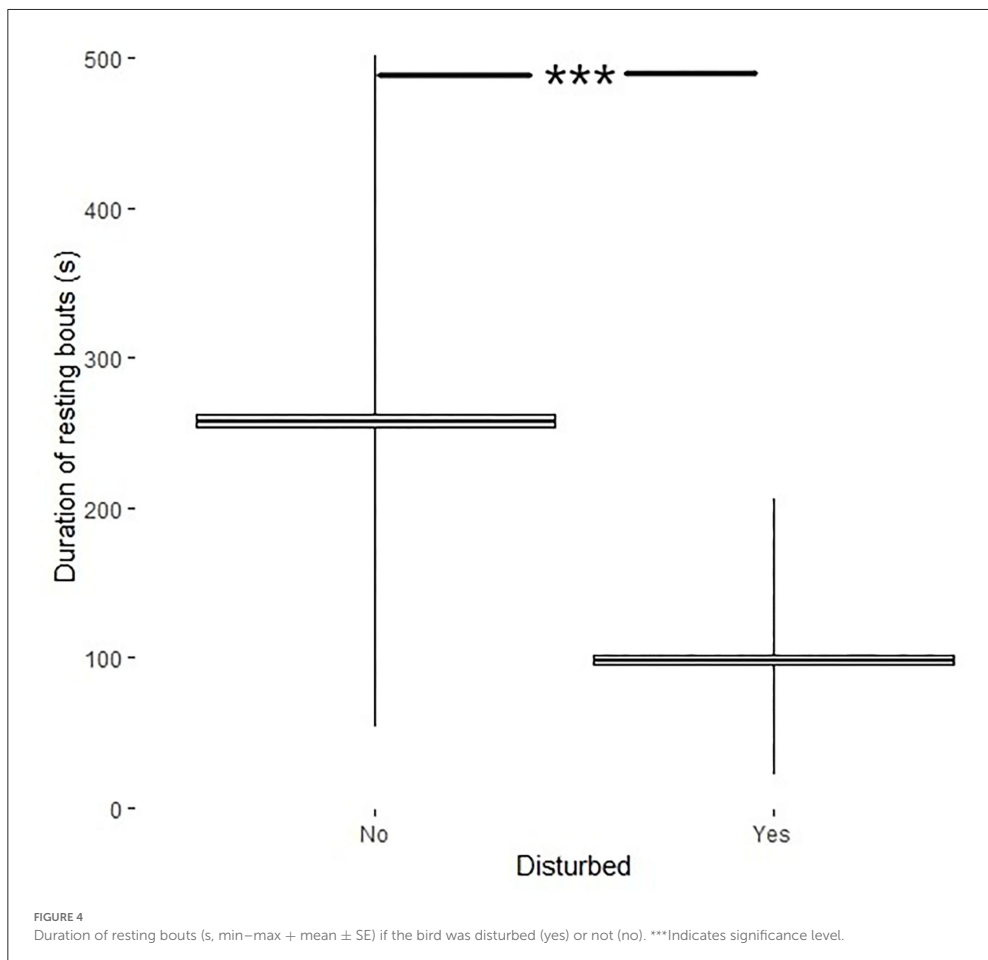
Chicks reared with brooders were seen under the brooders at all ages (Table 1).

Duration of resting bouts

There were no significant interactions between the explanatory factors treatment, age or period of the day for duration of resting bouts. A treatment effect was found on the duration of resting bouts ($df = 1, F = 375.0, p < 0.001$) where the treatment with brooders had longer resting bouts than the control treatment ($260.7 \pm 5.2s$ vs. $132.8 \pm 5.3s$). The position in the pen mattered for duration of resting bouts ($df = 2, F = 29.6, p < 0.001$). Resting bouts taking place under the artificial brooder ($329.4 \pm 8.4s$) were longer than in open areas ($176.5 \pm 6.3s$, Tukey's test $p < 0.001$) and near walls ($182.7 \pm 6.0s$, Tukey's test $p < 0.001$). Duration of resting bouts taking place in open areas did not differ from resting bouts taking place near walls ($p = 0.254$). The resting bouts were longer in the evening ($df = 1, F = 33.5, p < 0.001$) than in the morning (Evening vs. Morning: $229.1 \pm 6.4s$ vs. $190.0 \pm 6.4s$). The resting bouts were longer for older birds ($df = 1, F = 5.3, p = 0.02$) than younger birds (20 vs. 34 days of age: $202.3 \pm 6.4s$ vs. $216.8 \pm 6.5s$).

Duration of activity between resting bouts

There were no significant interactions between the explanatory factors treatment, age or period of the day for duration of activity between resting bouts. There was a treatment effect of the duration of activity between resting bouts ($df = 1, F = 21.85, p < 0.001$) where the treatment with brooders had longer activity than the control treatment ($49.2 \pm 2.4s$ vs. $40.0 \pm 3.3s$). The position in the pen while resting prior to becoming active also affected the duration of activity between resting bouts ($df = 2, F = 6.5, p < 0.01$) where birds resting in open areas ($37.7 \pm 2.7s$) were active for a shorter duration than birds resting under the artificial brooders ($52.2 \pm 4.2s$, Tukey's test $p = 0.013$) and near walls ($50.0 \pm 3.5s$, Tukey's test $p = 0.006$). Duration of activity after resting near walls did not differ from resting under brooders (Tukey's test $p = 0.935$).



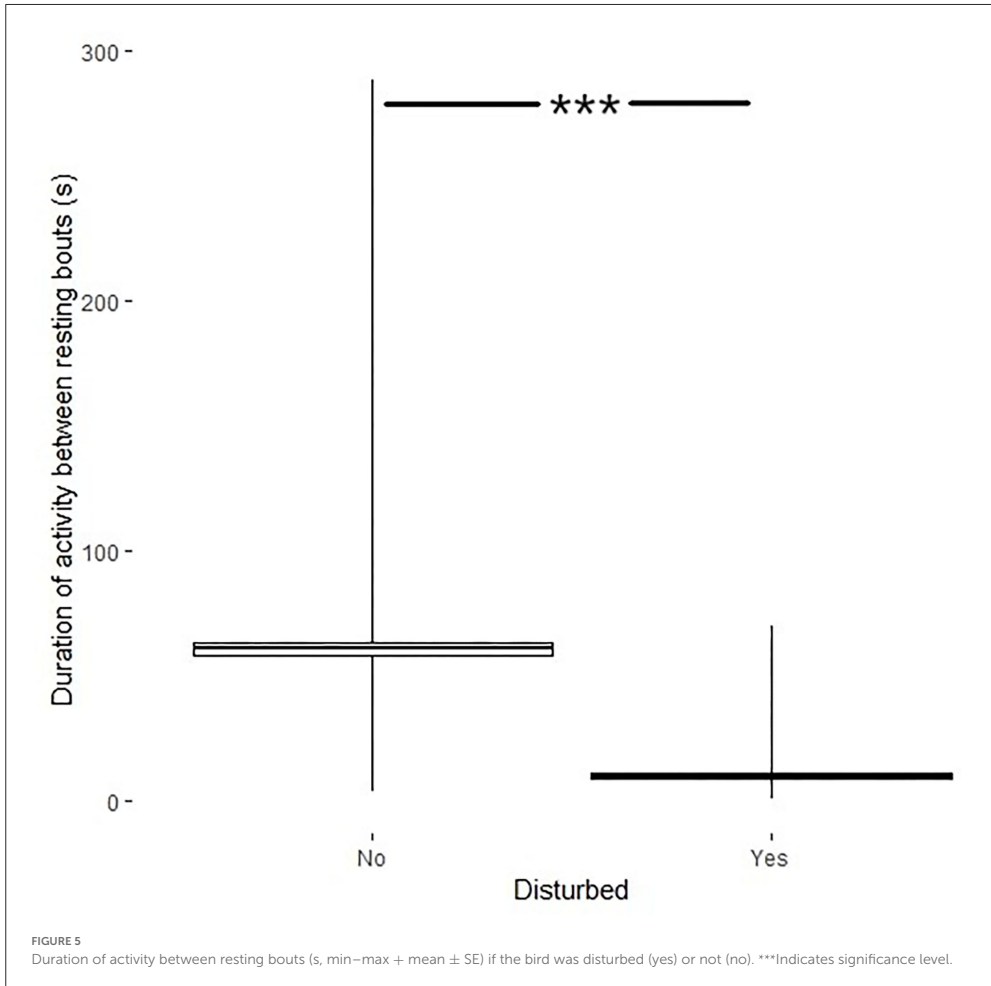
Proportion of resting bouts disturbed

There was a difference in the proportion of disturbances between the treatments both at 20 days of age ($df = 1, \chi^2 = 37.8, p < 0.001$) and at 34 days of age ($df = 1, \chi^2 = 12.2, p < 0.001$) with a lower proportion of resting bouts being disturbed in the treatment with brooders (0.15 and 0.25 disturbed) than in the control treatment (0.48 and 0.42 disturbed). There were no differences between positions within the treatments. At 20 days of age, the proportion disturbed in the treatment with brooders was 0.08 under the brooder, 0.23 in open areas and 0.13 near walls. At 34 days of age, the proportion disturbed in the treatment with brooders was 0.23

in open areas and 0.27 near walls. In the control treatment, the proportion disturbed in open areas was 0.55 and near walls 0.42 at 20 days of age. At 34 days of age, the proportion of disturbed in open areas was 0.53 and near walls 0.42 in the control treatment.

Influence of disturbances on durations of resting bouts and activity between resting bouts

The analyses of influence of disturbances on length of resting bouts and length of periods of activity between resting



bouts were performed on pooled data. Disturbances affected the duration of resting bouts ($df = 290, t = 23, p < 0.001$) where resting bouts of disturbed birds were shorter ($98.4 \pm 3.4s$) than the resting bouts if no disturbance occurred ($257.9 \pm 4.7s$) (Figure 4).

Disturbances also affected the activity between resting bouts ($df = 287, t = 25.6, p < 0.001$) where the duration of activity was shorter after a disturbance ($9.98 \pm 1.0s$) than the duration of activity when no disturbance had occurred ($61.0 \pm 2.4s$; Figure 5).

Spatial learning

The spatial learning task showed a difference between treatments in the proportion of birds successfully solving the task, i.e., leaving the start cage ($df = 1, \chi^2 = 10.2, p < 0.01$) where more birds from the treatment with brooders left the start cage (Table 2). No differences between treatments were found in latencies for the birds to leave the start cage ($df = 1, \text{Kruskal-Wallis chi-squared} = 0.20, p = 0.65$) or reach the companion cage ($df = 1, \text{Kruskal-Wallis chi-squared} = 2.03, p = 0.15$), for birds that left the start cage (Table 2).

TABLE 2 Proportion of chickens leaving the start cage in a spatial learning task and latencies (s) to either leave the start cage or reach the companion cage.

| Treatment | Proportion of chickens leaving start cage | Latency to leave start cage | Latency to reach companion cage |
|-----------|---|-----------------------------|---------------------------------|
| Brooders | 0.5a | 197.5 ± 36.5a | 227.4 ± 36.8a |
| Control | 0.27b | 203.1 ± 24.6a | 229.6 ± 24.7a |

Different letters within parameter indicate significant treatment differences ($p \leq 0.05$).

Discussion

This study shows in general that there are frequent physical disturbances causing individual chickens to end resting bouts throughout the day. Disturbances were common in both treatments. Similarly, in a study by Yngvevsson et al. (10), 53% of the 45-day old focal birds were disturbed during resting at daytime. Also, Forslind et al. (8) found that a high proportion of resting bouts ended due to the chickens being disturbed by other individuals, both during night and day, again suggesting that physical disturbances are common. Artificial brooders have been suggested to provide a separate resting place where chicks can go to rest, away from active chicks (13). Since the number of disturbances was lower under the brooders compared to the rest of the pen and compared to the control, they seem to some extent to fulfill the suggested hypothesis of being a separate resting place. Similarly, elevated platforms seem to provide a resting place, away from active individuals, as it has been shown that elevated platforms reduce the proportion of disturbances among birds resting on them, when observed at days 20 and 34 (8).

As both treatments had the same conditions after 21 days of age due to the removal of brooders, one could expect to find less differences in the behavior of the chickens at 34 days of age. However, the brooder treatment caused a lower number of disturbances even after the removal of the artificial brooders compared to the control treatment. Also, the duration of resting bouts were longer in the brooder treatment than in the control treatment even after the removal of the brooders. This means that we see a long term effect of using the brooders early in the rearing, which has previously been shown on other behavioral aspects in layers, e.g., reduction of feather pecking and fear (e.g., 21, 22). The mechanisms of the long-term effect seen in resting behavior need to be further studied.

A certain period of undisturbed sleep is necessary to reach specific sleep stages like Rapid Eye Movement (REM) and since poultry also show REM-like sleeping patterns (20), a longer period of undisturbed sleep is likely to be important. In the present study, the resting bouts were longer, both if the birds were disturbed or not, in comparison with the resting

bouts observed in Forslind et al. (8) where broilers were kept at a stocking density of 40 kg/m² with access to elevated platforms or at a stocking density of 34 kg/m² without access to elevated platforms. There are several differences between the studies, but one major difference is the stocking density. In the present study, the stocking density was kept at 20kg/m² whereas in Forslind et al. (8), the stocking density was kept as in commercial practice for Danish conditions, i.e. 40 kg/m² (or 34 kg/m² for the low-density treatment). The expectation was that with additional space birds can move about with less physical contact, reducing the need for birds to run over each other. Indeed, Dawkins et al. (21) showed that the disturbances increased with stocking densities where differences were found between a stocking density of 30 and 42 kg/m² or higher. Another reason for choosing a low stocking density was for the birds to all fit under the brooders, until 3 weeks of age. To gain this with a higher stocking density would imply covering more of the pen in brooders, which would reduce the opportunities for chicks to choose a resting place away from the brooders.

To get an impression of the motivation to rest, and thereby of the impact of disturbances, the duration of activity between two resting bouts was observed. When a resting bout was followed by only a short phase of activity, the chicken can be considered to have high motivation to continue resting. In the current study, the average time the chicks spent active was 40–50 s. In a study with elevated platforms, where resting bouts were found to be shorter and the proportion of resting bouts being disturbed to be higher, the activity between resting bouts was very short, around 10–15 s (8). This might be interpreted as that either brooders or the lower stocking density or the combination, gave the chickens a better quality of rest as the motivation to continue resting after becoming active was lower.

During the spatial learning task, twice as many birds with access to artificial brooders were successful in solving the task and leaving the start cage than birds reared without brooders. As our assumption was that birds that sleep better also have better cognitive skills, the lower proportions of disturbances and longer resting bouts within the treatment with brooders could have affected the outcome of the test. That would be supported by Tartar et al. (18) who showed that rats perform better in a water maze if not exposed to fragmented sleep. Also, Johnsson et al. (22) showed that sleep-deprived magpies performed worse in a reversal learning task and had lower motivation to complete the task. Sleep fragmentation could possibly be a reason for the results, as sleep fragmentation affect learning and memory (16), but it is unknown in the current study whether the chicks were experiencing sleep fragmentation. However, there could also be an effect of the occlusion by the brooders in the pen, since birds reared with brooders may have experienced situations where walking around the brooders was needed in order to find companions. Freire et al. (19) saw that chicks reared with the option to walk out of sight from an imprinted mother also

performed better in a spatial learning task. The spatial learning task has limitations (e.g., it is only one test, not all individuals from each treatment were tested and it is unknown if the tested individuals from the brooder treatment used the brooders), thus preferably another spatial learning task should be done to confirm the results in future studies.

A main reason for the high frequency of disturbances in both treatments is likely the lack of behavioral synchronization. When resting, chickens seek each other's company and when not synchronized in behavior they continuously enter and leave resting groups and areas, disturbing resting birds. Riber (14) showed that artificial brooders could somewhat act as a cue for social synchronization, specifically for inactive phases (23–25). Additional measures to better synchronize behavioral patterns would be needed. This could potentially be intermittent lighting programs, which could act as a signal for the chicks to initiate resting phases and therefore possibly synchronize resting behavior in the flock.

Conclusion

In this study, the frequency of disturbances and duration of resting bouts showed that individuals experience difficulties in achieving undisturbed rest. The introduction of artificial brooders provided an opportunity for somewhat longer and less fragmented rest. However, disrupted rest was common in all situations suggesting that more measures than adding an artificial brooder are needed to further reduce disturbances. Increased synchronization of behavioral patterns could possibly be such a measure that further reduces disturbance, but more research is needed to determine how to induce it in broiler flocks and to evaluate its potential effects on quality of sleep.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Ethics statement

The animal study was reviewed and approved by Uppsala djurförsöksetiska nämnd, Jordbruksverket, Sverige.

Author contributions

SF, CH, AR, HW, and HB contributed in the planning of the project, both theoretical and practical. SF conducted the experiment, data collection, analysis, and wrote the manuscript with input from CH, AR, HW, and HB. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Rest and sleep are important for the welfare of animals but rarely mentioned and considered in broiler production settings. This thesis investigated three possible treatments to improve the quality of resting behaviour; elevated platforms, artificial brooders and intermittent lighting. The results showed that all three treatments improve the resting behaviour and thus quality. Altering the broilers' environment to promote more natural resting patterns have positive effects on the resting behaviour and thus likely also on their sleep.

Sara Forslind received her doctoral education at the Department of Animal Environment and Health, Swedish University of Agricultural Sciences. Her undergraduate degree was received at Stockholm University.

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