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The effect of brooders on the synchronization of behaviour in broilers

Effekten av värmetak på synkronisering av beteende hos slaktkycklingar

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Abstract

For the first weeks of their life chickens in nature sleep and rest on the ground under the brooding hen. However, broilers in conventional production are kept in very barren environment with high stocking densities which does not encourage sleep and rest. It has been hypothesized that the absence of a broody hen leads to the chickens being less synchronized in their behaviour and that this increases the risk of resting chicks being disturbed by other active chicks. The aim of this study was to investigate if access to brooders would influence synchronization of behaviour and, because of potentially better sleep, make the chickens less fearful and have a more lasting memory. 600 broilers of the fast-growing genetic line Ross 308 were raised in 10 floor pens with 60 birds in each pen. There were four pens with heated brooders (HB), three pens with cold brooders (CB) and three pens without brooders (C). The chickens behaviour was recorded in the home pens on 7 and 21 days of age to measure their synchronization. Fourteen chickens from each treatment were subjected to a reversal learning test at 2 weeks of age and at 9 and 19 days of age five chickens from each pen were put through a tonic immobility test. At 7 days of age chickens from the HB and CB groups were more synchronized in their comfort behaviours and the HB groups also had a tendency to be more synchronized in their standing behaviour. At 21 days of age chickens from the HB groups were more synchronized in their resting behaviour. However, chickens from the CB and C groups were more synchronized in their explorative behaviour. In the reversal learning test chickens from the CB groups made more right choices and less wrong choices compared to the other groups. There were no differences found between the treatments in the tonic immobility test. The results from this study indicate that access to heated brooders for the first three weeks of life made the chickens more synchronized in their resting behaviour. However, more synchronized resting was not found to have an effect on their fearfulness nor on their performance in a reversal learning test. Further research would be needed to evaluate what effect access to heated brooders, and what effect synchronization, has on sleep disturbances.

Keywords: broilers, sleep, resting, synchronization, brooders

Sammanfattning

Under de första veckorna av sina liv sover och vilar kycklingar i naturen på marken under hönan. Men i konventionell slaktkycklingproduktion hålls kycklingarna i en mycket karg miljö med hög beläggningsgrad vilket inte uppmuntrar till sömn och vila. Det har hypotiserats att frånvaron av hönan leder till att kycklingarna blir mindre synkroniserade i sina beteenden vilket ökar risken för att vilande kycklingar blir störda av aktiva kycklingar. Syftet med den här studien var att undersöka om tillgång till värmetak påverkar synkroniseringen av kycklingars beteende och därmed påverka kycklingarnas rädsla och minne. 600 slaktkycklingar av linjen Ross 308 hölls i 10 boxar med 60 kycklingar i varje box. Fyra av boxarna hade varma värmetak (HB), tre boxar hade kalla värmetak (CB) och tre boxar var utan värmetak (C). Kycklingarnas beteende i boxarna observerades vid 7 och 21 dagars ålder för att undersöka deras synkronisering. Fjorton kycklingar från varje behandling deltog i ett *reversal learning test* vid två veckors ålder och vid 9 och 19 dagars ålder fick 5 individer från varje box delta i ett *tonic immobility test*. Vid 7 dagars ålder var kycklingarna från HB och CB grupperna mer synkroniserade i sina komfortbeteenden och HB grupperna hade också en tendens att vara mer synkroniserade i sitt stå beteende. Vid 21 dagars ålder var kycklingar från HB grupperna mer synkroniserade i sitt vilobeteende. Däremot var kycklingar från CB och C grupperna mer synkroniserade i sitt undersökande beteende. I reversal learning testet gjorde kycklingarna från CB grupperna mer rätta val och färre fel val jämfört med de andra grupperna. Det var ingen skillnad mellan de olika behandlingarna i tonic immobility testet. Resultaten från denna studie indikerar att tillgång till varma värmetak under de tre första veckorna av livet ledde till att kycklingarna blev mer synkroniserade i sitt vilobeteende. Dock verkade inte mer synkroniserad ha någon effekt på deras rädsla eller deras minne. Ytterligare forskning behövs för att kunna utvärdera vad för effekt värmetak, och synkronisering av vila, har för slaktkycklingars sömn och vila.

Nyckelord: slaktkyckling, sömn, vila, synkronisering, värmetak

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1 Introduction

Through an intensive genetic selection, broiler chickens have become the fastest growing farmed species (Meluzzi & Sirri, 2009). In conventional housing they are raised in deep litter systems without any environmental enrichment and a very barren environment (Bergmann *et al*., 2017). They are normally kept in large flocks of 10000-20000 birds with high population densities and grow to slaughter weight of 1.5-2.5 kg in about 5-6 weeks (Broom & Fraser, 2015). However, the commercial broiler husbandry conditions may disturb sleep and sleeping patterns. After hatching the chickens are commonly held with continuous light for the first few days (Malleau *et al*., 2007). Thereafter, they have to be kept with a minimum of 6 hours darkness, whereof four hours continuous (European Commission, 2007). Chickens diurnal rhythm, sleep and synchronized behaviour patterns are influenced by the photoperiod (Meluzzi & Sirri, 2009). It is possible that 6 hours darkness is not enough to meet the broilers need during their growth and rest periods (Olanrewaju *et al*., 2006). Furthermore, since there are no perches or other structures dedicated to resting the chickens sleep on the floor, which increases the risk of the chickens being disturbed by other active chickens (Malleau *et al*., 2007; Yngvesson *et al*., 2017).

1.1 Natural resting behaviour in poultry

The ancestor of modern domestic poultry (*Gallus gallus domesticus*) is the red jungle fowl (*Gallus gallus*) which roost in trees when resting (Collias & Collias, 1967; Arshad & Zakaria, 2009). This is considered to be an evolutionary adaptation that reduces the risk of predation (Olsson & Keeling, 2002). Both laying hens and broiler chickens have been shown to have a motivation to perch (Olsson & Keeling, 2000; 2002; Bokkers & Koene, 2003; Bailie *et al*., 2018). But while laying hens will use the perches for night-roosting as well as during the day (Duncan *et al*., 1992; Lambe & Scott 1998;), broiler chickens have been found to decrease their time spent perching with age (Pettit-Riley & Estevez, 2001; Bokkers & Koene, 2003). One explanation for this is that since broilers are much heavier it might make it difficult for them to get up on, or stay on, perches (Pettit-Riley & Estevez, 2001). This is supported by the fact that while red jungle fowl and laying hens prefer perches that are high up (Olsson & Keeling, 2000; Newberry et al., 2001; Arshad & Zakaria, 2009), broilers tend to favor perches that are closer to the ground (Norring *et al*., 2016).

Norring *et al*. (2016) found that broiler chickens were more likely to use elevated platforms over perches, however the usage of the platforms declined with age. Also, they used the platforms mainly during the light period rather than during their dark period. It is possible that broiler chickens are too young to be motivated to night-time roost. For the first weeks of their life chickens rest on the ground under the brooding hen (Workman & Andrew, 1989). At about one-month of age chicks of feral domestic fowls start to roost during the day and at around 6 weeks of age they start to night-roost (McBride *et al*., 1969). Layer chickens have been found to start perching during the day before they perch during the night and to prefer to rest under heating lamps instead of perching at 6 weeks of age (Heikkilä *et al*., 2006).

1.2 Synchronization of behaviour

Under natural conditions, flocking animals synchronize their behaviour either through social facilitation or diurnal rhythm (Collins *et al*., 2011). Social facilitation is when the sight of one individual performing a behaviour enhances the motivation of others to perform that behaviour (Colgan 1989). The existence of an ultradian rhythm, which is when a biological rhythm has a period that is shorter than a day, of activity has been reported in broody jungle fowl hens (Hogan-Warburg *et al*., 1993). This cycle is thought to be driven by the chicks since the brooding, and brooding cycle, disappears from the hen’s repertoire as soon as the chicks are removed (Hogan-Warburg *et al*., 1993). From a fitness perspective synchronization can increase the probability of finding resources and reduce the risk of predation (Keeling *et al*., 2017). Behavioural synchrony has been found to be important for laying hens, which is most apparent when it comes to egg-laying, feeding, perching and dust-bathing (Lill, 1968; Mclean et al., 1986; Hughes, 1971). Feral chickens have also been found to synchronize a lot of their behaviours such as resting, eating, walking and preening (Savory *et al*., 1978) A lack of synchronized behavioural patterns in the flock can lead to resting chicks having their sleep repeatedly disturbed by active chicks (Schwean-Lardner *et al*., 2014).

Malleau *et al*. (2007) hypothesized that the mother hen helps the chicks to synchronize their rest periods. Therefore, the absence of a broody hen is expected to lead to increased social desynchronization (Riber *et al*., 2007). The absence of the mother hen, combined with the long periods of light and the high density of birds, may contribute to the chicks not getting sufficient rest and sleep (Malleau *et al*., 2007). The domestic fowl is a precocial species and the chickens are able to be reared away from the mother hen (Malleau *et al*., 2007). However, for the first weeks of life they are incapable of fully regulating their body temperature (Hayne *et al*., 1986; Tazawa *et al*., 2004). Under natural conditions the chickens will stay under the brooding hen when they are inactive to keep warm (McBride *et al*., 1969; Wood-Gush & Duncan, 1976). Under commercial conditions the temperature is held at a consistent high level, however the chickens still show a ultradian pattern in their rest-activity rhythms (Riber *et al*., 2007; Nielsen *et al*., 2008). This pattern has been found to be non-random for the first 3-6 days of life in chickens that are reared without a broody hen (Broom, 1980; Nielsen *et al*., 2008). Wauters *et al*., (2002) found that brooded and non-brooded chickens had very similar time budgets, although brooded chicks had longer bouts of activity. However, while the same study found that brooded chicks expressed a precise ultradian rhythm, this was not observed in the non-brooded chicks.

1.3 The function of sleep

Siegel (2008) defined sleep as a rapidly reversible state of immobility and greatly reduced sensory responsiveness. The author also states that rest should be distinguished from sleep and defines rest as a state of reduced activity without loss of consciousness or greatly reduced responsiveness. Sleep occurs in all mammals and birds, however the function of sleep is not entirely known (Siegel, 2008; Rial *et al*., 2010; Libourel & Herrel, 2016). The primary functions appear to be physiological recuperation of the body in terms of energy conservation and tissue restoration and growth (Adam & Oswald, 1977; Malleau *et al*., 2007). Hence, it is possible that sleep disruptions may lead to undesirable disruptions of these physiological systems (Malleau *et al*., 2007). In rats, disruption of sleep has been found to results in pathological stress-like symptoms (Rechtschaffen *et al*., 1983). It has also been hypothesized that rest and sleep may originally have been evolutionary beneficial to minimize the danger of predation since an immobile individual is less likely to be detected (Broom & Fraser, 2015). Sleep is homeostatically regulated, meaning that when an individual does not get to sleep it leads to an increased drive for sleep and when allowed to sleep the individual will make up for this by significantly increasing sleep time. (Siegel, 2008). Therefore, it is of utmost importance to take rest and sleep cycles into consideration when evaluating the design and management of different husbandry systems.

1.3.1 The effect of sleep on learning and memory

Research on both humans and animals suggest that sleep deprivation adversely affects cognitive functions such as learning acquisition, working memory and reference memory (Alkadhi *et al*., 2013). Sleep mainly appears to support consolidation of memory (Vorster & Born, 2015). Consolidation refers to the stabilization of a memory and enables the retention of a memory over time (Vorster & Born, 2015). However, research has shown that sleep appears to mainly enhance memories involving the prefrontal-hippocampal memory system. For example, Smith & Rose (1996) found that rats that had been trained in a Morris water maze with a hidden platform and thereafter were subjected to sleep deprivation during specific time periods had disrupted memory consolidation. However, in the same study, rats that were trained with a visible platform in a Morris water maze, which is hippocampus-independent, were not affected by sleep deprivation. Inostroza *et al*. (2013) found that sleep in rats significantly enhanced retrieval in episodic-like memory task, object-place recognition and temporal memory tasks which critically rely on the hippocampus. In the same study there was no benefit of sleep on novel-object recognition memory which is not hippocampus dependent. In another study by Graves *et al*. (2003) mice that were sleep deprived after context fear conditioning had an impaired fear response. Whereas mice subjected to cued fear conditioning, which is amygdala dependent, was not affected by sleep deprivation. It is possible that the function of sleep varies between species and across the lifespan (Siegel, 2008). Like mammals, sleep in birds has been linked to putative memory functions. However, there are morphological differences in brain structure so it is likely that memory consolidation in birds functions differently (Vorster & Born, 2015).

1.3.2 The effect of sleep on fearfulness

In humans the quality and amount of sleep is known to influence the way we react to events and deprivation of sleep makes us more sensitive to emotional and stressful stimuli (Vandekerckhove & Cluydts, 2010). For example, it has been found that sleep deprivation is associated with anxiety in humans as well as anxiety-like behaviours in mice (Kumar & Garg, 2009; Alkadhi *et al*., 2013). However, there are contradicting results, for example sleep deprivation has been found to decrease fearfulness in rats and mice (Moore *et al*., 1979; Pokk, & Väli, 2001).

1.3.3 The importance of sleep in young animals

Sleep and rest appear to be particularly important for young animals (e.g. zebra finches, Derégnaucourt *et al*., 2005; chickens, Malleau *et al*., 2007; humans and rodents, Hagenauer, & Lee, 2013). For example, Jackson *et al*. (2008) found that undisturbed sleep was necessary for the formation of long-term memory when it came to imprinting in domestic chicks. Sleep has also been found to be critical for developmental song learning in juvenile zebra finches (Derégnaucourt *et al*., 2005). It was also found that in the initial phase of the song learning period sleep had an acute deteriorating effect on the birds performance, which is a similar effect to what has been seen on consolidation of memories in human children (Derégnaucourt *et al*., 2005; Fischer *et al*., 2007). However, the birds that had the highest degree of song deterioration after sleep achieved a better final imitation. (Derégnaucourt *et al*., 2005). This is different from adult birds and mammals where sleep usually results in an immediate enhancement in memory performance (Fischer *et al*., 2007; Brawn *et al*., 2010; Inostroza *et al*., 2013).

1.4 Aim and hypotheses

The aim of this study was to investigate if there was a difference in the synchronization of behaviour in chickens when raised with or without access to brooders. And if a better synchronization (and expected better sleep) will make the chickens less fearful and perform better in a learning and memory test.

1.4.1 Hypotheses

(1) Chickens raised with heated brooders will be more synchronized in their behaviours compared to chickens raised with cold brooders and the control group.

(2) Chickens raised with heated brooders will have a higher proportion of right choices and a lower proportion of wrong choices and not making a choice in a reversal learning test compared to chickens raised with cold brooders and the control group.

(3) Chickens raised with heated brooders will have a shorter time latency for all behaviours in a tonic immobility test compared to chickens raised with cold brooders and the control group.

2 Material and methods

This study was performed as a pilot study of a larger project investigating the effects of heated brooders on the synchronization and quality of chickens resting behaviour.

2.1 Animals and housing

600 day-old chicks of the fast-growing genetic line Ross 308 were obtained from a commercial hatchery and housed at a research facility in Uppsala, Sweden. They were allocated in 10 floor pens (4.0m x 3.0m per pen) with 60 birds in each pen (5 birds/m^2). The walls of each pen were 0.7 meters high and made of metal screen mesh, which meant the chickens had visual contact with neighboring pens. The floors of the pens were covered with wood shavings. Each pen contained 2 feeders and 6 drinking nipples. During the entire study, the birds had ad libitum access to water and feed. In order for the different treatments to have different temperatures (see below) the stable was divided by a movable wall. 6 pens were located on the left side of the stable and 4 pens on the right side (Fig 1). There were separate doors to each section of the stable. The first two days the chickens were kept with continuous bright light. On day 3 the chickens had a dark period of 2 hours, on day 4 they had a dark period of 4 hours and on day five they had a dark period of 6 hours and light period of 18 hours which was kept for the remainder of the experiment.

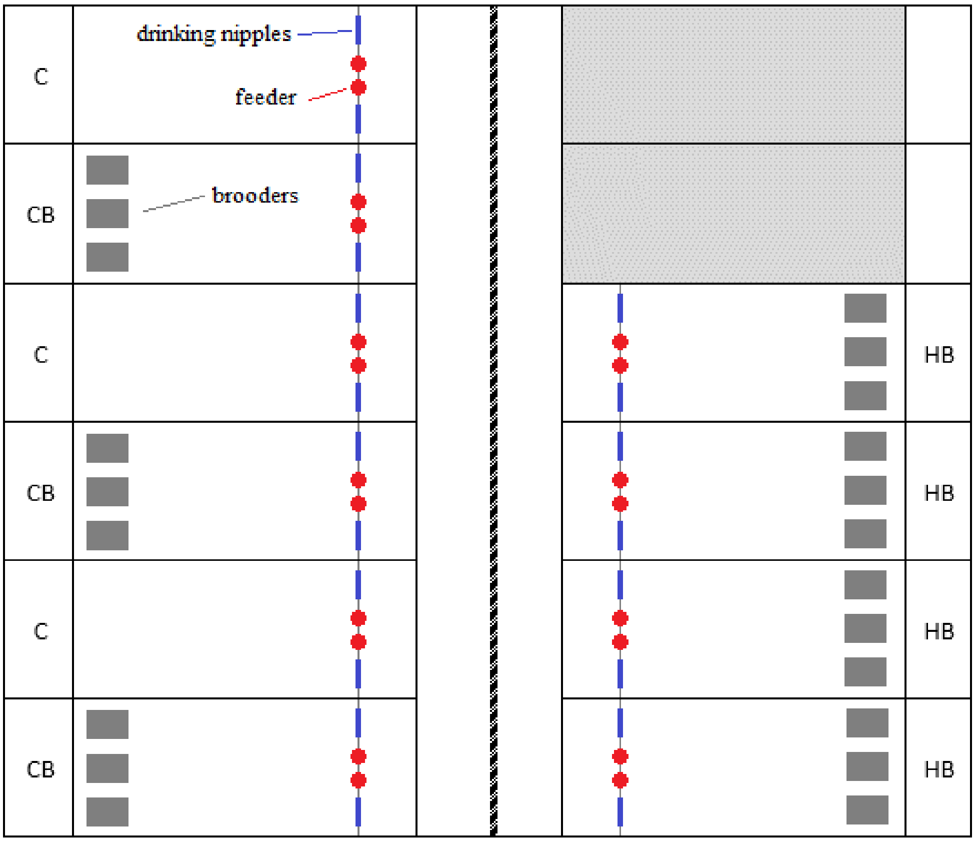

Fig 1. Overview of the layout in the facility were the study was carried out.

2.2 Treatments

2.2.1 Heated brooders (HB)

The 4 pens on the right side of the stable had three heated brooders each (Fig 1). For the first 3 days the brooders were located next to the feeders and were then moved to the back of pen for the remainder of the experiment. The brooders were 60 cm x 40 cm with an adjustable height. The first 3 days the height of the brooders was set at 10 cm, they were then set at the highest possible height of 15 cm for the remainder of the experiment. Strings of tarp were glued around the edges of the roof to make it dark underneath the brooders. The temperature in this part of the stable was 25.5°C (±4°C) on day 1 and was then gradually reduced until day 12 when it reached 20°C (±4°C) which was then kept for the remainder of the experiment. The temperature under the brooders was set at 34°C for the first 3 days, then at 31°C when the height was raised and then finally to 28°C at 2 weeks.

2.2.2 Cold brooders (CB)

Three of the pens on the left side of the stable had three cold brooders (Fig 1). For the first 3 days the brooders were located next to the feeders and were then moved to the back of pen for the remainder of the experiment. The temperature in this part of the stable was kept at 33.5 (±4°C) on day 1 and was then gradually reduced until it reached 23°C (±5°C) at the end of the experiment.

2.2.3 Control (C)

Three of the pens on the left side of the stable were used as controls, no brooders were provided (Fig 1). The temperature was the same as for the CB treatment.

2.3 Behavioural observations in the home pen

On 7 and 21 days of age the chickens were video recorded in their home pen for 24 hours. Five cameras (Sony SNC-CH120) were placed in the ceiling, each camera filming two pens. One camera (either Garmin VIRB XE or Gopro) was also placed at the side of the middle brooder for 2 hours in each pen. From the video recordings of the home pen the number of birds performing each behaviour was recorded every 5 minutes between 6-8 AM and 20-22 PM on both days (Table 1). From the video recordings underneath the brooders the number of birds performing each behaviour was also recorded every 5 minutes for the 2 hours recorded on both days.

Table 1. Ethogram of the behaviours observed in the home pen

Behavioural category	**Description**
Resting	Sitting with bent legs and abdomen in contact with ground and head held upright or lying on abdomen or side with neck relaxed without any other activity
Standing	Standing on one or both feet without any other activity
Locomotion	Walking/running, jumping or hopping without performing any other type of behaviour
Eating	Beak in or above feeder
Drinking	Beak raised towards water nipple (including pauses between swallows)
Explorative behaviour	Pecking with beak or scratching with claws at the litter/ground while standing or sitting
Comfort behaviour	Grooming of own feathers with the beak, stretching out one wing and/or one or both legs, manipulation of the litter with head and wings while lying down (including pauses)
Other	Behaviour that does not fit into the description of any of the above listed

2.4 Behavioural tests

2.4.1 Reversal learning (T-maze)

The T-maze consisted of a corridor (80 x 15 cm) linked to two perpendicular arms (40 x 20 cm) (Fig 2). At the end of each arm was a wire mesh cage (20 x 20 cm). The chickens had to choose between one of the two arms of the maze, one of the arms was empty and the other contained a reward in the form of two conspecifics in the wire mesh cage and mealworms placed at the edge of the wire mesh cage. The individuals used as rewards were the same individuals that participated in the reversal learning test. The first training session was conducted on 8 and 9 days of age with seven individuals from each group. Each individual got five trials. Half of the groups had the reward in the left arm and half in the right arm. If the chicken would not walk when placed in the maze the experimenter would gently push the chicken with their hand to coach it into movement. If the chicken didn't walk to the arm where the reward was the experimenter again would gently push the chicken with their hand in the right direction. A second training session was conducted on 12 and 13 days of age. Because of time constraints only 6 groups were used, two groups from each treatment. Each individual got five trials with the same procedure as in the first training session. This was directly followed by the first test where the chicken had to choose the correct arm three times in a row to pass and move on to the final test. One chick from group 6 (C) and one chick from group 9 (HB) didn't succeed within 10 trials and these individuals were excluded from the final test. The final test was conducted on 15 and 16 days of age. In this test the side with the reward was changed and each individual got ten trials. If the chicken would not come out of the corridor when placed in the maze the experimenter would gently push the chicken with their hand to coach it into movement. For the first and second training session, if the chicken would walk to the correct arm on its own or need to be directed by the experimenter was recorded. For the first test and the final test, it was recorded if the chicken chose the arm with the reward, the arm without the reward or didn't make a choice.

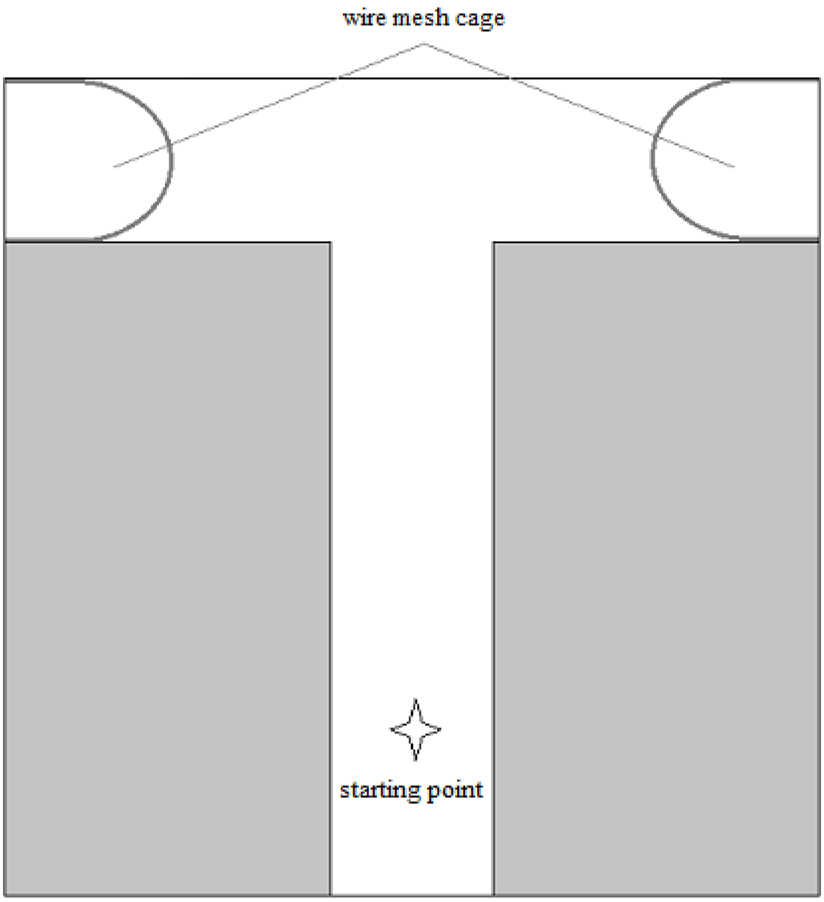

Fig 2. Overview of the T-maze used in the reversal learning test

2.4.2 Tonic immobility (TI)

Tonic immobility is a restraining test that is commonly used in poultry and it is considered that the duration of the tonic immobility response is a measure of the level of fear (Forkman *et al*., 2007). On 9 and 19 days of age, a tonic immobility test was performed with five individuals from each group. The chickens used in the test were marked to ensure that different individuals were used in the test on the different days. All five chickens from each group was collected from the home pen and kept together in a crate outside of the room where the test was carried out. All the tests were carried out by the same observer. During the test, the chicken was placed on its back on a table and the observer held one hand over the bird's head and the other hand gently pushed on the bird's stomach for 10 seconds. If TI was not successfully induced the procedure was repeated up to three times. When TI was successfully induced a timer was started and time to the first vocalization, first head movement and when the chicken jumped up was recorded. If the chick had not jumped up after 10 minutes the test was terminated and a maximum score of 600 seconds was given.

2.5 Statistical analysis

The data from the behavioural observations and the behavioural tests were summarized in Microsoft Excel 2019 and statistical analyses were performed using R 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

2.5.1 Home pen observations

Because of the placement of the cameras the feeders and drinkers were not completely visible on video and therefore the behaviours eating and drinking were not included in the statistical analysis. Also, since there were very few observations for the behaviour 'other' this was also not analyzed. Since there were only cameras under one of the brooders in each pen the observations from each brooder was multiplied with 3 and the sum was then added to the observations from the pen. There were a few instances when there was a disturbance in the form of people either in the observed pen or one of the adjacent pens (4 observations from HB and 1 from CB on day7; 6 observations from HB, 1 from CB and 1 from C on day 21). These observations were excluded from the analyses. To calculate how synchronized the different behaviours were the number of registrations for each variable's observation was divided with the total number of observed individuals for that observation. A Shapiro-Wilk test was then used on each variable to check for a normal distribution. Because the variables were found to not have a normal distribution a Kruskal-Wallis test was used. If a difference was detected then a pairwise comparison using Wilcoxon rank sum test was performed to see which of the treatments there was a difference between. There was a difference in the synchronization between the two days for all variables except for resting, therefore each day was analyzed separately.

2.5.2 Reversal learning

First a Shapiro-Wilk test was used on to check for a normal distribution. Since none of the variables were found to have a normal distribution a Kruskal-Wallis test was performed. The effect of the different treatments on the number of right choices, wrong choices and times no choice was made was analyzed. When a difference was detected a pairwise comparison using Wilcoxon rank sum test was performed to see which of the treatments there was a difference between.

2.5.3 Tonic immobility

Because of a low number of chicks performing vocalization behaviour during the test this variable was not included in the analyzes. A Shapiro-Wilk test was used on each variable to check for a normal distribution. Since the variables were found to not have a normal distribution a Kruskal-Wallis test was performed on the time until the first head movement and until the chicken jumped up.

3 Results

3.1 Behavioural observations in the home pen

3.1.1 Synchronization on 7 days of age

There was no difference in synchronization for resting behaviour, locomotion behaviour and explorative behaviour (Table 2). There was a tendency for difference in standing behaviour and there was a difference in synchronization of comfort behaviour (Table 2). Chickens from the heated brooders treatment had a tendency to be more synchronized in their standing behaviour than the chickens from the cold brooders (P=0.094) and the control treatments (P=0.094) (Fig 3). Both the chickens from the heated brooder and the cold brooders treatment were more synchronized in their comfort behaviour than the chickens from the control (P=0.002) (Fig 3).

Table 2. Values for the variables from the behavioural observations on 7 days of age

Variable	Treatment	Mean	Standard error	Chi-square	P-value
Resting	C	0.580	0.011	4.016	0.134
	CB	0.571	0.010		
	HB	0.598	0.010		
Standing	C	0.026	0.002	5.306	0.070^{T}
	CB	0.027	0.003		
	HB	0.034	0.003		
Locomotion behaviour	C	0.049	0.003	0.423	0.809
	CB	0.049	0.004		
	HB	0.048	0.003		
Explorative behaviour	C	0.172	0.007	0.936	0.626
	CB	0.182	0.007		
	HB	0.176	0.007		

Comfort behaviour	C	0.040	0.003	14.29	<0.001*
	CB	0.056	0.003		
	HB	0.064	0.004		

T = tendency, * = significant difference

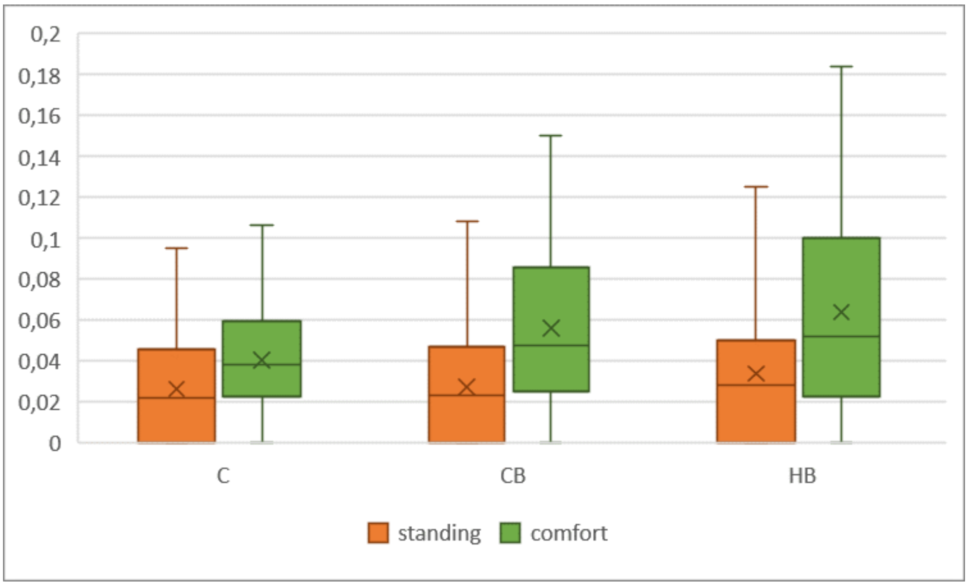

Fig 3. Boxplot of the differences in standing and comfort behaviour between the control treatment (C), the cold brooders treatment (CB) and the heated brooders treatment (HB) at 7 days of age.

3.1.2 Synchronization at 21 days of age

No difference was found between any of the treatments in standing behaviour, locomotion behaviour or comfort behaviour (Table 3). There was a difference in the synchronization for resting and explorative behaviour (Table 3). Chickens from the heated brooders treatment were more synchronized in their resting behaviour compared to the chickens from the cold brooders and control treatment (P<0.001) (Fig 4). Chickens from the cold brooders and the control treatment were more synchronized in their explorative behaviour compared to chickens from the heated brooders treatment (P=0.032; P<0.001, respectively) (Fig 4).

Table 3. Values for the variables from the behavioural observations on 21 days of age

Variable	Treatment	Mean	Standard error	Chi-square	P-value
Resting	C	0.538	0.012	39.369	<0.001*
	CB	0.565	0.014		
	HB	0.636	0.009		

Standing	C	0.028	0.003	3.685	0.158
	CB	0.024	0.024		
	HB	0.029	0.029		
Locomotion behaviour	C	0.034	0.003	0.672	0.715
	CB	0.034	0.003		
	HB	0.032	0.002		
Explorative behaviour	C	0.171	0.007	15.409	<0.001*
	CB	0.152	0.006		
	HB	0.137	0.005		
Comfort behaviour	C	0.058	0.004	4.133	0.127
	CB	0.058	0.004		
	HB	0.074	0.005		

* = significant difference

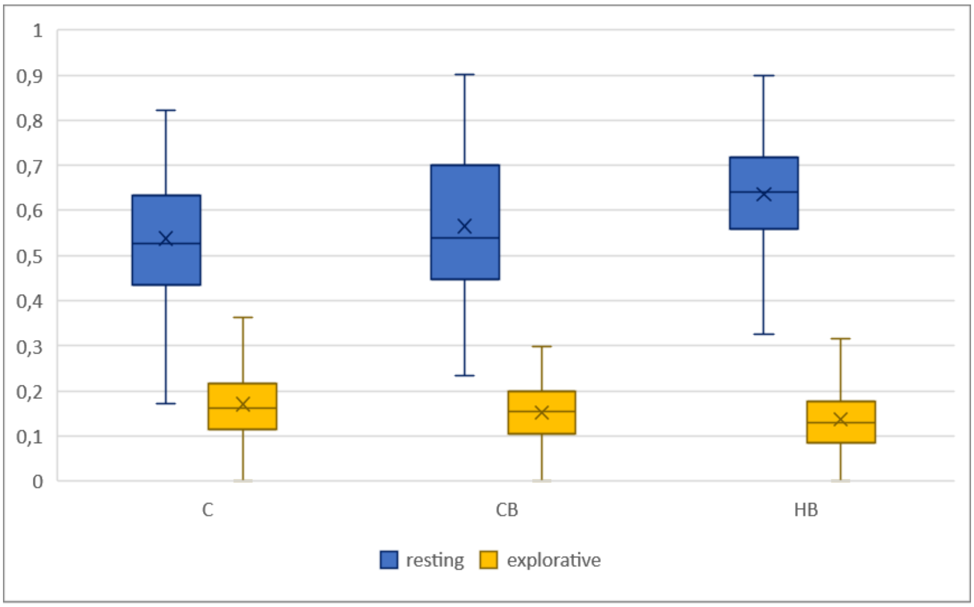

Fig 4. Boxplot of the differences in resting and explorative behaviour between the control treatment (C), the cold brooders treatment (CB) and the heated brooders treatment (HB) at 21 days of age.

3.1.3 Usage of brooders

There were no statistical analyses done on the difference in usage of the heated brooders compared to the cold brooders. However, there were a lot more observation recorded under the heated brooders (744) compared to the cold brooders (184).

3.2 Reversal learning

There was no difference in the number of times the chickens didn't make a choice between the treatments (Table 4). There was a tendency for difference between the number of right choices (Table 4). However, when comparing the treatments it showed that chickens from the cold brooders treatment made more right choices compared to the control treatment (P=0.044) (Fig 5). There was a difference in the number of wrong choices (Table 4). Chickens from the cold brooders treatment made less wrong choices compared to chickens from the heated brooders treatment (P=0.037) and the control treatment (P=0.008) (Fig 5).

Table 4. Values for the variables from the reversal learning test

Variable	Treatment	Mean	Standard error	Chi-square	P-value
Right choice	C	5.769	0.810	5.352	0.069^{T}
	CB	8.286	0.244		
	HB	6.846	0.799		
Wrong choice	C	2	0.506	10.403	0.006*
	CB	0.429	0.137		
	HB	1.231	0.281		
No choice	C	2.231	0.810	0.365	0.833
	CB	1.286	0.286		
	HB	1.923	0.720		

^{T} = tendency, * = significant difference

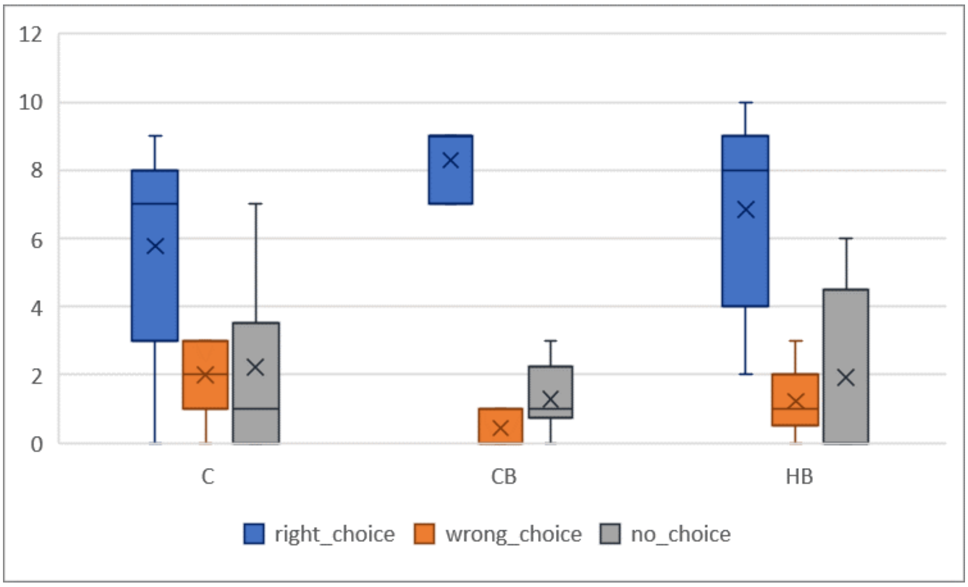

Fig 5. Boxplot of the differences in the number of right choices, wrong choices and no choice being made between the control treatment (C), the cold brooders treatment (CB) and the heated brooders treatment (HB) in the final reversal learning test.

3.3 Tonic immobility

There was no difference between the treatments in the time it took until the chickens jumped or in the time until the first head movement (Table 5).

Table 5. Values for the variables from the tonic immobility test

Variable	Treatment	Mean	Standard error	Chi-square	P-value
Head movement	C	82.172	19.627	1.323	0.516
	CB	80	12.276		
	HB	66.487	9.894		
Jump up	C	249.133	30.264	4.229	0.121
	CB	192.967	25.236		
	HB	171.075	20.769		

4 Discussion

For the first weeks of their life chickens in nature sleep and rest on the ground under the brooding hen (Workman & Andrew, 1989). But in conventional broiler production the chickens are raised in a very barren environment that may lead to disturbed sleep and sleeping patterns. It has been hypothesized that the absence of a broody hen leads to the chickens being less synchronized in their behaviour and that this, in combination with the long periods of light and the high density of birds, may contribute to the chickens not getting sufficient rest and sleep (Malleau *et al*., 2007). The aim of this study was to investigate if there was a difference in the synchronization of behaviour in chickens when raised with or without access to brooders as well as if a better synchronization (and expected better sleep) will make the chickens less fearful and have a more lasting memory.

4.1 Behaviour in the home pen

Chickens rest on the ground under the brooding hen for the first weeks of their life (Workman & Andrew, 1989). This is thought to aid in thermoregulation as well as help the chickens synchronize their resting behaviour (Malleau *et al*., 2007). Wauters *et al*., (2002) found that brooded chickens had a precise ultradian rhythm which was not observed in non-brooded chicks. Previous research on synchronization in broilers has mostly been focused on the effect of lighting programs and light intensity (Malleau et al., 2007; Alvino *et al*., 2009; Schwean-Lardner *et al*., 2014). Malleau *et al*. (2007) investigated if the presence of a 'surrogate mother', in the form of four feather dusters tied together, would aid in synchronizing rest in chickens. However, very few chickens rested under the surrogate mother. The authors hypothesize that this might be because the surrogate mother was separate from the heat source and the chickens may find heat more important than tactile stimulation. Therefore, the hypothesis was that the chickens raised with access to heated brooders would be more synchronized in their behaviours compared to the chickens raised with cold brooders and chickens raised without access to brooders.

At 7 days of age chickens that had access to either cold or heated brooders were more synchronized in the comfort behaviour compared to chickens that had been housed without access to brooders. There was also a tendency for chickens who had access to heated brooders to be more synchronized in their standing behaviour. This would seem to indicate that having access to brooders had some effect on the chickens' synchronization. The reason for why chickens raised with heated and cold brooders were more synchronized in their comfort behaviour could be that they were more influenced by social facilitation. Broiler chickens tend to stay near the walls of the pen which has been suggested to be in order to avoid disturbances (Buijs *et al.*, 2010). Since the chickens with access to brooders tended to stay under and around the brooders rather than be spread out along the walls of the pens this could have led to them having more social interactions. Webster & Hurnik (1994) found that preening was synchronized within and between groups of laying hens held in battery cages. This indicates that the synchronization might have been caused by non-social factors common to all cages. However, social influences on the synchronization cannot be ruled out since the hens could see and hear the hens in the other cages.

The reason for why there was no difference in synchronization in the chickens resting behaviour at 7 days of age could be because the stocking density was very low and the group sizes small compared to what it would be in a production system. Stocking density is known to affect broilers behavioural activities such as locomotion, preening, scratching and resting (Meluzzi, 2009). Hall (2001) found that the frequency of lying was greater and the lying bout length, walking, ground pecking and preening bout length decreased at higher density. They also found that the number of disturbances increased at a higher density and with age. It is possible that the chickens in this study at 7 days of age had enough space that having their rest disturbed was not a problem and that this helped them be more synchronized in all the treatments.

This is supported by the fact that at 21 days of age chickens that had access to heated brooders were more synchronized in their resting behaviour compared to the chickens with cold brooders and without brooders. However, the chickens without access to brooders and the chickens with access to cold brooders were more synchronized in their explorative behaviour compared to chickens with access to heated brooders. This could be a sign that the chickens without brooders and cold brooders spent less time resting and therefore spent more time in active behaviours. The fact that there was no difference in synchronization in other behaviours could be because broilers tend to have a high degree of inactivity.

Because of access to a limited number of cameras we were only able to record under two brooders at the same time. This meant that there was only behavioural observations from one of the brooders in each pen and the brooders were recorded at different time periods. While the chickens didn't appear to favor one brooder over another, it is still possible that certain individuals favored one of the two brooders that wasn't recorded. Also, because of the placement of the cameras there was some part of each pen that was not in view. This in combination with two of the brooders not being filmed means that there were individuals for each observation whose behaviour was not recorded. Therefore, the results may not be entirely accurate.

It is difficult to determine if an individual is sleeping without using electrodes to measure brain activity. In behavioural studies rest in poultry is usually divided into two substates. Dozing, which is when a chicken is sitting or standing with its neck more or less withdrawn, and sleeping, were the chickens is sitting or standing with its head tucked into the feathers above the wingbase or behind the wing (Blokhuis, 1984). Because the behaviour observations in the pens were made from video recordings that had been filmed from above the pens it was not possible to differentiate between the resting positions. Also, in this study there were no observations done during the dark period. Presumably, the synchronization of resting would be higher at such a time than during the light period (Yngvesson *et al*., 2017). These factors could also have had an effect on the results.

4.2 Reversal learning (T-maze)

Sleep is known to support consolidation of memory (Vorster & Born, 2015) and sleep deprivation adversely affects learning and memory (Alkadhi *et al*., 2013). The hypothesis was that chickens who were more synchronized in their resting behaviour would have less sleep deprivation and therefore perform better in a reversal learning test. However, chickens raised with access to cold brooders made more right choices compared to chickens without access to brooders. They also made fewer wrong choices compared to chickens raised with heated brooders and chickens without access to brooders. Because the chickens with heated brooders were the ones who had a better synchronization in their resting behaviour (at 21 days of age) it doesn't appear that less sleep deprivation is the reason why the chickens with cold brooders performed better in the reversal earning test.

They were more synchronized in their explorative behaviour, however it is unclear why this would lead them to have a better memory. One theory would be that the synchronization of explorative behaviour was an indication that there was more social facilitation in the cold brooders treatment and that they were therefore more motivated to find and join their conspecifics in the reversal learning test.

However, this seem unlikely since the chickens without brooders also were more synchronized in their explorative behaviour yet did not perform better in the reversal learning test. Also, there was no difference in the number of times the chickens didn't make a choice between the three treatments, which indicates that there wasn't a difference in motivation between treatments. There were a lot more observation recorded under the heated brooders (744) compared to the cold brooders (184). This indicates that it might not have been the access to brooders that is the cause of the cold brooder treatment performing better in the reversal learning test.

Because not all the chickens showed an interest in mealworms a combination of conspecifics and access to mealworms were used as a reward in this reversal learning test. However, because of the design of the arena used this meant that it was possible for the chickens to see where the reward was when they exited the corridor. It is therefore possible that the chickens' didn't actually learn which direction to go and instead simply relied on visual cues. If that is the case this test might be more suitable to be seen as a measurement of motivation to reach the reward rather than a measurement of the chickens' ability to learn and remember.

4.3 Tonic immobility

The quality and amount of sleep is known to influence the reaction to stimuli and sleep deprivation is associated with anxiety in mammals (Kumar & Garg, 2009; Vandekerckhove & Cluydts, 2010; Alkadhi *et al*., 2013). Therefore, the expectation was that chickens who were more synchronized in their resting behaviour, and thereby had less sleep deprivation, would be less fearful and have a shorter time latency for all behaviours in a tonic immobility test. However, there was no difference between the time until the first head movement or the time until the chickens jumped up between the three treatments. This indicates that a more synchronized resting behaviour didn't lead to the chickens being less fearful. However, the tonic immobility tests were performed at 9 and 19 days of age and the data from the two days was analyzed together. Since there was no difference in the synchronization of resting behaviour at 7 days of age it's possible that the first tonic immobility test was performed before the treatments had had an effect on the chickens behaviour and fearfulness.

4.4 Future research

In this study the chickens were held in groups of 60 individuals. This is a big difference from a production system were the groups can consist of 10000-20000 individuals (Broom & Fraser, 2015). It is unclear how this might influence their synchronization and resting behaviour. In future studies the effect of brooders at different group sizes and stocking densities would be beneficial to see if it could have an effect in a commercial setting.

While the chickens with heated brooders were more synchronized in their resting behaviour at 21 days of age it is possible they were not synchronized enough to reduce disturbances and lead to better sleep. Future studies should be performed to evaluate what effect access to brooders, and what effect synchronization, has on sleep disturbances. Yngvesson *et al*. (2017) found that chickens who rested on the floor were frequently disturbed by other chicks and no disturbances were observed when the chickens rested on perches. Therefore, studies assessing the effect of a combination of heated brooders and some form of platform might be interesting.

4.5 Conclusion

The aim of this study was to investigate if access to heated brooders would lead to better synchronization and if better synchronization would make the chickens less fearful and have a more lasting memory. The results from this study indicate that access to heated brooders for the first three weeks of life made the chickens to be more synchronized in their resting behaviour. However, more synchronized resting was not found to have an effect on their fearfulness nor on their performance in a reversal learning test.

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Popular scientific summary

Sleep occurs in all mammals and birds and appears to be especially important for young animals. Sleep has been found to have an effect on learning and memory and a lack of sleep can lead to an individual being more fearful. Therefore, it is important to take an animal species rest and sleep behaviour into consideration when evaluating the design and management of different husbandry systems. In today's broiler production the chickens are raised away from the mother hen in very large groups. This leads to the chickens not being very synchronized in their resting behaviour and active chicks disturb resting chicks. This is notably different from the wild where the chickens sleep under the hen on the ground for the first few weeks of their life. In this study we wanted to evaluate if access to brooders would help the chickens synchronize their behaviour, and lead to better sleep, and thereby improve their memory and make them less fearful. To test this three different treatments were used on 10 pens with 60 chickens in each pen. In four pens chickens were raised with access to heated brooders, three pens had cold brooders and in three pens the chickens were raised without any brooder. Their behaviours in the pens were observed on 7 and 21 days of age to measure how synchronized they were in their behaviours. At 2 weeks of age 14 individuals from each treatment got to partake in a reversal learning test. In this test the chickens got to walk through a T-maze and learn that on one side there was a reward in the form of other chicks and mealworms. When the chickens had learned the reward was moved to the other side of the T-maze and it was recorded how many tries the chickens needed to learn where the reward now was. At 9 and 19 days of age five individuals from each pen got to partake in a tonic immobility test to measure their fearfulness. In this test the chickens were placed on their backs to induce tonic immobility and the time until their first head movement and until they jumped up was recorded. At 7 days of age the chickens that had access to cold or heated brooders were more synchronized in their comfort behaviours and the chickens with heated brooders also had a tendency to be more synchronized in their standing. At 21 days of age the chickens that had access to heated brooders were more synchronized in their resting, however the chickens raised with cold brooders and those raised without brooders were more synchronized in their explorative behaviours. In the reversal learning test the chickens that had access to cold brooders were made more right choices and less wrong choices compared to the other treatments. There were no differences found in the tonic immobility test. These results indicate that access to heated brooders for the first three weeks of life can make chickens be more synchronized in their resting. However, this did not appear to have an effect on their memory or their fearfulness. Further research is needed to evaluate if the chickens being more synchronized in their resting disturb each other less.