An investigation of nest characteristics and social factors affecting pre-laying behaviour and nest choice in laying hens

Inauguraldissertation
der Philosophisch- naturwissenschaftlichen Fakultät
der Universität Bern

vorgelegt von

Nadine Ringgenberg

von Leissigen

Leiter der Arbeit:
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SUMMARY **5**

CHAPTER 1, GENERAL INTRODUCTION **7**

Pre-laying behaviour 7
Laying hen welfare and artificial nests 9
Social factors 13
Thesis objectives 15

CHAPTER 2, DOES NEST SIZE MATTER TO LAYING HENS? **23**

Abstract 24
Introduction 25
Materials and methods 26
Results 31
Discussion 34

CHAPTER 3, NEST CHOICE IN LAYING HENS: EFFECTS OF NEST PARTITIONS AND SOCIAL STATUS **43**

Abstract 44
Introduction 45
Materials and methods 46
Results 51
Discussion 55

CHAPTER 4, EFFECTS OF VARIATION IN NEST CURTAIN DESIGN ON PRE-LAYING BEHAVIOUR OF DOMESTIC HENS **67**

Abstract 68
Introduction 69
Materials and methods 71
Results 79
Discussion 84

CHAPTER 5, GENERAL DISCUSSION **95**

Conclusions 99

ACKNOWLEDGEMENTS **101**

CURRICULM VITAE **105**

SUMMARY

The aims of this thesis were to investigate the preference of laying hens for specific characteristics of group-nests – size and a partition in the nest – and to examine whether changes in nest curtain appearance affect egg-laying location and pre-laying behaviour. As a result of the current trend to increase the size of nests in aviary systems we performed two experiments, using preference tests, to assess the pre-laying behaviour and the selection of laying hens for nests differing in size and in the presence of a partition. Both experiments involved small groups of hens with free access to two group-nests that looked identical from the outside but differed in one characteristic.

In the first experiment, we found a relative preference for the small nest as hens laid more eggs, performed fewer nest visits per egg and remained longer in that nest. Furthermore, sitting behaviour was observed more frequently in the small nest while standing behaviour did not differ between the nests, suggesting that the hens explored both nests but preferred the small one. Hens may have preferred the smaller nest due to a greater feeling of enclosure.

In the second experiment, we found a relative preference for the partition nest as more eggs and fewer nest visits per eggs were counted in that nest. Hens were also consistent in their egg-laying location. However, after a nest position switch, the hens kept laying their eggs in the familiar location and there was no longer a preference for the nest with the partition and the number of visits per egg no longer differed between the nests. In addition, the social status of the hens resulted in changes in pre-laying behaviour with subordinate hens performing more nest visits and laying their eggs later than more dominant hens although nest choice itself was not affected. The use of a partition in nests could be a practical solution to providing more cover without decreasing nest size. The preferences of hens for smaller nests and nests with partitions should be taken into account when designing group-nests.

The objective of the third experiment was to investigate whether visual stimuli on nest curtains influence nest selection and the distribution of eggs among nests. Groups of hens were assigned to pens with a row of six identical nests or to pens with a row of six nests differing in the curtain appearance (different colours and symbols). Nest position effects, including side of pen entrance, were also investigated with regards to pre-laying behaviour. The hens with access to the nests with the visual stimuli visited fewer nests than hens with identical nests, and this difference increased with age. We found no other evidence that the two treatment groups differed in their pre-laying behaviour and hens were found to be inconsistent in their nest selection. Corner nests and especially nests beside the pen door were preferred compared with the middle nests, this was most marked at the beginning of lay. Nest occupancy status was also an important factor affecting the type and quantity of nest visits. Overall, the visual cues used on nest curtains had little influence on pre-laying behaviour and nest selection while nest position, entrance side, age and whether there were hens in the nests had greater effects.

Chapter 1

GENERAL INTRODUCTION

In 1964, Ruth Harrison described the conditions in which most farm animals were kept in England in her book "Animal machines", which not only raised public awareness on the issue of animal welfare but also set legal reforms concerning captive animals in motion. Around the same time, animal welfare gained acceptance as a scientific discipline; it contradicted the then widespread view that animals did not have feelings (Broom, 2011). The pre-laying behaviour of laying hens was a topic that was investigated early on and evidence was found for frustration prior to egg-laying in battery cages (Wood-Gush, 1972, 1975). However, the improvement of animal welfare was to be a case of "evolution rather than revolution" with legislation to ban battery cages only coming into effect on a large scale in 2012 in the European Union (CEC, 1999), although in Switzerland, laying hens could no longer be kept in cages after 1991 (Swiss Animal Welfare Ordinance, 1981). Today, the two predominant alternative systems to battery cages for laying hens are aviary systems and furnished cages. The introduction to this thesis explores pre-laying behaviour, specific aspects of nests used in different housing systems, and social factors affecting nest use in the domestic hen.

Pre-laying behaviour

Poor welfare can arise when an animal is highly motivated to behave in a certain way but is prevented from doing so (Duncan, 1998; Gonyou, 1994; Weeks and Nicol, 2006). A sub-optimal environment may result in the animal repeatedly attempting to perform a behaviour, and if it can be shown that animals are willing to pay a price to perform this activity, it is considered to be a behavioural priority (Dawkins, 1990; Weeks and Nicol, 2006).

A key priority for laying hens is the performance of pre-laying behaviour (Cooper and Appleby, 2003; Duncan, 1998; Weeks and Nicol, 2006). Hens that do not have access to a defined nest site show signs of severe frustration before egg-laying (Wood-Gush, 1972, 1975) and they will work very hard to access nest boxes by pushing heavy doors (Cooper and Appleby, 2003; Kruschwitz et al., 2008) or squeezing through narrow gaps (Cooper and Appleby, 1995, 1996, 1997). Hens will even work as hard to gain access to a defined nest site before oviposition as for feed after a 4 hour deprivation (Cooper and Appleby, 2003). Both the presence of a nest box and the performance of pre-laying behaviour are important.

Pre-laying behaviour is triggered by ovulation, which occurs about 24 h before egg-laying, and the release of estrogen and progesterone from the post-ovulatory follicle (Wood-Gush and Gilbert, 1973). Hens normally start performing pre-laying behaviour 1 to 2 hours prior to oviposition during which time the egg continues to develop and moves down the oviduct (Mench, 2002). Pre-laying behaviour in domestic hens with access to a nest site is characterized by an increase in locomotor behaviour when hens presumably search for a nest site. This is then followed by visiting nest sites and finally by remaining in a nest where the hens settles down and perform nesting behaviour (manipulating the floor with beak and feet) before sitting in preparation for egg-laying (Appleby and McRae, 1986; Cooper and Appleby, 1996; Duncan et al., 1978; Hughes et al., 1989).

Natural settings

The purpose of pre-laying behaviour is to find a nest site that will allow the successful development and hatch of the eggs (Duncan, 1998). From observations of the Red Junglefowl (*Gallus gallus*), the primary ancestor of the domestic chicken, in South Asia, Collias and Collias (1967) described that hens separate themselves from their flock to lay their eggs in hollows in the ground lined with leaves and feathers. Breeding and nesting usually occur once a year in the springtime (Romanov and Weigend, 2001). Typical clutch size in these birds ranges from 4-7 eggs with subsequent clutches of 3-4 eggs if the first eggs are not hatched successfully. Duncan et al. (1978) reported on the pre-laying behaviour of a small flock of domestic hens released on an unpopulated island. As the Red Junglefowl, these feral hens separated themselves from the flock to go lay their eggs but nest selection could not be observed due to the very secretive behaviour leading up to egg-laying. Nest sites were characterized by concealment and some sort of cover though they were variable in terms of features (open sites with vegetative covers, patches of rushes, etc.) suggesting that hens have different preferences in terms of nest sites.

Successive clutches were laid in nests in close proximity to the previous ones but never in the same nests. During egg-laying, the hens spent 1-2 hours in the nest which is similar to hens in pens (Stämpfli et al., 2011) and clutch size varied between 7-12 eggs.

Commercial settings

After a hen has laid her clutch, incubation behaviour is triggered in most domestic and wild fowl by the release of the prolactin hormone which also causes egg-laying to stop (Mench, 2002). However, incubation behaviour or broodiness has been almost completely eliminated in hybrid strains of laying hens such as the White Leghorn. This is a consequence of direct selection against broodiness but also an indirect response to the selection for high egg production which dramatically increased clutch size (Romanov, 2001). Today, hybrid strains of hens used for egg production will lay up to 320 eggs during 52 weeks of lay. Hens lay their eggs in sequences with an egg laid per day for a number of consecutive days before a pause of one or more days which terminates the sequence (Icken et al., 2008; Johnston and Gous, 2007). The length of laying sequences varies between 10 and 20 days with some hybrid hens having laying sequences of up to 150 days (Icken et al., 2008). Eggs are laid in the morning shortly after daybreak, the lag between successive ovipositions decreases towards the middle of a laying sequence then increases towards the end of a sequence (Johnston and Gous, 2007; Miyoshi et al., 1997). At the peak rate of lay most hens are in the middle of long sequences and the eggs are predominantly laid early in the morning (Johnston and Gous, 2007). This extremely high egg production rate also means that pre-laying behaviour occurs almost every single day in the reproductive life of a domestic laying hen.

Laying hen welfare and artificial nests

Nests in furnished cages

Furnished cages are improved battery cages that provide slightly more space per hen, they also provide resources such as perches, an enclosed nesting area and a dust bath or a scratching area. Many designs of furnished cages exist, and they accommodate small groups of 5-10 hens as well as larger groups of up to 100 hens (Appleby et al., 2002; Fröhlich et al., 2012; Hunniford et al., in press; Shimmura et al., 2008). Nests in furnished cages are usually located in a corner; they often have a perch running through it and are surrounded by plastic curtains and/or solid walls. One or two nests are usually provided per cage. It is questionable whether hens see this area as a proper nest and whether it provides adequate opportunities for nest exploration and nest selection. Although furnished cages undoubtedly improve the welfare of hens compared with battery cages, the hens are still restricted in their behavioural expression (Lay et al., 2011; Rodenburg et al., 2008; Shimmura et al., 2010).

Nests in aviary systems

The first aviary systems were brought about by a modification of littered floor pens by incorporating elevated perches and slatted floors at different levels (Fröhlich et al., 2012; Niebuhr, 2007). Like in cage systems, conveyor belts underneath the slatted floors allow for automatic manure removal, thereby minimizing the spread of infections. The use of the third dimension allows the hens to perform specific activities in separated areas: elevated perches for resting and withdrawing, nest entry platforms and enclosed nests for pre-laying behaviour and egg-laying and litter on the floor for pecking, foraging and dustbathing (Bessei, 1997; Channing et al., 2001). Good management is crucial to a working system, both in terms of animal welfare and production (Kaufmann-Bart, 2009; WPSA, 2012).


Fig. 1. Typical group-nest, shown in a small pen setting.

Aviary systems function on the basis that hens are highly motivated to lay their eggs in enclosed nest sites, rather than on the littered floor, which allows for automatic egg collection. Commercial group-nests are enclosed on three sides by solid walls; they have a plastic roof and plastic curtains in the front with one or two openings (Fig. 1). They are arranged in long rows to accommodate the egg-laying of groups of thousands of hens. The floor is slanted so that the eggs roll onto a conveyor belt and covered with astroturf® or rubber pimples. A nest entry platform allows the hens to walk in front of the nests and easily enter and exit them although some systems have perches in front of the nests. In Switzerland and countries of the European Union there must be at least 1 m^2 of nest surface area available per 100 and 120 hens respectively (Animal Welfare Ordinance, 2008; CEC, 1999). In addition, in Switzerland, all commercial farm animal housing systems or equipment (such as nests) must be approved by the authorities before they can be sold to producers (Swiss Animal Welfare Act).

Nest attractiveness

The number of eggs laid outside of nests (floor or mislaid eggs) can be used as a crude measure of nest attractiveness. Floor eggs may be a welfare problem if the hens laying these eggs do not find the nests attractive and are not able to fully carry out pre-laying behaviour (Cooper and Appleby, 1996). Most floor eggs are usually laid at the beginning of lay when hens are learning to use nests (Appleby, 1983). Floor eggs are a production problem as they have to be collected by hand in loose-housing systems and they are often cracked or dirty reducing their commercial value. In furnished cages, floor eggs (or eggs laid outside of the nests) also result in decreased egg quality but they are collected automatically.

In well-established aviary systems in Switzerland and Austria, very low levels of floor eggs are reported: from less than 1 % (Lentfer et al., 2013; Niebuhr, 2007) to less than 5 % (Lentfer et al., 2011; Stämpfli et al., 2013). In studies of commercial aviaries in Sweden and the UK, low levels of floor eggs are also reported (< 5 %) (Donaldson and O'Connell, 2012; Odén et al., 2005). These values are similar to floor eggs in small experiments. In an investigation of new commercial group-nests in small experimental pens, between 1 and 6 % of floor eggs were reported (Buchwalder and Fröhlich, 2011; Stämpfli et al., 2011, 2012). Similarly in small wooden nests, with or without litter, where the eggs remained in the nests, between 3 and 5 % of eggs were mislaid (Clausen and Riber, 2012; Cooper and Appleby, 1996; Riber, 2010). On the other hand, when looking at nests in furnished cages, the percentage of floor eggs reported is much more variable with 8 to 23 % of floor eggs in Hunniford et al. (in press), 32 to 57 % in Guesdon and Faure (2004) and 13 to 55 % in Wall (2011) although Tauson (2005) reported that close to 100 % of eggs were laid in nests in furnished cages. These numbers demonstrate that from a welfare point of view, nests in aviary systems are likely more attractive than nests in furnished cages, or that hens have better access to them. The small percentage of floor eggs reported in aviaries and the fact that it is comparable to floor eggs reported in small floor pens suggest that commercial group-nests are attractive and answer to the requirements for pre-laying behaviour. However, floor eggs are not enough to assess how attractive a nest is and pre-laying behaviour should be assessed in order to compare different nest (Buchwalder and Fröhlich, 2011).

Two major developments concerning group-nests in aviary systems have occurred recently: the first is the integration of nests inside of the aviary rack itself rather than alongside the wall of the barns and the second is the rather large increase in the size of these nests. Lentfer et al. (2011) found that integrated nests did not negatively influence the pre-laying behaviour of the hens nor the number of floor eggs compared with wall placed nests. In terms of size, group-nests have retained a relatively constant depth of 50 to 60 cm over the years but their width has increased from approximately 1 m in the early group-nests up to 3-4 m in some systems today. Some barn builders even remove the walls between every second nest, which doubles their intended size. Therefore we set out to study whether nest size was a factor that influenced nest attractiveness (Chapter 2). An alternative to decreasing nest size, namely to use partitions in the middle of nests, was investigated in Chapter 3.

Given that many nests are provided in loose-housing systems, the nest searching phase of pre-laying behaviour is accommodated for as the hens can visit multiple nests before choosing the most appropriate one. However, with the exception of nest position and whether there are already hens in the nest, hens must choose a nest site among dozens of identical nests. Nest position influences nest choice with hens often preferring nests located in pen corners or at the edges of nest rows (Clausen and Riber, 2012; Lentfer et al., 2013; Niebuhr, 2007). Given that aviaries contain long rows of identical nests, it has been suggested that the use of different nest types or nest appearances could be used to reduce this strong preference for corner nests and the risk of overcrowding (Appleby, M.C., McRae, 1986; Lentfer et al., 2011). Chapter 4 of this thesis presents a study to assess the effects of using variations in nest curtain appearance on nest selection.

Social factors

Other than nest characteristics, a central theme in this thesis is the influence of social factors (social status and gregarious nesting) on pre-laying behaviour and nest choice in laying hens.

Social status

When hens first encounter each other, they show high levels of aggression before a stable hierarchy gradually forms which is characterized by low levels of aggression (Guhl, 1986; Rushen, 1982; Siegel and Hurst, 1962). Initial agonistic interactions bring about benefits in the future as dominants hens will have preferential access to resources once conflicts arise (Banks et al., 1979; Lee and Craig, 1982; Rushen, 1984). Social status can be based on individual recognition or on the "confidence" or "loser" effect. The first scenario occurs when a hen decides on whether to fight or retreat based on her past interactions with winning or losing against that specific individual (Dawkins, 1995; Pagel and Dawkins, 1997). On the other hand, if social status is based on the "confidence" or "loser" effects, each individual decides on whether to fight or retreat based on its own winning and losing experiences (Barnard and Burk, 1979; Chase et al., 1994). From a theoretical model Pagel and Dawkins (1997) argued that dominance hierarchies break down in large groups because there is no benefit in using individual recognition to form hierarchies; they further argued that dominance hierarchy formation is not limited by the number of animals hens are able to recognize but by the probability of re-encountering the same hen numerous times. D'Eath and Keeling, (2003) experimentally showed that hens in groups of 120 individuals are not nearly as aggressive when encountering unfamiliar hens for the first time as animals from smaller groups of 10 individuals. Thus, hens in large groups seem to be less aggressive and base their social system on the "confidence" or "loser" effect rather than on past experiences with recognized individuals. However, hens with a high social status have advantages in any group such as better access to feed (Banks et al., 1979; Collias and Collias, 1967) and space (Keeling and Duncan, 1989) while there are some conflicting results on whether perches

(Cordiner and Savory, 2001) and nests (Freire et al., 1996; Rietveld-Piepers et al., 1985) are resource over which competition occurs.

Freire et al. (1998) reported that subordinate hens in small groups of four with one nest site (hollow in the ground) showed more locomotion and less resting behaviour in the 10-15 minutes prior to oviposition while Rietveld-Piepers et al. (1985) found no relation between social status and nest use using small group-nests. Competition for nest use has also been studied in furnished cages. Hunniford et al. (in press) suggested that there is competition for the nest site in furnished cages as high levels of aggressive interactions occurred in the cage during the time period when most hens laid their eggs. Furthermore, Shimmura et al. (2008) reported that lower ranking hens in large furnished cages spent less time sitting, more time standing and walking and showed more escape behaviour than higher ranking hens when inside the nest, but nest use was not specifically linked to the egg-laying period and only four groups of hens were observed. Comparisons of the behaviour of hens in cages and loose-housing systems are problematic due to the large differences in nest characteristics, group size and overall freedom of movement. We therefore also investigated whether access to a preferred nest is influenced by social status in Chapter 3.

Gregarious nesting

Gregarious nesting refers to hens choosing to enter nests that are already occupied by one or more hens when empty nests are available and is considered to be an unwanted behaviour as it may increase the risk of overcrowding in nests (Riber, 2010, 2012a). This phenomenon occurs even when laying hens are kept in semi-natural conditions (Riber, 2012a) and when housed in pens with access to group-nests (Riber, 2012b). Riber (2012b) suggested that such "gregarious nesting" may be an anti-predator strategy, although other explanations such as individual preferences for specific nests or nest positions cannot be excluded. However, these studies were conducted with nests that were not of a rollaway type meaning that once laid, the eggs remained in the nests. This is a confounding factor since we know that hens are highly attracted to eggs. Therefore there is a need to gain a better understanding of this behaviour in rollaway group-nests, similar to the ones used in commercial loose-housing systems (Chapter 4).

Thesis objectives

We aimed to evaluate specific nest characteristics thought to influence nest attractiveness in laying hens, namely size, internal structure and nest curtain appearance and study their effects on pre-laying behaviour.

Specifically, the objectives were:

1) To investigate the pre-laying behaviour and relative preference of hens with access to two group-nests differing in size.

2) To examine the pre-laying behaviour and relative nest preference of hens given access to two group-nests differing in the presence of a small partition and to explore the effects of social status on pre-laying behaviour and nest choice.

3) To investigate the effects of distinctive visual stimuli on nest front curtains on pre-laying behaviour, egg distribution and gregarious nesting.

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Chapter 2

DOES NEST SIZE MATTER TO LAYING HENS?

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Abstract

Laying hens in loose housing systems have access to group-nests which provide space for several hens at a time to lay their eggs. They are thus rather large and the trend in the industry is to further increase the size of these nests. Though practicality is important for the producer, group-nests should also cater to the egg-laying behaviour of hens to promote good welfare. One of the factors playing a role in the attractiveness of a nest is the amount of enclosure: hens prefer more enclosure when having a choice between different nest types. The aim of this study was to investigate if hens prefer smaller group-nests to lay their eggs given that they may seem more enclosed than larger nests.

The relative preference of groups of laying hens for two nest sizes—0.43 m2 vs. 0.86 m2—was tested in a free-access choice test. The experiment was conducted in two consecutive trials with 100 hens each. They were housed from 18 to 36 weeks of age in five groups of 20 animals and had access to two commercial group-nests differing in internal size only. We counted eggs daily as a measure of nest preference. At 28 and 36 weeks of age, videos were taken of the pens and inside the nests on one day during the first 5 hours of lights-on. The nest videos were used to record the number of hens per nest and their behaviour with a 10 min scan sampling interval. The pen videos were observed continuously to count the total number of nest visits per nest and to calculate the duration of nest visits of five focal hens per pen.

We found a relative preference for the small nest as more eggs, fewer nest visits per egg and longer nest visit durations were recorded for that nest. In addition, more hens—including more sitting hens—were in the small nests during the main egg-laying period, while the number of standing hens did not differ. These observations indicate that even though both nests may have been explored to a similar extent, the hens preferred the small nest for egg-laying.

Keywords

Laying hens, nest size, group-nests, nest choice, preference test, animal welfare

Introduction

Humans feel safer in spaces perceived as having more enclosure, which is the degree to which spaces are visually defined by surrounding surfaces (Alkhresheh, 2007; Stamps, 2005). And small spaces give a greater feeling of enclosure compared with large spaces (Alkhresheh, 2007). Similarly, in laying hens, a smaller nest may provide a greater sense of protection than a larger one given that the main purpose of a nest is to provide the hens with an isolated and safe place to lay their eggs (Duncan, 1978). Hens are also more motivated to gain access to enclosed nest sites compared with open nest sites (Appleby and McRae, 1986; Zupan et al., 2008). However, the current trend in the industry is to increase the size of group-nests (for example through removal of side walls) as these are cheaper to build (E. Fröhlich, personal communication).

Commercial rollaway group-nests used in free-run housing systems range in floor surface area from approximately 0.5 to 1.8 m2, with a relatively constant depth of 0.5 to 0.6 m and a width of up to 3 m. Legal requirements exist for group-nests in a few countries but they only pertain to the maximum number of hens allowed per m2 of nest surface area: 100 hens per m2 in Switzerland (Animal Welfare Ordinance, 2008) and 120 hens per m2 in the EU and New Zealand (CEC, 1999; NAWAC, 2012). In Switzerland, commercial farm animal housing systems or equipment, including nests for laying hens, must be approved by the Federal Veterinary Office before they can be sold to producers (Wechsler, 2005). Therefore, various nest properties have recently been examined experimentally (Buchwalder and Fröhlich, 2011; Kruschwitz et al., 2008; Stämpfli et al., 2011; Stämpfli et al., 2012). Buchwalder and Fröhlich (2011) used preference tests to compare commercial group-nests with simple wooden rollaway group-nests (with only a thin plastic mat on the nest floor) and found smaller nests often preferred by the hens for egg-laying. Similarly, Holcman et al. (2007) reported that broiler breeder hens laid more eggs in smaller individual nests than larger group-nests. In captive-reared partridges given a choice between three nest types, a preference was shown for nests providing the least amount of internal space and resembling natural conditions the most (Robles et al., 2001). However, the results from the previous three studies are confounded as many characteristics differed between the nest types; it is unclear whether nest size affected the choice of the hens. The relationship between nest size and nest use, predation rate and reproductive characteristics has been investigated in studies of wild birds (ex: Lambrechts et al., 2011; Soler et al., 1998; Weidinger, 2004). But it is difficult to draw relevant conclusions from these studies for domestic laying hens as they are held in artificial conditions, are provided with formed nests and do not reproduce.

Our aim was to test the hypothesis that hens prefer smaller over larger group-nests as a site to lay their eggs. Commercial group-nests were used and hens were tested in groups to mimic commercial housing systems. Thus, groups of hens were given a free choice between two identical group-nests that differed in size only. We expected that hens would lay more eggs, show fewer nest visits per egg, spend more time, and sit more in the smaller nests given that such effects are characteristic for preferred nests (Kruschwitz et al. 2008; Struelens et al., 2008).

Materials and methods

Animals and housing

The relative preference for nest size was assessed in two consecutive trials, each with a different batch of a commercial strain of laying hens (Lohmann Selected Leghorns) in the winter of 2011/2012 and in the spring of 2012. For each trial, non-beak trimmed day-old chicks were purchased from a commercial hatchery. They were reared in a pen (18 m2) until 9 weeks of age at which time they were split into two groups of 120 animals (2 pens of 18 m2) with unrestricted access to water, commercial feed, perches and sawdust bedding. At 18 weeks of age, 100 hens were randomly chosen from the 240 animals, moved to the experimental barn and assigned to five pens in groups of 20.

The experimental pens were of identical size (3 × 3 × 2 m, length × width × height) and arranged in two rows (Fig.1a). The hens had access to sawdust bedding, three perches (0.3 m apart horizontally; at 0.6, 1.3 and 1.6 m high), ad libitum commercial layer mash feed from a round feeder and water from 8 nipple drinkers. There were visual barriers up to a height of 1.6 m between the pens. Two group-nests differing in internal size only were placed opposite each other on either side of the door in each pen (Fig. 1a). Their position was counterbalanced across pen and trial. The hens had access to both nests at all times.

The group-nests were of a rollaway type commercially available in Switzerland. The large nest was the unmodified version with internal dimensions of 0.60 × 1.44 m and the floor of the small nest was half of this size with internal dimensions of 0.60 × 0.72 m (Fig. 1a). The small nest was modified by adding two internal walls and closing off the front edges of the nest. The walls of both nests were made up of plywood which was painted black. Both nests looked identical from the outside and were closed on three sides with a roof, two red curtains in the front (0.60 × 0.45 m, width × height) with an entry of 0.25 m in the middle and a platform to access the nest made up of a metal grid (0.30 × 1.44 m, width × length). They had a floor covered in brown AstroTurf® and divided in two with both parts slanting towards the middle (Fig. 1b). The front floor was higher than the rear to allow eggs to roll onto the egg collection belt. The light intensity on the floor in the rear of the nest was 0.7 ± 0.1 lux in the large nest and 0.6 ± 0.1 lux in the small nest in both trials.

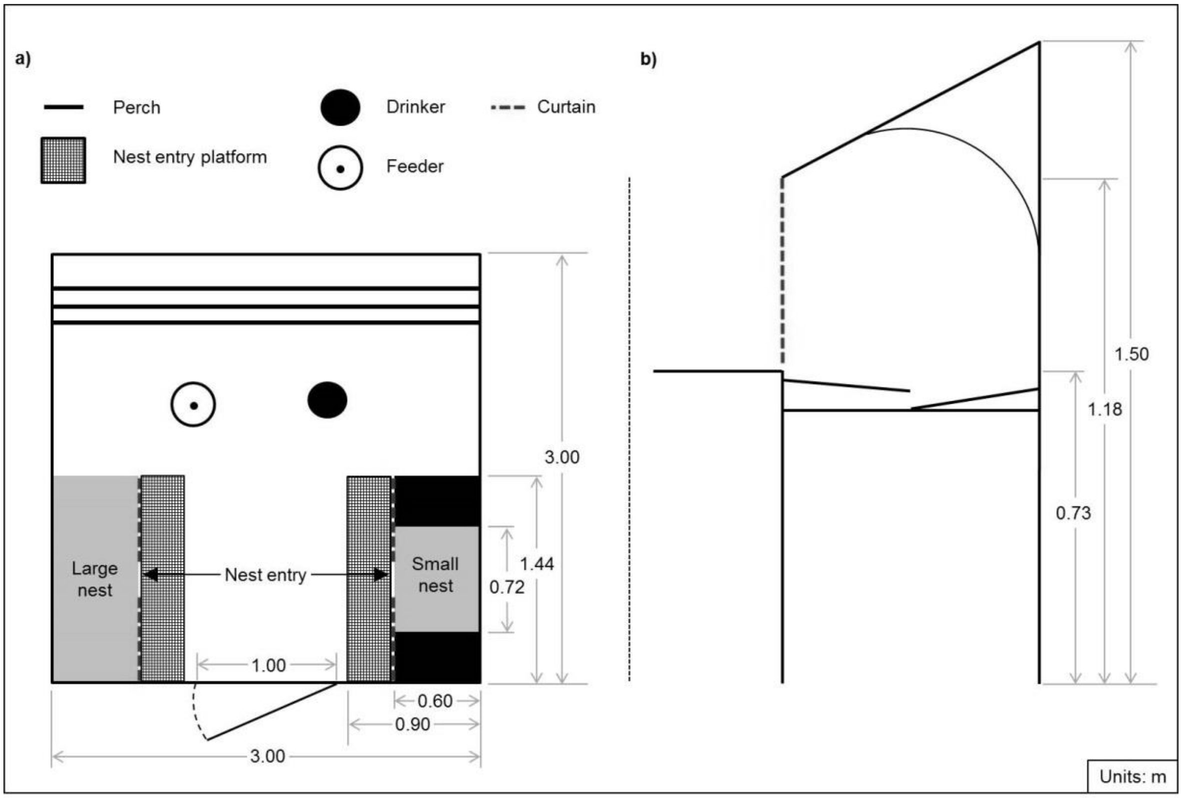

Fig 1. (a) top view of an experimental pen for a group of 20 hens, (b) cross section of nest, slopes of 10 % for front floor and 15 % for rear floor.

From the first day of age until the end of the experiment, artificial light was used to prevent seasonal effects of natural daylight on egg-laying behaviour. The photoperiod followed standard commercial practice. At 18 weeks of age, the hens had 10 hours of light from 6:30 to 16:30 h with a 15 min twilight phase at the beginning and end of the day. Light exposure was then gradually increased by 30 min each week until 15 hours of light was reached in week 28 of age (1:30 to 16:30 h); the photoperiod then remained constant until the end of the study. In the experimental barn the average light intensity at bird height on the pen floors was 7.8 ± 1.0 lux and temperature was maintained at 17.7 ± 4.3 °C in trial 1 and 18.9 ± 3.2 °C in trial 2. The hens were kept in the experimental barn until 37 weeks of age and were then sold to local farmers.

Data collection

The number of eggs laid per nest was our primary outcome variable used to assess nest preference, based on previous studies also having used egg number as the main criterion for nest attractiveness in choice tests (Buchwalder and Fröhlich, 2011; Cooper and Appleby, 1996; Duncan and Kite, 1989). Nest and floor eggs were collected and recorded once daily between 9:00 and 13:00 h from 18 until 36 weeks of age given that by this age most hens have gone through several egg-laying sequences (Icken et al., 2008). We also recorded behavioural data, which were our secondary outcome variables, to gain additional information on nest preference. To observe the hens during the egg-laying phase, two infra-red digital video cameras (Conrad, BP258IR) were mounted in each nest. Additionally, a digital video camera (Samsung, SCC-C4305P) was installed above each pen providing a complete outside view of the nests. Videos of the pens and inside the nests were taken for a 5 hour period after the lights were turned on once during the 28th week of age and once during the 36th week of age. This time frame was chosen as most hens lay their eggs within the first 5 hours of the day (Lentfer et al., 2011; Riber, 2010) and verified during both trials: more than 95 % of eggs were laid by the time the lights had been on for 5 hours.

Videos from inside the nests were analyzed with a 10 min scan sampling method using the behaviours described in Table 1. The pen footage was observed continuously during both 5 h periods and all nest visits were recorded to calculate the number of nest visits per egg. In addition, five hens per pen were randomly chosen and marked with a blue animal marker spray (Raidex GmbH) on their back at 23 weeks of age for individual identification. These focal hens were observed continuously to calculate individual nest visit durations (time of nest exit - time of nest entry) (Table 1). To speculate on which nest the focal hens laid their eggs in, the two longest nest visits per day were extracted from the data set. If both were in the same nest or if the longest visit was 50 % greater in duration than the other, we assumed that this visit was the one during which the egg was laid. We were however unable to confirm this as it was very difficult to see the hens laying their eggs due to crowding on the rear nest floors.

Table 1
Behaviour and location of hens recorded from nest and pen videos (adapted from Lentfer et al., 2011; Struelens et al., 2008).

Videos	Observation	Description	
	Front floor	Hen is on the front floor with the head and at least one leg	
Nest [a]	Rear floor	Hen is on the rear floor with the head and at least one leg	
	Standing	Hen is standing up (including walking)	} Total hens
	Sitting	Hen has keel bone touching the ground, both legs are under the body	
Pen [b]	Nest visit	Hen enters nest (both legs in nest) → Nest visits per egg	
Pen [c]	Nest entry	Hen enters nest (both legs in nest)	} Nest visit duration
	Nest exit	Hen exits nest (both legs out nest)	

[a] 10 min scan sampling on all hens, [b] continuous sampling on all hens, [c] continuous sampling on focal hens

All behavioural data were viewed and analyzed using the behavioural observation software package INTERACT (Mangold International GmbH, 2011, Version 9, Arnstorf, Germany). Blinding the researcher to treatment was impossible when collecting eggs and analyzing videos inside nests but the videos taken from above the pen could be analyzed blindly since the nests looked identical from the outside.

The Cantonal Veterinary Office approved this experiment (Bern, Switzerland, Approval BE110/11) and we followed the ethical guidelines of the International Society of Applied Ethology.

Statistical analysis

All statistical analyses were performed with R (version 3.0.1) and R Studio (version 0.97.551). P-values below 0.05 were considered significant for all analyses and the function lme in the R package nlme (Pinheiro et al., 2013) was used to fit linear mixed effects models. The assumptions of normally distributed errors and homogeneity of variance were examined graphically with the use of the Normal plot (residual quantiles versus quantiles of a normal distribution) and the Tukey-Anscombe plot (residuals versus estimates). To satisfy these assumptions, data on the number of nest visits per egg and the number of nest visits for focal hens were square-root transformed; data on mean number of standing hens per nest and on mean nest visit duration were log-transformed. Results shown are untransformed means.

The proportion of eggs in the small nest was compared with 50 % in the model since the distribution of eggs in both nests was not independent. Week of age was included in the model as a fixed effect. Data reported for eggs are mean proportion of eggs per nest per day, averaged over week. To investigate the role of nest size on the mean number of nest visits per egg per day, nest size, week of age and their interactions were specified in the model as fixed effects. For the mean number of hens in the nests per scan averaged over hour (for standing, sitting and total hens), nest size, week of age, hour and all two-way interactions were included in the model as fixed effects. Finally, for the mean duration of nest visits per focal hen per day, nest size, week of age, hour and all two-way interactions were in the model as fixed-effects. In all models trial and pen were included as random effects (as well as hen for the focal animal data).

The full models were reduced using the function stepAIC in the R package MASS (Venables and Ripley, 2002) that performs stepwise backward model selection using Akaike's information criterion. When there was a statistically significant interaction, the models were run separately for each hour and the interaction terms were removed.

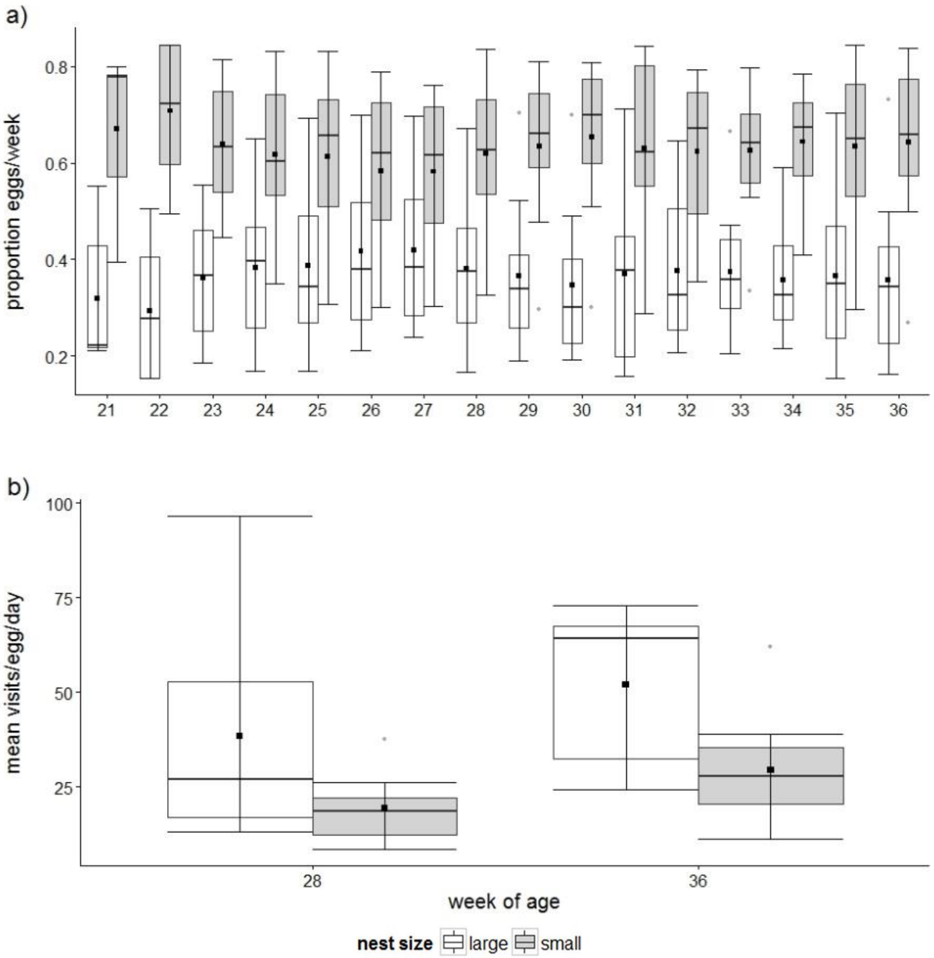

Fig 2. (a) Mean proportion of eggs per nest from week 21 to 36 of age (nest size: $F_{1,149} = 11.54$, $P < 0.001$, week of age: $F_{1,149} = 0.34$, *ns*), (b) mean number of nest visits per egg per nest for one day during week 28 of age and one day during week 36 of age (for the first 5 hours of lights-on, nest size: $F_{1,24} = 11.18$, $P = 0.003$, week of age: $F_{1,24} = 4.67$, $P = 0.044$). Boxplots: boxes represent 1^{st} and 3^{rd} quartile, the thick lines are the medians, the squares represent means, whiskers extend to most extreme data points (within 1.5 × interquartile range) and grey dots represent outliers.

Results

Egg numbers and nest size

The hens started laying eggs during their 19th week of age and as expected reached 50 % production by 21 weeks of age. We collected a total of 10’002 eggs from the nests from the beginning of egg-laying at 19 weeks of age until the end of the experiment at 36 weeks of age

(6’157 eggs from the small nests and 3’845 eggs from the large nests). The proportion of eggs laid on the floor was 3.3 % in trial 1 and 4.1 % in trial 2. During early egg-laying from 19 until 20 weeks of age, we found no evidence of nest preference (proportion of eggs to total eggs in nests: 0.37 ± 0.06 in the small nest vs. 0.31 ± 0.04 in the large nest, $F_{1,9} = 1.85$, $P = 0.21$). Between 21 and 36 of age, the hens laid a greater proportion of eggs in the small nests compared with the large nests and we found no evidence that the age of the hens in that period influenced this egg-laying pattern (Fig. 2a).

Behaviour of all hens and nest size

More nest visits per egg occurred in the large nest than in the small nest and at 36 weeks of age than at 28 weeks of age (Fig. 2b). For the video observations inside the nests, we pooled the data for the number of hens on the front and rear floors as few hens were observed sitting on the front floor of the nests. Out of the total number of hens sitting per scan, only 4.66 ± 0.93 % of hens sat on the front floor of the small nest and only 5.50 ± 0.98 % sat on the front floor of the large nest. The total number of hens per scan ranged from 0 to 9 hens in the small nest and from 0 to 7 hens in the large nest (sitting hens + standing hens). We found an interaction between nest size and hour ($F_{4,176} = 3.15$, $P = 0.02$, Fig. 3a), indicating that nest size affected the total number of hens in nests, though the number depended on the hour: there were more hens in the small nest during hour 3 and 4 after lights-on than in the large nest. We also found an interaction between week of age and hour ($F_{4,176} = 3.15$, $P = 0.02$, Fig. 3b) with more hens in the nests during the first 2 hours of lights-on during week 36 of age compared with week 28 of age.

For the number of sitting hens in the nests, we found an interaction between nest size and hour ($F_{4,181} = 2.55$, $P = 0.04$, Fig. 3c), with more hens sitting in the small nest during hour 3 and 4 after lights-on than in the large nest, but we found no evidence that week of age affected the number of sitting hens ($F_{1,181} = 0.34$, ns, Fig. 3d).

There was no evidence that the mean number of hens standing in the small nest (0.93 ± 0.55) differed from the mean number of standing hens in the large nest (0.95 ± 0.72, $F_{1,179} = 0.25$, ns, Fig. 3c). But there was an interaction between week of age and hour ($F_{4,179} = 3.37$, $P = 0.01$), with more hens standing in the nests during the first 2 hours of lights-on when they were 36 weeks of age compared with 28 weeks of age (Fig. 3f).

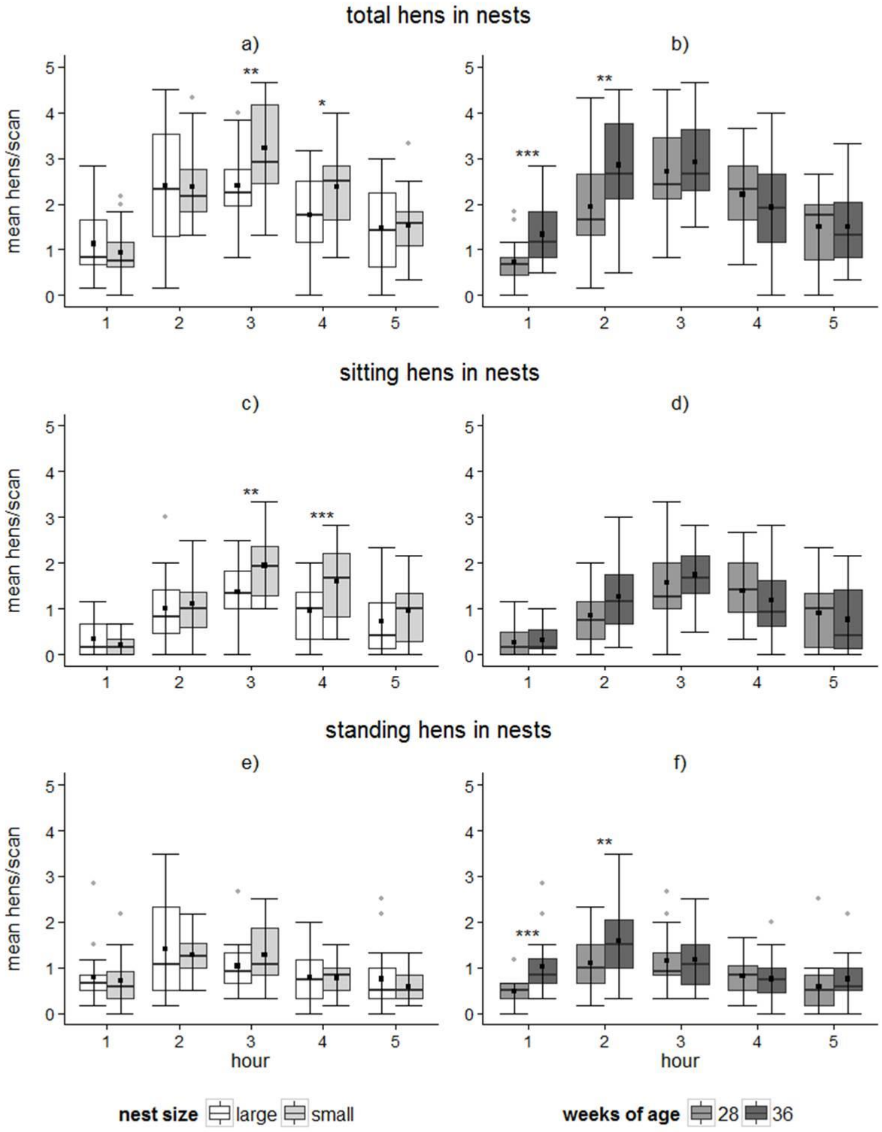

Fig 3. Mean number of hens in nests per hour after lights-on and nest (a, c, e) and week (b, d, f). Stars represent significant differences between nest sizes or weeks of age: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Boxplots: boxes represent 1st and 3rd quartile, the thick lines are the medians, the squares represent means, whiskers extend to most extreme data points (within 1.5 × interquartile range) and grey dots represent outliers.

Behaviour of focal hens and nest size

Of the 50 focal hens, we excluded two from the analysis (one died during the experiment and the other did not enter the nests on the days of observation). Most focal hens visited both nests on both days of observation (77.1 % of focal hens) while 16.7 % visited two nests on only one day and 6.2 % visited one nest only on both days. Hens visited the small nest an average of 16.11 ± 2.23 and the large nest an average of 13.29 ± 1.72 times per day (neither nest size nor week of age affected these numbers: $F_{1,124} = 0.59$, ns and $F_{1,124} = 0.34$, ns, respectively)

Focal hens showed a longer mean nest visit duration in the small nest (8.31 ± 1.65 min) compared with the large nest (3.69 ± 0.79 min, $F_{1,129} = 7.49$, $P < 0.05$). For the longest visit durations, data from nine hens was excluded (similar maximum nest visit duration in large and small nest). We found that at 28 weeks of age, 37.8 % of focal hens had their longest nest visit in the large nest and 62.2 % had their longest nest visit in the small nest. At 36 weeks of age, 37.2 % of hens had their longest nest visit in the large nest while for 62.8 % of hens it was in the small nest. Of these hens, 77.1 % had their longest nest visit in the same nest on both days. We found no evidence that the longest nest visit duration was affected by nest size (small nest: 31.23 ± 3.69 min, large nest: 23.23 ± 2.97 min, $F_{1,38} = 0.32$, ns).

Discussion

We report here that hens show a relative preference for smaller group-nests. The increased proportion of eggs in the small nest points to a preference for that nest as egg-laying is the final purpose of nest-seeking and nesting behaviour. In addition, behavioural data from two days during the peak egg-laying period reinforces this conclusion. The increased number of nest visits per egg in the large nest implies that it was the less attractive nest as the hens required more nest visits to lay one egg than in the small nest. A high number of nest visits per egg has also previously been associated with less preferred group-nests (Buchwalder and Fröhlich, 2011). We also found more hens overall, more sitting hens and longer nest visit durations in the small nest. Although the increased numbers of sitting hens only occurred during hour 3 and 4 after lights-on, this is the time during which most hens lay their eggs (Lentfer et al., 2011). The similar number of standing hens in both nests and the focal hen data suggest that hens explored both nests prior to egg-laying. Other studies found that preferred nest sites resulted in less locomotion, fewer nest visits, longer nest visit durations and more sitting behaviour (Buchwalder and Fröhlich, 2011; Cooper and Appleby, 1995; Freire et al., 1996; Kruschwitz et al., 2008). Kruschwitz et al. (2008) reported that laying hens performed less exploratory behaviour prior to choosing nests with a greater degree of cover and more nesting behaviour in such nests than in more open nest sites. Similarly, nest boxes in furnished cages with plastic curtains received fewer nest visits per egg and resulted in longer nests visits than nests with open fronts (Struelens et al., 2005).

The focal hen data demonstrates that although hens spent more time overall in the small nest, the longest nest visit duration did not differ between nests which supports our prediction that this was the nest visit in which eggs were laid. These results agree with Stämpfli et al. (2011) who reported that nest visits in which hens laid an egg lasted between 10 and 90 minutes. Most focal hens were consistent in their nest choice even though a majority of them did visit both nests each day. However, we were unable to assign all individual hens to their eggs and to infer whether or not all hens were exclusive in their choice of nest.

The Oxford Dictionary defines size as "the relative extent of something; a thing's overall dimensions". We also use the term "nest size" in a relatively broad sense to define the space available in a nest which inherently included differences in floor surface area, wall surface area and curtain surface area between the small and large nest. Since the nests only differed in size, we imply that the hens preferred the small nest due to this characteristic. But there could be explanations for this nest choice other than size. Social factors may have influenced this preference as hens were tested in groups to mimic commercial conditions. Rietveld-Piepers et al. (1985) reported that dominant hens come into lay before subordinate hens, thus the nest choice of dominant hens may influence the choice of the other hens. Furthermore, familiarity of nest position, rather than preference alone, may have affected nest choice once the hens were older and accustomed to egg-laying (Duncan and Kite, 1989). Both of these explanations are however unlikely as 77 % of focal hens visited both nests on both days of observation, so we assume that most hens made an active nest choice throughout the experiment. Nests may also be entered for purposes other than egg-laying behaviour such as hiding from other hens, but since we used egg number as the primary criterion to assess preference and recorded behaviour only during the main egg-laying period of the day this should not affect our conclusions.

Even though the hens laid more eggs in the small nest, some also laid eggs in the large nest. At least three possible reasons exist for choosing this nest. First, as Kruschwitz et al. (2008) suggested, laying hens may have different needs when it comes to an appropriate nest site; some hens may simply have preferred the large nests for egg-laying due to its size or due to the lower hen density. In fact, three focal hens only entered the large nest on both days of observations. Second, we cannot exclude the possibility that they chose the large nest due to lack of space in the small nest as not all 20 hens could fit in the small nest, especially since all of the hens tried to fit on the rear floor. We counted a maximum of nine hens in the small nest, which was then full, whereas the large nest contained a maximum of seven hens only. Thirdly, social factors also likely played a role in the selection of the large nest. Freire et al. (1998) showed that subordinate hens were more active prior to egg-laying and were displaced from the nest site more often than dominant hens. In our experiment some hens may have been displaced from the small nest and had to use the large nest, however we were unable to assess dominance status in this experiment as the hens were not all individually identified. But the large nest was probably not unattractive enough to disregard it as a nest or to delay the timing of oviposition which laying hens are to some extent able to control (Reynard and Savory, 1999).

Older hens entered the nests earlier in the day than when they were younger, which is consistent with the work of Riber (2010), and suggests that once the hens were more experienced they were faster in choosing a nest. And yet, there were more nest visits per egg when the hens were older. This unexpected result and the relatively high number of nest visits per egg compared with other studies is difficult to explain (for example, less than 15 visits per egg in Stämpfli et al., 2011 and 2-17 nest visits per egg in Buchwalder and Fröhlich, 2011). However, one of the nests tested by Buchwalder and Fröhlich (2011) was of the same type as the one used in this study and a similar high number of nest visits per egg was reported (40.29 ± 11.38 nest visits per egg compared with 34.86 ± 3.67 nest visits per egg in our experiment). Thus, the nest type itself may be responsible for the hens performing such a high number of nest visits. This is supported by our observations: the hens predominantly used the rear floor of the nest for sitting while the front floor was used for standing or for sitting only when the rear floor was occupied. Such a split floor nest design may be unattractive to laying hens.

Conclusion

From this study, we conclude that nest size does matter to laying hens, at least in a small group setting. The hens showed a relative preference for the small group-nest—even though it was half the size of the large nest—as demonstrated by the greater proportion of eggs. The hens may have found the small nests to offer more protection and enclosure than the large nest. Therefore, when designing attractive group-nests their size should be taken into account.

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Chapter 3

NEST CHOICE IN LAYING HENS: EFFECTS OF NEST PARTITIONS AND SOCIAL STATUS

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Abstract

Nest choice in loose-housed laying hens is influenced by many factors including nest characteristics, position and social factors. We examined the relative preference of laying hens for two group-nests differing in the presence or absence of a partition in the middle of the nest depending on social status. We hypothesized that hens would prefer the partitioned nest as it provides more enclosure or cover, and that social status would affect nest choice.

Relative preference for the nests was assessed in a free choice preference test conducted in two consecutive trials with eight groups of 20 hens each from 18 to 31 weeks of age. The hens were individually marked and had access to two commercial group-nests (49 × 114 cm), one of which contained an internal wooden partition (30 × 7 cm) which divided the nest in two equal halves. At 28 weeks of age, the position of the nests was switched. The number of eggs laid was recorded daily throughout the trials. On one day each at 24 and 28 weeks of age (after the nest switch) video recordings were made of the first 5 h of daylight. From these videos we recorded the number of nest visits per egg per nest and the number of nest visits per hen per nest. On one day each at 24 and 27 weeks of age we also recorded videos from within the nests to assess individual nest choice for egg-laying. In addition, we recorded aggressive interactions between individual hens during the first hour of light on one day each at 18, 24 and 27 weeks of age to calculate David's score which was used to establish social status.

We found a relative preference for the partition nest with a greater proportion of eggs laid in these nests as well as fewer nest visits per egg. The hens were also consistent in their egg-laying location over the two days of observation. After the nest switch, however, the hens did not switch egg-laying location and the number of visits per egg no longer differed between nests suggesting that the preference for the partitioned nest was only important at the beginning of lay. In addition, although social rank had no impact on preference of nest type, lower ranking hens performed more nest visits and laid their eggs slightly later on one day of observation compared with higher ranking hens.

Keywords: laying hen, group-nests, nest choice, preference test, pre-laying behaviour, egg-laying

Introduction

Non-cage systems for laying hens take advantage of the motivation of hens to lay their eggs in an enclosed area in order to allow automatic egg collection. To ensure a working system, attractive nests must be provided to avoid floor eggs and to improve animal welfare by allowing hens to perform highly motivated pre-laying behaviour (Cooper and Appleby, 1995; Duncan and Kite, 1989; Kruschwitz et al., 2008a). The attractiveness of a nest is influenced by various factors including physical characteristics (Duncan and Kite, 1989; Kruschwitz et al., 2008b; Stämpfli et al., 2011), position (Huber-Eicher, 2004; Lentfer et al., 2011; Riber and Nielsen, 2013) and presence of other hens (Riber 2010, 2012). Individual hen factors likely play a role as well, such as hormonal status, age, and social rank.

In response to the current industry trend of increasing the area of group-nest, we compared two group-nests differing in size (0.43 m2 vs. 0.86 m2) in a previous study and found that hens laid more eggs and performed fewer nest visits per egg in the smaller nests (Ringgenberg et al., 2014a). However, in large aviaries smaller group-nests may be associated with an increased risk of overcrowding in preferred locations like the ends of rows (Clausen and Riber, 2012; Lentfer et al., 2013; Niebuhr, 2007; Riber and Nielsen, 2013). An alternative to smaller nests would be to partition larger nests internally into smaller areas, thereby increasing nest attractiveness without promoting overcrowding.

The use of partitions in nests has not been investigated before but Duncan (1978) reported that hens kept in natural conditions had their nest sites well concealed, most utilizing a vegetative cover. Knowing that hens are highly motivated to lay their eggs in an enclosed area (Appleby and McRae, 1986; Zupan et al., 2008), we hypothesized that a central partition would increase nest attractiveness.

Pre-laying behaviour and nest site selection are also affected by social factors, including the presence of hens (Riber, 2012), the identity of those hens (Ringgenberg et al., 2014a), and their relative social status (Freire et al., 1998). There are however conflicting results on whether social status affects nest site choice; i.e. whether higher ranking hens have preferential access to the most attractive nesting locations (Freire et al., 1998; Rietveld-Piepers et al., 1985). Social status affects priority access to other resources such as feed (Banks et al., 1979; Collias and Collias, 1967) and we hypothesized that if there was a preferred nest, social status would affect nest choice.

Therefore, we used a free choice preference test to assess relative preference among two group-nests, differing only in the presence or absence of a partition, and to examine the pre-laying behaviour of laying hens kept in small groups. Furthermore, we explored the social status of the hens, using David's score (David, 1987), in terms of nest choice and timing of egg-laying. We expected hens to show a relative preference for nests offering a greater degree of enclosure and structural complexity, i.e., the partitioned nest. We also expected that hens with a higher social rank would lay more eggs in the preferred nests and would perform fewer nest visits than subordinate hens.

Materials and methods

Animals, housing and treatments

The Cantonal Veterinary Office had approved this experiment (Bern, Switzerland, Approval BE27/13) and we followed the ethical guidelines of the International Society of Applied Ethology. The experiment was conducted over two consecutive trials, the first conducted from May to August 2013 and the second from September to December 2013. For each trial, 160 LSL non beak-trimmed laying hens were reared in one group with ad libitum access to water, feed, perches and sawdust bedding from 1 to 17 weeks of age. At 18 weeks of age, the birds were moved into the experimental barn and randomly assigned to eight pens in groups of 20 animals. The hens were individually marked with numbered PVC plates (8 × 6 cm) mounted on the back of the hens with two straps that went under the wings (as in Harlander Matauschek et al., 2010). Daigle et al. (2012) studied the behaviour of hens outfitted with similarly mounted sensors and found that aggressive interactions were not affected and that the hens habituated to the tags within two weeks in terms of resource use.

The pens were arranged in rows (two rows of three pens and one row of two pens) and were identical in size (3 m × 3 m) with sawdust bedding, three perches and ad libitum access to feed and water. To prevent seasonal effects of daylight, only artificial light was provided. At 18 weeks of age, the hens had 10 hours of light from 6:30 to 16:30 h with a 15 min twilight phase at the beginning and end of the day. Light exposure was then gradually increased by 30 min each week until 15 hours of light was reached at week 28 of age (1:30 to 16:30 h); the photoperiod then remained constant for the remainder of the study (until week 33 of age). The mean temperature was 21.3 ± 4.5 °C in the first trial and 16.1 ± 1.9 °C in the second trial. After the experiment, the hens were sold to local farmers.

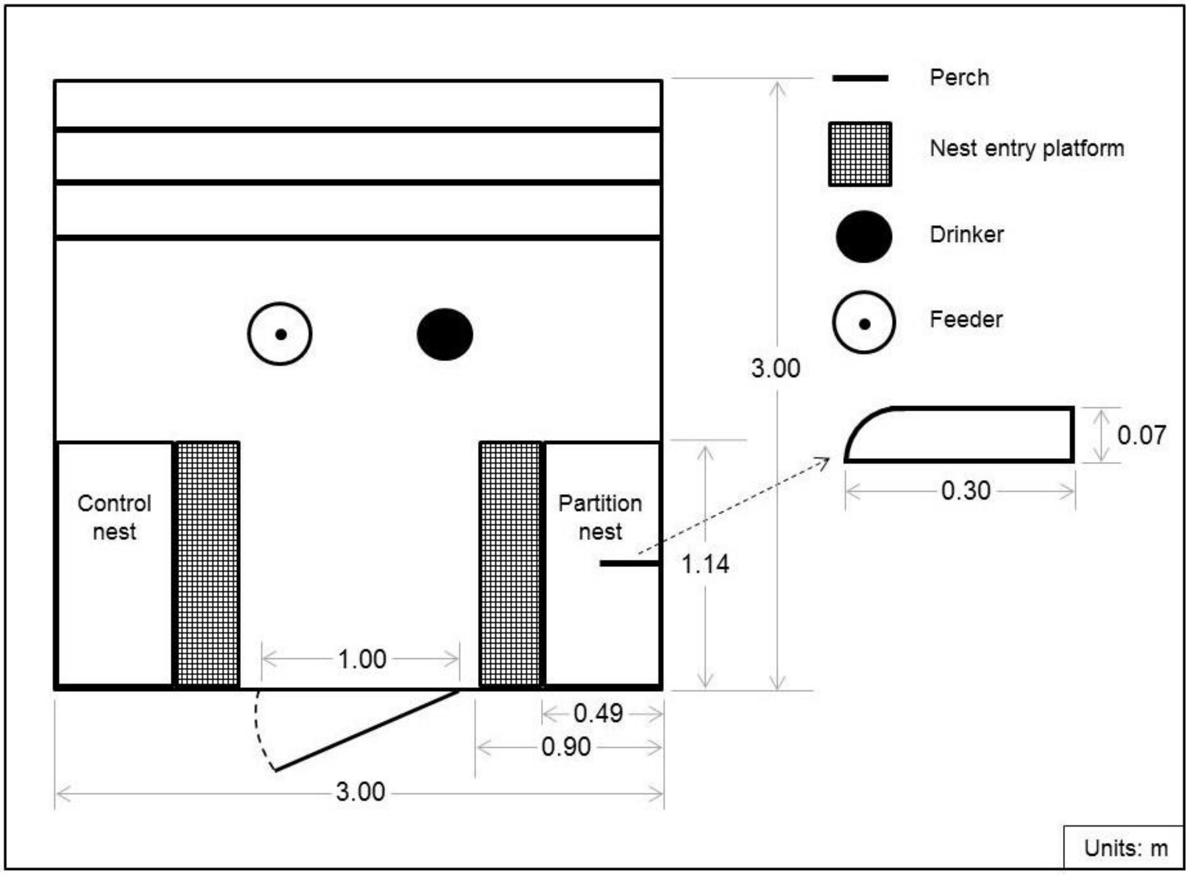

Fig.1. Top view of an experimental pen for a group of 20 hens and cross section of the partition. Height of nest entry platform: 0.70 m; height of perches: 0.60, 1.30, 1.60 m.

The hens had access to two commercial group-nests positioned across from each other on either side of the door (Fig. 1). The nest positions (left or right of the door) were balanced across pen and trial. The nests were identical with the exception that the partition nest contained a wooden partition 10 cm high and 1 cm wide in the middle of the nest (Fig. 1). The nests were of a rollaway type with a green Astroturf® covered floor sloping towards the front, allowing for manual egg collection underneath the nest entry platform. The nests were closed at the front with a plastic red curtain with two openings (width = 24 cm, height = 30 cm). The outer appearance of the nests was identical. The small width of the partition only marginally affected the actual surface area and volume of the two nest types (0.54 % less surface area and 0.1 % less volume in the partition nest compared to the control).

During week 28 of age, after the hens had been in full lay for four weeks, we switched the position of the nests in order to determine if hens would follow their preferred nest for egg-laying. In order to control for the effect of the nest vs. the partition, we swapped only the partition in half of the pens and the entire nest in the other half of the pens.

Data collection

The number of eggs per nest was recorded daily for the entirety of the experiment. A digital video camera (Samsung SCO-2080R) was located above each pen to provide a complete view of the nest exterior and surrounding floor; two infra-red video cameras (Conrad, BP258IR) were mounted in each nest.

Videos inside of the nests were taken on one day during week 24 of age and on one day during week 27 of age. Using continuous recording and focal animal sampling, we determined the timing of egg-laying of individual hens before possible disturbances associated with the nest switch.

Videos of the pen surroundings were taken on one day during week 24 of age and on one day during week 28 of age (after the nest switch). Using continuous recording and focal animal sampling, the number of nest visits of individual hens was assessed during the first 5 h of daylight (Table 1). Based on these observations, we further calculated the number of nest visits per egg for each nest (total number of visits per day/number of eggs per day) and the mean number of nest visits per hen per day.

Table 1 Ethogram of behaviours recorded for individual hens (adapted from Cordiner and Savory, 2001; Lentfer et al., 2011; O'Connor et al., 2011; Struelens et al., 2008)

Behaviour	Definition
Enter nest	Hen enters the nest, whereby the head and at least one foot are inside the nest
Exit nest	Hen moves out of the nest, whereby the head and at least one foot are outside of the nest
Aggressive interaction[a]	Rapid peck(s) between two hens, the retreating hen was defined as the loser of the interaction

[a] If there was no clear winner, the interaction was recorded but not used to calculated the David's Score

To assess the relative social status of individual hens, we used continuous recording and focal animal sampling for the first hour after lights-on on three separate days: the second day after the hens were moved to the experimental pens during week 18 of age (to allow for some time to become accustomed to the backpacks), and one day each within weeks 24 and 27 of age. All aggressive interactions between hens were observed during that time (Table 1), in a similar manner to other authors who had assessed the social status of hens in small groups (Cordiner and Savory, 2001; Freire et al., 1998; O'Connor et al., 2011; Schütz and Jensen, 2001). Based on these observations, a David's score was calculated for each hen (David, 1987) using SOCPROG 2.5 (Whitehead, 2009). Unlike other methods, this score takes the relative success of the opponent into account when determining the overall success of an individual (Gammell et al., 2003). The David's score has previously been used in studies on laying hens (O'Connor et al., 2011; Riedstra et al., 2013) as well as other birds (ex. Aplin et al., 2013; Bonisoli-Alquati et al., 2011). No assumptions of a linear hierarchy were made and hens were categorized using the David's score as either high (> 0.5 × SD), medium (0.5 to -0.5 × SD) or low (< -0.5 × SD) ranking (as in Markham et al., 2014).

All behavioural data were viewed and analysed with the behavioural observation software package INTERACT (Mangold International GmbH, 2011, Version 9, Arnstorf, Germany). The observer (N.R.) was blind to nest type when analyzing pen videos as the nests looked identical from the outside. For the egg collection and analysis of videos inside the nests blinding was not possible as the partition was clearly visible.

Statistics

We performed all statistical analyses with R Studio (version 0.98.507, Racine, 2012) as the user interface for R (version 3.0.1, R Development Core Team, 2012). Our primary outcome variable to assess relative nest preference was the proportion of eggs laid per nest per week. The secondary outcome variables were the number of visits per egg, the number of nest visits per hen, the number of hens observed laying eggs in the nests, the timing of egg-laying and the number of aggressive interactions.

To verify normal distribution of errors and homogeneity of variance, we examined plots of residual quantiles vs. quantiles of a normal distribution, the Tukey-Ascombe plot (residuals vs. estimates) and a histogram of the residuals. To satisfy these assumptions, data on the number of nest visits per egg were log-transformed. F-test statistics were used for models with normally distributed errors and likelihood-ratio tests were used for models based on the binomial distribution.

The mean proportion of eggs per week was analysed from week 21 of age (50 % production) to the end of the experiment (week 31 of age). A linear mixed effects model was fitted to the egg data with the function lme from the R package nlme (Pinheiro et al., 2013). Given that the distribution of eggs in the two nests was not independent, the outcome variable was the proportion of eggs in one nest minus 0.5 in order to test if it differed from zero, the null hypothesis being that the proportion of eggs in that nest did not differ from 0.5. To test the effect of the nest switch, phase (before switch, after switch) was included in the model as a fixed effect. The random term was pen nested in trial. The function stepAIC from the R package MASS (Venables and Ripley, 2002) was used to perform automatic stepwise backward model selection with Akaike's information criterion.

The mean number of nest visits per egg in weeks 24 and 28 of age was analysed with the function lme and model selection was performed as above. The random terms were pen nested in trial. Fixed terms were nest, week of age and nest switch phase as well as all two way interactions (except nest switch:week of age). The timing of egg-laying and the number of nest visits per hen during weeks 24 and 27 of age was also analysed as above. The random term was hen nested in pen nested in trial. The fixed terms were social rank, week of age, and their two-way interaction.

The number of hens observed laying their eggs in the nests at 24 and 27 weeks of age was analysed with a generalised mixed effects model with a binomial distribution (logit link function) with the function glmer from the R package lme4 (Bates et al., 2013). The random terms were trial, pen and an observation level term to control for overdispersion. Model selection was performed manually with a stepwise backward model selection based on the AIC (there is no method for using stepAIC on a model with a binomial distribution). The number of agonistic interactions was analysed in the same manner with pen nested in trial as fixed effects and week of age as a random effect. For the same data, repeatability estimates (R) (varying from 0 to 1) were calculated according to Nakagawa and Schielzeth (2010) to estimate the consistency of location of egg-laying. The function rpt.binomGLMM.multi for binary data from the R package rptR (Schielzeth and Nakagawa, 2013) was used with 1000 permutations and hen as the group identity.

Results

Eggs

From week 21 of age (50 % production) until week 27 of age (before the nest switch), hens laid 6062 eggs in the control nests and 8135 eggs in the partition nests. For the last 4 weeks of the experiment, after the nest switch, hens laid 3663 eggs in the control nests and 2757 eggs in the partition nests. Hens only laid 156 eggs on the floor, which is less than 1 % of the total number of eggs laid throughout the experiment.

We found evidence that a greater proportion of eggs were laid in the initial position of the partition nest than expected by chance ($F_{1,160} = 6.34$, $P = 0.013$) (Fig. 2a). Phase (before and after nest switch) was not included in the best model, therefore the hens continued to lay their eggs in the same nest position as before the switch.

The number of nest visits per egg varied according to week of age, with more nest visits in control nests than partition nests before the nest switch but not after the nest switch (interaction between nest and week of age: $F_{1,41} = 5.91$, $P = 0.02$) (Fig. 2b).

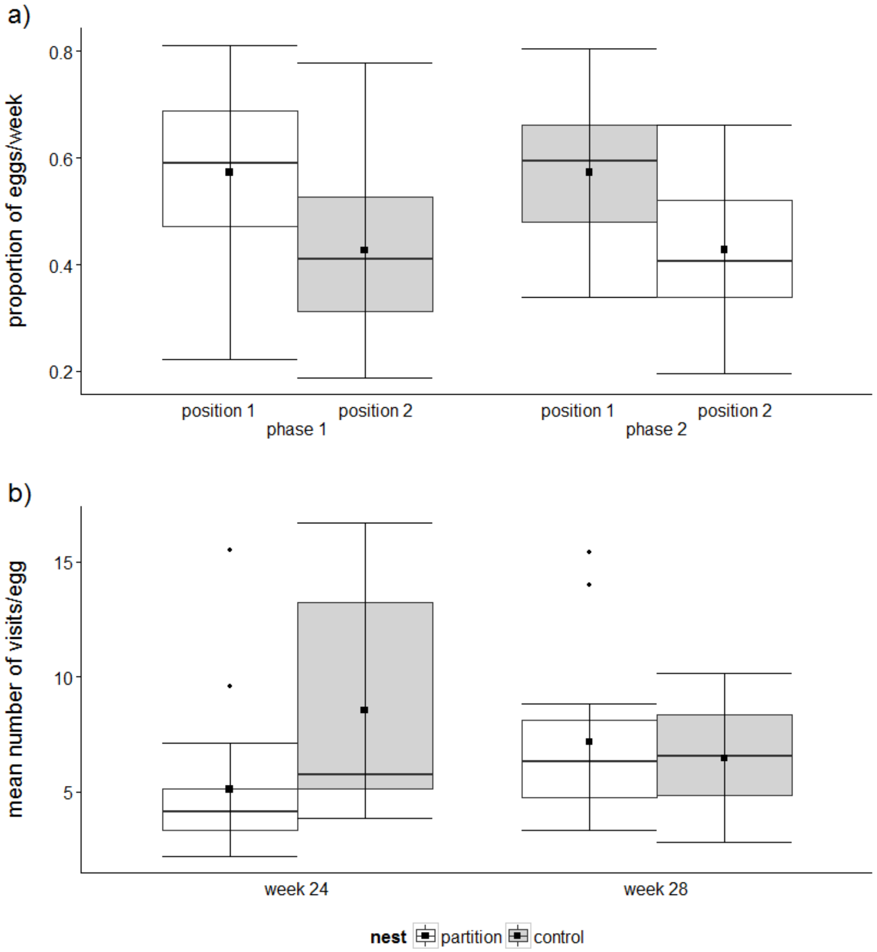

Fig. 2. (a) Mean proportion of eggs per nest in phase 1 (week 21 to week 27 of age) and phase 2 after the nest switch (week 28 to week 32 of age), position 1 refers to the initial position of the partition nest and position 2 refers to the initial position of the control nest. (b) Mean number of visits per egg (ratio of nest visits to eggs laid per nest) before (week 24 of age) and after the nest switch (week 28 of age). Boxplots: boxes represent 1st and 3rd quartile, the thick lines are the medians, the squares represent means, whiskers extend to most extreme data points (within 1.5 × interquartile range) and grey dots represent outliers.

The best model for the mean number of visits per hen per pen included the following fixed effects: the mean number of visits per egg tended to be affected by week of age with more visits in week 28 than 24 ($F_{1,160}$ = 3.33, P = 0.07), social rank with lower ranking hens making more nest visits ($F_{2,160}$ = 3.33, P = 0.017), and nest type with more nest visits in the control nests ($F_{1,160}$ = 5.53, P = 0.02) (Fig. 3).

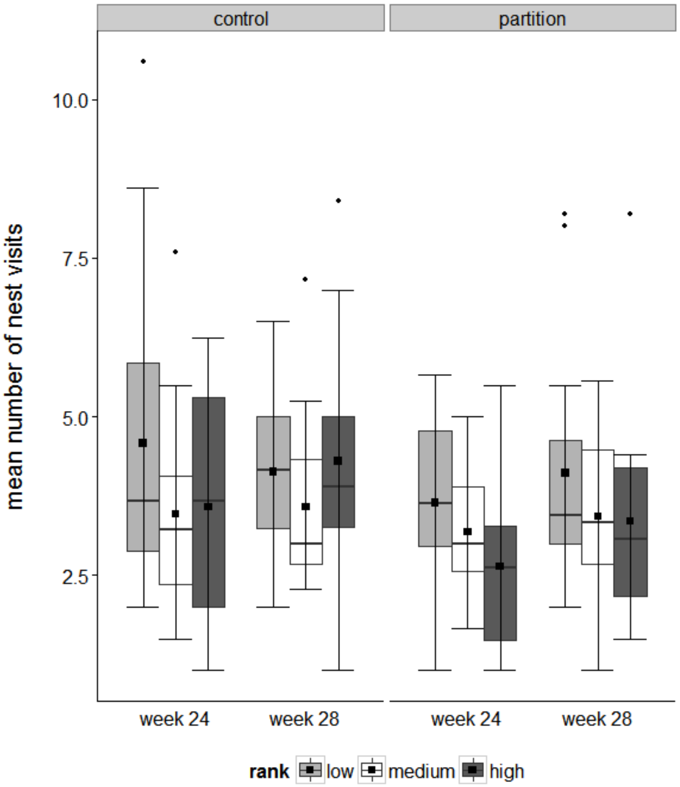

Fig. 3. Mean number of visits per hen per pen for control and partition nests, according to week of age and social rank. Boxplots: boxes represent 1st and 3rd quartile, the thick lines are the medians, the squares represent means, whiskers extend to most extreme data points (within 1.5 × interquartile range) and grey dots represent outliers.

We found a main effect of nest (X^2_2 = 13.42, P < 0.001) on the number of hens observed laying their eggs in the nests but the interaction between nest and social rank was not in the best model. There were more hens laying their eggs in the partition nest than the control nest on both days (partition nest: 8.67± 3.06 (mean ± SD), control nest: 6.20 ± 2.9; Z = 2.44, P = 0.01). In addition, more hens were laying their eggs in the partition nest on both days than in both nest types (partition nest: 8.67± 3.06, both nest types: 3.14 ± 1.70; Z = 5.83, P < 0.001). There were also more hens laying their eggs in the control nests on both days than in both nest types (control nest: 6.20 ± 2.9, both nest types: 3.14 ± 1.70; Z = 3.57, P < 0.001).

The hens observed laying their eggs on both days of observations (1 day during week 24 of age and 1 day during week 27 of age) (n = 273, pens = 15) were consistent in their choice of nest (R = 0.73, CI = [0.51, 0.75], P = 0.001).

The timing of egg-laying was affected by the interaction between social rank and week of age ($F_{2,214}$ = 3.00, P = 0.05) (Fig. 4): during week 24 of age, there was no evidence that rank affected the timing of egg-laying, but in week 27 of age, low ranking hens laid their eggs later in the day compared with the higher ranked hens. In addition, timing of egg-laying occurred later in the day in week 27 of age than in week 24 of age for all ranks.

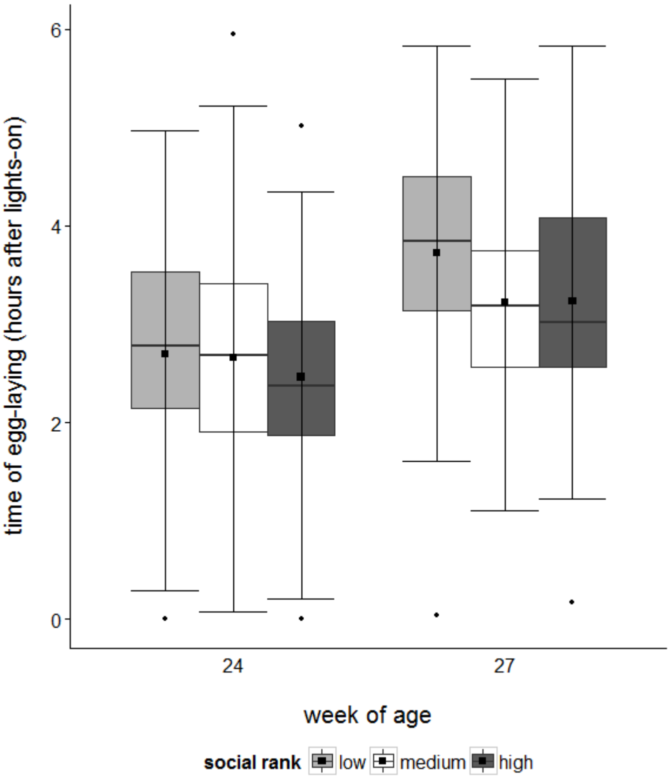

Fig. 4. Time of egg-laying (hours after lights-on) for the hens seen laying their eggs on both days of observation (n = 273, pens = 15). Boxplots: boxes represent 1st and 3rd quartile, the thick lines are the medians, the squares represent means, whiskers extend to most extreme data points (within 1.5 × interquartile range) and grey dots represent outliers.

The number of agonistic interactions decreased with age (X^2_2 = 770.92, $P < 0.001$) with an initial high mean number of 85.00 ± 39.81 interactions per pen during week 18 of age (mean ± SD), 30.00 ± 10.60 interactions during week 24 of age and 22.06 ± 7.69 interactions during week 27 of age.

Discussion

We report that laying hens in small groups showed a relative preference for group-nests with a partition as they laid a greater proportion of eggs and performed fewer nest visits per egg in these nests; however, this was the case only before the positions of the nests were switched. Unlike expected, social status did not seem to influence nest choice but we found that low ranking hens laid their eggs slightly later in the day and performed more nest visits compared with higher ranking hens.

Pre-laying behaviour and initial nest choice

From the assessment of our main outcome measure, proportion of eggs per nest, we conclude that hens showed a relative preference for the partitioned nest, at least until the nests were switched. In addition, the lower number of visits per egg in the partitioned nests clearly points to a preference for these nests. This measure – number of nest visits per egg – has previously been shown to be increased in least preferred nests (Buchwalder and Fröhlich, 2011; Ringgenberg et al., 2013, 2014b) but only if there were also fewer eggs in the least preferred nest (Stämpfli et al., 2011, 2013). The number of visits per egg gives us information on the nest choosing phase of pre-laying behaviour, which begins 1-2 h prior to oviposition (Cooper and Appleby, 1995; Duncan and Kite, 1989; Kruschwitz et al., 2008a). The increased number of nest visits per egg in the control nest points to an extended nest choosing phase and may be indicative of frustration (Wood-Gush, 1972; Yue and Duncan, 2003).

Hens likely preferred the partitioned nests due to the added cover that the partition provided, similarly, the use of structural elements in pens is known to be attractive to chickens (Cornetto and Estevez, 2001; Rodriguez-Aurrekoetxea et al., 2014). In this experiment, the level of enclosure was likely higher in the partition nest, and as in our previous study (Ringgenberg et al., 2014a), hens preferred nests offering a greater degree of enclosure. However, although the proportion of eggs laid in the partition nest was significantly higher than 0.5, hens in all pens also laid eggs in the control nest. Therefore, the control nests were either still attractive enough for egg-laying or the partitioned nests were too small for all hens. In addition, there were four pens in which hens preferred the control nests both in terms of proportion of eggs and number of visits per egg. Although these pens were all located in corners of the barn, the side of the pen and the side of the barn on which the control nest was varied in each pen.

Nest position effects

We performed the nest switch in order to check whether hens showed a strong enough preference for the partition to switch egg-laying location after the swap. Contrary to our expectations, the hens did not switch nests. Multiple explanations can explain this nest position consistency. Firstly, the control nests were not unattractive enough to warrant changing egg-laying position, an important point considering that we used commercial nests that were already shown to be attractive (nest CN1 in Buchwalder & Fröhlich, 2011) and some hens consistently laid their eggs in the control nest. Secondly, the nest partition was only important at the beginning of lay and once the hens were accustomed to laying their eggs in a nest on a specific pen side, they did not switch again seemingly preferring familiarity over greater structural complexity. Thirdly, some hens may not have noticed that the nests were swapped. Riber et al. (2013) found that after moving groups of hens to different pens with alternate nest arrangements, some hens were nest conservative (they continued to lay their eggs in the most isolated nest) while others were location conservative (they continued to lay their eggs in the same location). These results may also support the first explanation, that our control nest was not unattractive enough. In Riber et al. (2013), the most isolated nests were more attractive even after the pen switch, however, the nests were not rollaway nests so the hens may also have been attracted by eggs previously laid in the nests. In the present study, sixty-five percent of the hens visited both nests on the day after the nest switch which is slightly more than during week 24 of age (57 %) and suggests that at least some hens noticed the nest switch. It must however be noted that the initial preference for the partition nest may still be important as an attractive nest at the start of lay is of particular importance to reduce floor eggs.

The number of visits per egg followed a similar pattern to the distribution of eggs before the nest switch (preference for partition nests); however, there was no difference between nest types in the number of visits per egg after the nest switch. This measure may therefore be more informative than the proportion of eggs and tells us that the hens no longer found a nest more attractive than the other. Alternatively, the hens that chose the nest with the partition before the switch may have performed more nest visits after the switch because they suddenly had the least preferred nest in their usual nest position, whereas hens choosing the control nest before the switch now had a more attractive nest in its place and performed fewer visits. Interestingly, unlike in other experiments (Ringgenberg et al., 2014a; Ringgenberg et al., submitted for publication) there was no increase in the number of visits per egg over time although this may be because the current experiment did not go beyond week 28 of age and/or more attractive nests were used.

Social status and nest choice

The number of agonistic interactions decreased sharply over time with most of the aggression occurring soon after re-grouping at 18 weeks of age, as expected from other studies (O'Connor et al., 2011; Cordiner et al., 2001; Rushen 1982). We found no evidence that social status as measured by David's score had an effect on nest choice. As Rietveld-Piepers et al. (1985) suggested, the lack of an effect may be because nests in the wild would not be in short supply and are thus not a resource over which hens compete. These authors also found no relation between social status and nest choice, nor between social status and floor eggs. In captivity, however, nests that are more attractive may be in short supply (i.e. there are only two corner nests) and competition over them likely occurs especially considering the high density of hens per nest (100 and 120 hens per m2 of nest surface area in Switzerland and the EU, respectively) (Animal Welfare Ordinance, 2008; CEC, 1999). Hunniford et al. (in press) observed increased levels of aggression during the peak egg-laying time in furnished cages, suggesting competition over the designated nest site. However, it is difficult to compare the behaviour of hens housed in furnished cages and non-cage housing systems due to the differences in nest enclosure, space allowance, substrate availability and freedom of movement.

Although social status did not affect nest choice in the present study, we found that low ranking hens performed more nest visits and laid their eggs slightly later in the day compared with medium and high ranking hens. Similarly, Freire et al. (1998) reported that subordinate hens in small groups of four in a round pen with no nest boxes (all hens laid their eggs in one hollow) showed more locomotion and less resting behaviour in the 10-15 minutes prior to oviposition. Unlike Freire et al. (1998), we saw very little overt aggression in the nest sites although subtle dominance displays may have been missed. The prolonged searching phase (characterized by many nest visits) that the low ranking hens showed in our study was perhaps due to their inability to settle in the nests because of more dominant hens occupying them. Although this was only the case on the second day of observation in week 27 of age, the subordinate hens likely delayed their timing of egg-laying because of their prolonged searching phase. It is known that hens can delay the timing of egg-laying, especially when acutely stressed (Hughes et al., 1986; Reynard and Savory, 1999). We also found that all hens laid their eggs slightly later in the day at 27 weeks of age than at 24 weeks of age which is likely due to the increasing interval between eggs in the same egg sequence (Icken et al., 2008).

Conclusion

Small groups of hens with access to two commercial group-nests differing only in their internal structure (partition vs. no partition) showed a relative preference for the partitioned nest as measured by the higher proportion of eggs and the lower number of visits per egg in the partition nests. However this preference was no longer seen after the position of the nests was swapped during the 28th week of age. Thus this initial nest preference likely became associated with nest location. We also show that a low social status, as measured by David's score, does not affect nest choice per se, but is associated with a prolonged searching phase and a slightly later timing of egg-laying compared with a higher social status. Nest partitions could be an easy addition to commercial group-nests in aviary systems to improve their attractiveness.

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Chapter 4

EFFECTS OF VARIATION IN NEST CURTAIN DESIGN ON PRE-LAYING BEHAVIOUR OF DOMESTIC HENS

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Abstract

Laying hens in loose-housing systems select a nest daily in which to lay their eggs among many identical looking nests and often prefer corner nests. We investigated whether heterogeneity in nest curtain appearance – via colours and symbols – would influence nest selection and result in a more even distribution of eggs among nests.

We studied pre-laying behaviour in groups of 30 LSL hens across two consecutive trials with eight groups per trial. Half of the groups had access to six identical rollaway group-nests, while the others had access to six nests of the same type differing in outer appearance. Three colours (red, green, yellow) and three black symbols (cross, circle, rectangle) were used to create three different nest curtain designs per pen. Nest position and the side of entrance to the pens were changed at 28 and 30 weeks of age, respectively, whereby the order of changes was counterbalanced across trials. Nest positions were numbered 1 to 6, with nest position 1 representing the nest closest to the pen entrance. Eggs were counted daily from week of age 18 to 33. Nest visits were recorded individually with an RFID system for the first 5 h of light throughout weeks 24-33.

Hens with nests differing in curtain appearance visited fewer nests than hens with identical nests, and this difference increased with age. We found no other evidence that curtain appearance affected nest selection and hens were inconsistent in their daily nest selection. A high proportion of eggs were laid in corner nests especially during the first three weeks of lay. The number of visits per egg depended upon nest position and age: it increased with age and was higher after the door switch than before in nest position 1, whereas it stayed stable over time in nest position 6. At 24 weeks of age, gregarious nest visits (hens visiting an occupied nest when there was at least one unoccupied nest) and solitary nest visits (hens visiting an unoccupied nest when there was at least one occupied nest) accounted for a similar amount of nest visits, however, after the door switch, gregarious nest visits made up more than half of all nest visits, while the number of solitary nest visits had decreased. The visual cues were too subtle or inadequate for hens to develop individual preferences while nest position, entrance side, age and nest occupancy affected the quantity and type of nest visits.

Keywords: laying hens, group-nests, nest selection, egg-laying, pre-laying behaviour

Introduction

Domestic hens have retained a high motivation to seek a protected nest site to lay their eggs even though they cannot isolate themselves from the flock (Cooper and Appleby, 1995, 2003). As an indication of this motivation, approximately one hour before oviposition, all hens, regardless of their environment, will attempt to perform pre-laying behaviour with the normal process occurring as follows: hens explore their environment before visiting various nest sites and choosing a site for oviposition (Cooper and Appleby, 1995; Duncan and Kite, 1989; Kruschwitz et al., 2008a). Pre-laying behaviour is performed to maximize the likelihood of successful development and hatch of the eggs (Duncan, 1998). It is triggered by internal stimuli and its manifestation depends on the environment (Duncan and Kite, 1989; Hughes et al., 1989). For example, hens without access to a nest pace repeatedly and attempt to escape prior to egg-laying, which is a sign of severe frustration (Cooper and Appleby, 1997; Duncan and Wood-Gush, 1972; Wood-Gush, 1975).

The physical characteristics of commercial group-nests, which are used in most non-cage systems, such as flooring (Duncan and Kite, 1989), enclosure (Kruschwitz et al., 2008b), size (Ringgenberg et al., 2014), curtain type (one-piece or striped) and floor slope (Stämpfli et al., 2011, 2012) influence nest choice in laying hens. In addition, social factors play a role in nest selection. For example, domestic hens often choose to enter occupied nests when empty ones are available (Riber, 2010, 2012a), even if housed in semi-natural conditions with outdoor access (Riber, 2012a). Riber (2012b) suggested that such "gregarious nesting" may be an anti-predator strategy, although other explanations such as individual preferences for specific nests or nest positions cannot be excluded. The latter is particularly relevant in systems where hens have to choose between identical nests arranged in long rows. In such an environment, a disproportionately high number of hens lay their eggs in the nests closest to the entrance of the barn or at the ends of rows, which can result in overcrowding (Clausen and Riber, 2012; Huber-Eicher, 2004; Lentfer et al., 2011, 2013; Niebuhr, 2007; Riber, 2010; Riber and Nielsen, 2013). Overcrowding not only results in welfare problems but also in economic losses due to cracked and/or dirty eggs. Given that domestic hens usually lay their eggs within the first 5 h of daylight (Lentfer et al., 2011; Riber, 2010) and that most producers provide just enough nest space to meet minimal requirements (120 and 100 hens per m2 of nest surface area in the EU and Switzerland, respectively), the problem of overcrowding may be exacerbated if hens are using nests unevenly (Animal Welfare Ordinance, 2008; CEC, 1999).

Appleby and McRae (1986) suggested that heterogeneous nests may reduce the risk of overcrowding, as it would help laying hens to distinguish between the nests. For example, variation in nest appearance, such as nest colour (Huber-Eicher, 2004), may result in a more even distribution of hens across nests. Rietveld-Piepers et al. (1985) reported that some hens were consistent in nest choice over a long period of time, others only within clutches, and others were inconsistent. Similarly, some hens appeared to prioritize the nest location (i.e. kept laying in the same location when the nest was moved), while others preferred the nest itself (i.e. followed the nest if it was moved) (Riber and Nielsen, 2013; Sherwin and Nicol, 1994). Therefore, we predict that nests differing in outer appearances will affect daily nest choice of laying hens by exploiting individual variation in nest preference.

Symbols on nest boxes have been used successfully with wild flycatchers (Ficedula albicollis) to study social learning (Jaakkonen et al., 2013; Seppänen and Forsman, 2007). In laying hens, studies have shown that they can differentiate between a black cross and circle (Railton et al., 2010) and between vertical and horizontal lines (Werner et al., 2005). They can also easily discriminate between the colours red and green (Foster et al., 1995; Patterson-Kane et al., 1997), while chicks can categorize the colours orange, green, red, and blue (Ham and Osorio, 2007). Laying hens also showed preferences for yellow painted nests compared with red, blue and green nests (Huber-Eicher, 2004). In addition, Railton et al. (2014) showed that laying hens easily discriminated between red and green objects of different shapes (triangular vs. rectangular) and transferred this discrimination to photographs of the coloured shapes. However, they were not able to do this when the different objects or photographs were of the same colours. Therefore, laying hens should be capable of differentiating between different symbols and colours on nest curtains.

We investigated the effects of distinctive visual stimuli on nest curtains on nest use in laying hens. We expected that these visual stimuli on nest curtains would result in a more even distribution of eggs among nests compared with groups of hens with access to identical nests. In addition, we expected that the pre-laying behaviour of hens with the visual stimuli nests would be characterized by fewer different nests visited, by fewer nest visits per egg, and by a higher consistency in nest selection compared with the pre-laying behaviour of hens with access to identical nests. We also predicted that the influence of conspecifics (i.e. gregarious nesting) would be more pronounced in pens with identical nests than in pens with the visual stimuli in terms of nest choice. Lastly, we investigated the effects of nest position relative to the pen entrance on pre-laying behaviour.

Materials and methods

Animals and housing

The experiment was conducted in two trials, from November 2012 to February 2013 and from April to July 2013 at the Research Centre for Proper Housing of Poultry and Rabbits in Zollikofen (ZTHZ), Switzerland. For each trial, 240 non-beak trimmed day-old chicks of the Lohmann Selected Leghorn breed were purchased from a hatchery. Chicks were reared in one pen (18 m2) until nine weeks of age when they were split into two groups of 120 animals in two pens of 18 m2 each in order to meet the minimal space requirements of the Swiss Animal Welfare Ordinance. The animals had ad libitum access to water and feed and each pen was equipped with two perches and sawdust bedding. At 18 weeks of age, the birds were moved into the experimental barn and randomly assigned to eight pens in groups of 29-31. The sample size was chosen based on a power analysis using estimates of variation for the proportion of eggs per nest from reported values in Clausen and Riber (2012) and Ringgenberg et al. (2014). Given that we were limited in space in our experimental barns, two consecutive trials were conducted to ensure an adequate sample size.

The experimental pens were arranged in two rows of four and were identical in size (3 × 4 m) with two 3 m long mushroom-shaped plastic perches at 0.9 m and 1.4 m from the ground and 0.4 m apart horizontally. Each pen contained sawdust for litter, six nests (see detailed description below), and a round feeder and drinker with eight nipples provided ad libitum commercial layer mash and water, respectively (Fig. 1a). Pens were visually isolated from each other with white tarps and had individual ventilation outlets.

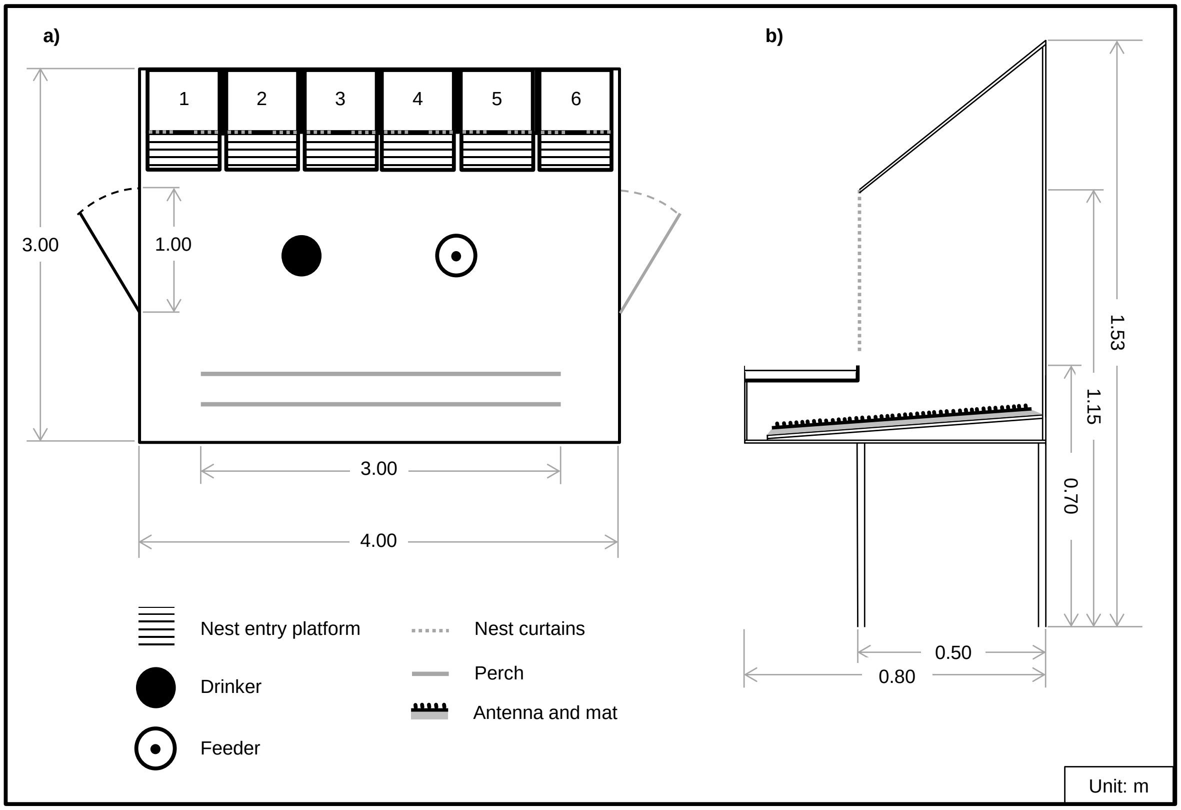

Fig. 1. (a) layout of pen with row of six rollaway nests and two entrance sides, numbers 1 to 6 represent the nest positions with nest position 1 always beside the entrance. (b) side view of one nest with RFID antennas on the nest floors, the internal nest surface area was 0.5 × 0.6 m.

Six wooden group-nests were placed in a row along one side of each pen (on the same side of every pen) (Fig. 1a). The nests were custom-built for this experiment to fit two radio-frequency-identification (RFID) antennas (Benzing, Gantner Pigeon System, GmbH) (Fig. 1b). The antennas made up the floor of the nests and were covered by green Astroturf® mats. The eggs rolled towards the front of the nest underneath the nest-entrance platform where they were manually collected. The nest entrance platform was 0.7 m above the ground and covered in a plastic grid. Each nest had two curtains on the front (0.45 × 0.30 m) with an opening of 0.20 m in between. The mean light intensity in the nests was 1.2 ± 0.3 lux. The nest surface area available per hen was 0.06 m2, which is six times the minimal legal requirement in Switzerland (Animal Welfare Ordinance, 2008).

To prevent seasonal effects of daylight, only artificial light was provided. Three 75-W Halogen light bulbs were hung equidistant above the nests in order to avoid different lighting conditions within the nests. At 18 weeks of age, the hens had 10 hours of light from 6:30 to 16:30 h with a 15 min twilight phase at the beginning and end of the day. Light exposure was then gradually increased by 30 min each week until 15 hours of light was reached at week 28 of age (1:30 to 16:30 h); the photoperiod then remained constant until the end of the study (week 33 of age). The average light intensity at bird height on the pen floors was 23.3 ± 0.3 lux in both trials and the average temperature was 16.2 ± 0.4 °C in trial 1 and 19.0 ± 3.8 °C in trial 2. After the experiment, the hens were sold to local farmers. We followed the ethical guidelines of the International Society of Applied Ethology (ISAE) and the Cantonal Veterinary Office (Bern, Switzerland, Approval BE 97/12) approved the study.

Treatments

Half of the pens were assigned to a control treatment with all nests being identical (red curtains), while the other half was allocated to a visual stimuli treatment with the nests having different colours and symbols on the curtains. Within each trial, the pen positions in the barn alternated between control and visual stimuli pens. The visual stimuli included three curtain colours (red, green, and yellow) and three black symbols with the same surface area (0.02 m2) (cross, open circle, and rectangle). Reflectance spectra of the colours used under the experimental conditions are presented in the appendix.

Coloured tarps were used for both the curtains and the symbols, which were sewn centrally on the curtains, in order to test materials that are easily available. The inside of all curtains, regardless of treatment, was red; this ensured that only one characteristic differed between nests. Nine different combinations of the colours (red, yellow, and green) and the symbols (cross, circle, and rectangle) created visual stimuli on the nest curtains of visual stimuli pens. We randomly picked three of these combinations for each pen. Since each pen contained six nests, we used each combination twice so that if hens clearly preferred a colour/symbol combination, that nest would not be overcrowded.

Given that each pen had two entrances (Fig. 1a), the pen configuration allowed for control of human access to the pens by blocking off entire corridors and accessing the pens either from the center corridor or from the side corridor. A nest position code was assigned for statistical analysis: position 1 was always the nest beside the entrance. The experiment was conducted in two distinct phases of nest position and the side used for researchers and caretakers to access the pen was counterbalanced in each trial:

- Trial 1 (Nov. 2012 - Feb. 2013, 240 hens): the positions of the nests were changed at the beginning of week 28 of age and the entrance side was changed at the beginning of week 30 of age.
- Trial 2 (Apr. - Jul. 2013, 240 hens): the position of the entrance was changed at the beginning of week 28 of age and the positions of the nests were changed at the beginning of week 30 of age.

The positions of the nests were changed in the following manner: all curtains were moved one position to the left (with the leftmost curtain moved to the opposite side) and the nests were moved in the following manner: nests 1, 2, 3 switched position with nests 4, 5, 6 (but staying in the same order in regards to entrance side).

Radio frequency identification system (RFID)

An RFID system from Gantner Pigeon System GmbH (Schruns, Austria) recorded nest use. Each hen had a RFID passive tag (0.4 × 2.2 cm, Hitag S, 2048 bits, 125 kHz) encased in a green plastic wing band (1.0 × 3.5 cm, Roxan) attached to the right leg using a yellow plastic coil leg ring. All nests were outfitted with two antennas (0.76 × 0.30 m, Benzing, Gantner Pigeon System GmbH, Model PLB 765) (Fig. 1b), covering the entire floor surface of the nest including the area underneath the nest entrance platform. To prevent the RFID antenna from capturing signals from the tags when the hens were standing on the nest entrance platform and not in the nest itself, a 2 mm thick metal plate was placed underneath the grid. When a hen stepped into a nest, the RFID tag was activated by the radio frequency field generated by the antennas and transmitted a uniquely encoded radio signal with its identification information to the antenna. The antennas were connected in groups of six to a network node (two nodes per pen) which decoded the signals, performed the synchronization between the antennas and sent the data to 15 electronic registration devices (Benzing M1, Gantner Pigeon System GmbH) through cables. The registration devices then stored the signals, while allowing logging of multiple signals at the same time, and then transmitted the data to two multiport hubs (Multiplexer, Com Server Moxa 8-port NPort 5650-8, Brea, U.S.A) connected to two personal computers. Each tag reading was recorded in a CSV file with the date, time (0.1 s), tag identification number and antenna number by a program created by the RFID manufacturer.

The RFID system was validated by Gebhardt-Henrich et al. (2011) for detecting the use of outdoor range by large flocks of laying hens and was shown to be an effective means of recording bird movement between specific areas. Prior to the current study, we tested the experimental setup in one pen with 10 individually marked hens and video recordings to ensure that antennas properly recorded each nest visit. This preliminary effort allowed us to identify some recording errors, which occurred when the hens entered a nest with only one leg (i.e., did not fully enter the nest). Therefore, only visits lasting 10 s or longer were kept in the dataset. Problems reported in Gebhardt-Henrich et al. (2011), such as the hens moving over the antennas too fast to be registered would be unlikely to occur in the current experiment given that the antennas were in the nests (as opposed to the entrance/exit to ranging areas). Prior to moving the hens into the experimental pens, we tested all tags and antennas to ensure all were functioning. For a single day at 24 weeks of age, videos were taken in four pens from which 15 min were analysed to compare each nest entrance and exit with the RFID data for validation purposes. For all pens, more than 90 % of all visits that were visually observed were correctly recorded by the RFID system with a time difference of ± 5 s. We detected no false positives. Seven days in the first trial were not used due to recording problems (adaptors for the antennas needed to be replaced) and a pen in trial 2 had to be removed due to antennas having hardware failures.

Data collection

Eggs laid in the nests and on the floor were collected and recorded daily between 10:00 h and 13:00 h from 18 weeks of age until the end of the experiment when the hens were 33 weeks of age.

Data on nest use were collected with the RFID system on each day of the experiment starting when lights came on until 5 h had elapsed from weeks 24 to 33 of age. We then calculated the number of visits per egg (the number of times hens entered a specific nest to lay one egg), the number of different nests visited, and the proportion of visits to nests according to occupancy status (Table 1). The last two parameters did not take nest position into account for ease of analysis.

Table 1 Classification of nest visits according to nest occupancy status (Riber, 2010). Each time a hen entered a nest, this visit was classified in one of four categories depending on the status of the visited and non-visited nests (occupied or not).

All occupied^a	all nests, including the one visited, were occupied
All unoccupied^a	all nests, including the one visited, were empty
Occupied^b	nest was occupied by one or more hens, but at least one other nest was empty
Unoccupied	the nest visited was unoccupied while at least one other nest was occupied

Statistical analysis

All statistical analyses were performed with R Studio (version 0.98.507, Racine, 2012) as the user interface for R (version 3.0.1, R Development Core Team, 2012). The primary outcome variable to evaluate nest use and pre-laying behaviour was the proportion of eggs laid per nest. Our secondary outcome variables were mean number of nest visits per egg, mean number of different nests visited per week and proportion of nest visits per nest occupancy status.

Model selection

Model selection was done with an information theoretic approach using the Bayesian Information Criterion (BIC) to objectively retain the model that describes the data in the most parsimonious way (Burnham and Anderson, 2004; Richards et al., 2011). We used the function dredge from the package MuMIn (Barton, 2013), which performs an automatic model selection with all subsets of the full model (down to the null model) and ranks each model by its BIC. This approach for model selection takes into consideration that more than one model may be equally good in explaining the data. Therefore, if the BIC between the highest ranked models differed by two or less and if the models were nested, we chose the simpler model (Burnham and Anderson, 2002; Raftery, 1996) as was the case for the outcome variable proportion of eggs.

In Table 2, we show the selected models based on BIC. In addition, we provide the BIC weight which is the probability that this model is the best fitting among all possible models where the sum of all model weights in the set is 1 (Symonds and Moussalli, 2010). We also report the relative likelihood of the chosen model vs. the null model, termed the evidence ratio (Burnham and Anderson, 2004).

Table 2 Explanatory variables in selected models for each outcome variable and associated selection criteria

Outcome variable	**Explanatory variables**[a]	**BIC**[b]	**Weight**[c]	**ER**[d]
Proportion of eggs	nest position × week of age, nest position × entrance change	44932	0.25	$3.0e^{92}$
Visits per egg	nest position × week of age, entrance change	1503	0.35	$5.1e^{37}$
Mean number of different nests visited	treatment × week of age	93.7	0.61	$3.7e^{20}$
Proportion visits per nest status	entrance change × occup, week of age × occup	875.3	0.90	$5.9e^{241}$

[a] final models also included main effects that are shown in interactions
[b] Bayesian Information Criterion
[c] BIC weight which is the probability of the given model to fit the data best within all possible combinations (e.g. a BIC weight of 0.50 means that there is a 50 % chance that this is the best model)
[d] Evidence Ratio between the chosen model and the intercept only model

To verify normal distribution of errors and homogeneity of variance, we examined plots of residual quantiles vs. quantiles of a normal distribution, the Tukey-Ascombe plot (residuals vs. estimates) and a histogram of the residuals.

The following planned contrasts were incorporated in the model:

- Nest position 1 compared with nest positions 2-6 (to test effect of the entrance)
- Nest position 6 compared with nest positions 2-5 (to test effect of corner nest we took nest position 1 away as it was confounded with the entrance effect)

Repeatability

Repeatability estimates are the most commonly used statistic to estimate behavioural consistency (Bell et al., 2009). They describe the proportion of variation that is consistent in repeated measurements of the same subject (Lessels and Boag, 1987; Nakawaga and Schielzeth, 2010). We calculated repeatability estimates according to Nakagawa and Schielzeth (2010) in order to evaluate the consistency of egg-laying location and the behavioural consistency of nest visits for individual hens (whether hens visited the same nests over time). Repeatability estimates range from 0 to 1 and when measurements from individuals or groups are consistent over time, then repeatability estimates are high and the measurement is repeatable (close to 1) (Bell et al., 2009).

A multiplicative overdispersion model for proportion data was used (function rpt.binomGLMM.multi in R package rptR (Schielzeth and Nakawaga, 2013)) with a logit link and 1000 permutations to calculate repeatabilities (R) for the following outcome variables: daily proportions of eggs per nest per pen and daily proportions of nest visits per nest per hen. They were calculated for the following time periods: 1) week 24-27, 2) week 28-29 and 3) week 30-33 of age to take account of nest and door changes. To assess the calculated repeatability estimates, we used confidence intervals: if they included zero, the null hypothesis (R = 0) could not be rejected. P-values are also provided, they represent the proportion of randomizations resulting in Rrandomized > Robserved (Nakawaga and Schielzeth, 2010).

To assess whether the repeatabilities differed between treatments (i.e. if hens were more consistent in nest choice in the visual stimuli treatment), the repeatability estimates were used as an outcome variable in a linear mixed effects model with treatment and time period and their interactions as fixed effects. Hen nested in pen nested in trial were the random terms.

Egg data specifics

The proportion of eggs laid in each nest per day was analysed from the beginning of egg-laying (week 18 of age) until week 33 of age. A generalised linear mixed effects model with a binomial distribution (logit link function) was fitted to the data with the function glmer from the R package lme4 (Bates et al., 2013). The random terms were pen nested in trial. Treatment (visual stimuli vs. control), week of age (continuous variable), nest position (1 to 6), entrance change (before, after) and nest change (before, after) were included as fixed effects (plus all two-way interactions except week of age × nest change and week of age × entrance change).

Behaviour data specifics

Linear mixed effects models were calculated for the behavioural data from weeks of age 24 to 33 using the function lme in the R package nlme (Pinheiro et al., 2013). Random effects were pen nested in trial. Model selection and examination of model assumptions were performed as for the egg data.

The mean number of nest visits per egg for each nest position (means per week) was log-transformed prior to analysis; the fixed effects in the full model were identical as for the egg data. For the mean number of different nests visited per week (means per hen per pen), the full model included the same fixed effects as the egg data but without nest position and nest occupancy status. The full model for the proportion of nest visits per nest occupancy status (averaged over pen and week) included the same fixed effects as above but with nest occupancy status instead of nest position. As non-binomial proportions data was the outcome variable it was logit transformed to fulfil the assumptions of linear modeling (Warton and Hui, 2011).

Results

Eggs

Throughout the study, we collected a total of 41 627 eggs from the first egg laid until week 33 of age, of which 148 were laid on the floor (0.36 %). As expected, hens reached 50 % production by week 21 of age and 100 % production by week 24 of age. The number of eggs per nest ranged from 0 to 13 in each week of age and the repeatabilities (R) of the proportions of eggs per nest were low for all time periods and both treatments (all $R < 0.1$, all confidence intervals included 0 and all p-values > 0.05). In weeks 18 and 19 of age, most of the eggs laid were in nest position 1 regardless of treatment (Fig. 2).

Table 3 Model estimates and associated 95 % confidence intervals before and after the entrance change for the proportion of eggs per nest (a) and for the proportion of nest visits (b)

	(a) nest position × entrance change					
	1	2	3	4	5	6
before	**0.20 (0.19,0.21)**	0.16 (0.15,0.17)	0.17 (0.17, 0.18	0.17 (0.16, 0.18)	0.14 (0.13, 0.14)	**0.16 (0.15, 0.16)**
after	**0.16 (0.15,0.17)**	0.14 (0.13, 0.15)	0.17 (0.17, 0.18)	0.19 (0.18, 0.20)	0.15 (0.14, 0.16)	**0.19 (0.17, 0.19)**
	(b) nest occupancy status × entrance change					
		occupied	unoccupied	all occupied	all unoccupied	
before		0.42 (0.39, 0.45)	0.35 (0.33, 0.38)	0.19 (0.17,0.21)	0.05 (0.05, 0.06)	
after		0.50 (0.47, 0.53)	0.31 (0.28, 0.33)	0.18 (0.16, 0.20)	0.03 (0.03, 0.04)	

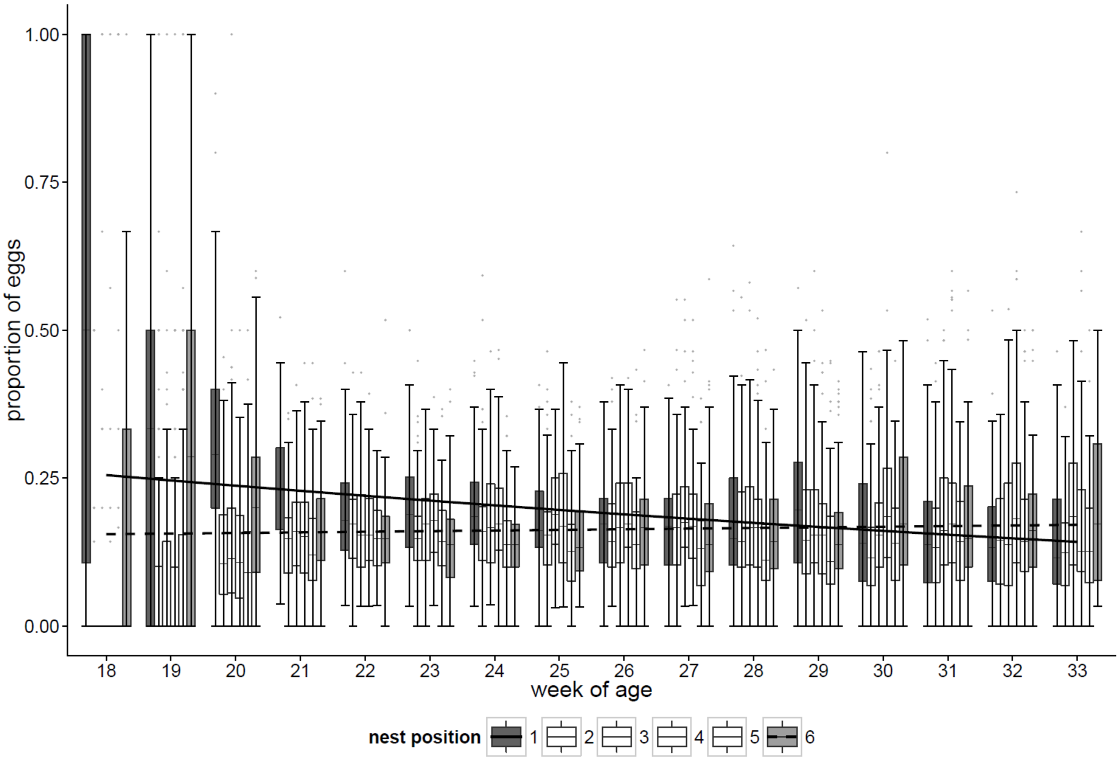

Fig. 2. Mean proportion of eggs per nest and week of age, the solid line is the model estimate for nest position 1 (nest closest to the entrance) and the dashed line for nest 6. Boxplots: boxes represent 1st and 3rd quartile, the lines in the boxes are the medians, whiskers extend to most extreme data points (within 1.5 × interquartile range) and grey dots represent outliers.

Over the course of the study, the proportion of eggs laid in nest position 1 decreased compared with the rest of the nest positions while the proportion of eggs laid in nest position 6 increased compared with nest positions 2-5 (interaction between nest position and week of age, Fig. 2). Similarly, after the entrance change, the proportion of eggs laid in nest position 1 decreased compared with the rest of the nest positions, while the proportion of eggs laid in nest position 6 increased (interaction between nest position and entrance change, Table 3a). Although treatment did not appear in the best fitting model, we show in figure 3 that the proportion of eggs per symbol and colour in visual stimuli pens was very similar.

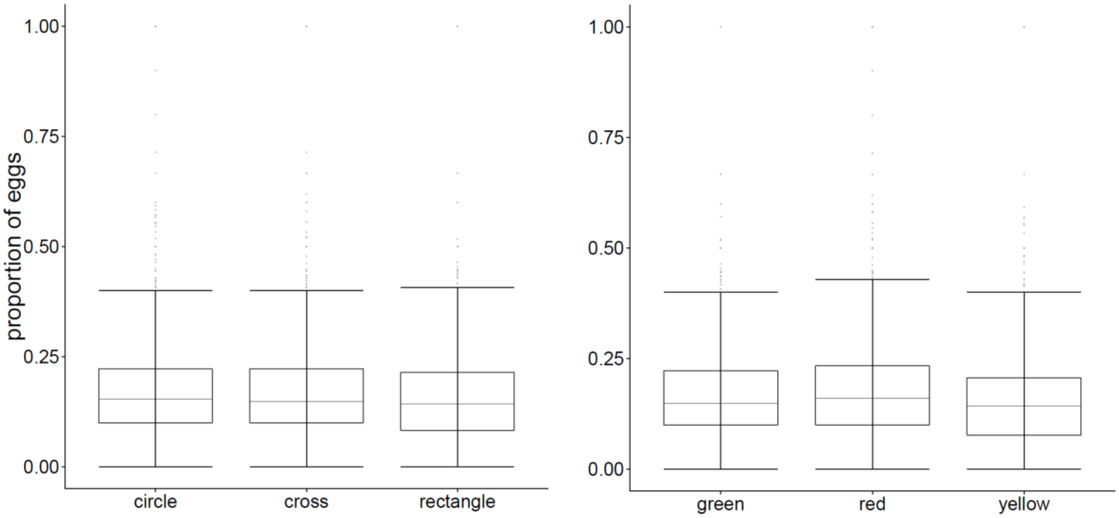

Fig. 3. Mean proportion of eggs per nest per symbol and colour used in the visual stimuli pens. Boxplots: boxes represent 1st and 3rd quartile, the lines in the boxes are the medians, whiskers extend to most extreme data points (within 1.5 × interquartile range) and grey dots represent outliers.

Nest visits per egg

Estimated effects for the number of nest visits per egg increased from 7.11 (95 % CI: 5.91, 8.57) before the entrance change to 11.63 (9.66, 14.0) after the entrance change. The number of visits per egg also varied with nest position and week of age (Fig. 4) with the number of visits to nest position 1 gradually increasing over the course of the study and the number of visits to nest position 6 remaining stable.

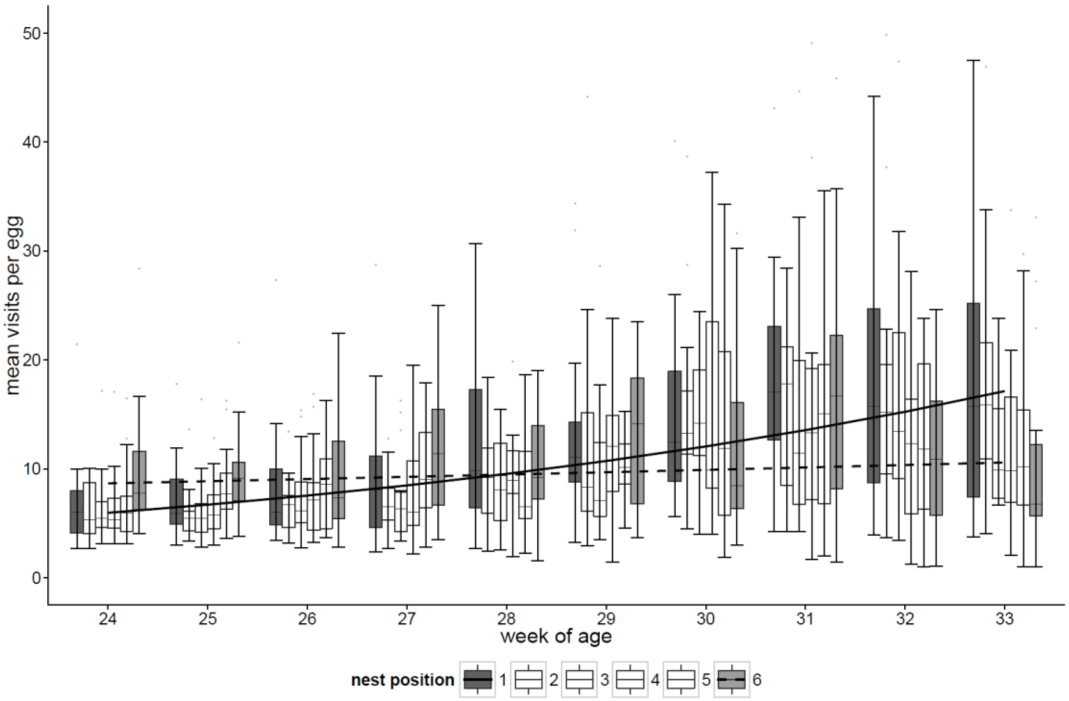

Fig. 4. Mean number of visits per egg and week. The solid line represents the model estimates for nest position 1 and the dashed line for nest position 6. Boxplots: boxes represent 1st and 3rd quartile, the lines in the boxes are the medians, whiskers extend to most extreme data points (within 1.5 × interquartile range) and grey dots represent outliers.

Number of nests visited

The best fitting model for the number of different nests visited included the interaction between treatment and week of age. Over the course of the experiment there was an increase in the number of different nests visited which was greater in the control treatment than in the visual stimuli treatment (Fig. 5). When looking at the repeatability of nest visits to each nest position for each hen over the three time periods, only 19 % of hens had confidence intervals that did not include zero, of those, the repeatabilities were significant but low (< 0.35 for visual stimuli pens and < 0.25 for control pens, p-values < 0.05), there was no evidence that repeatabilities differed between treatments or across time as neither treatment nor time period were in the best fitting model.

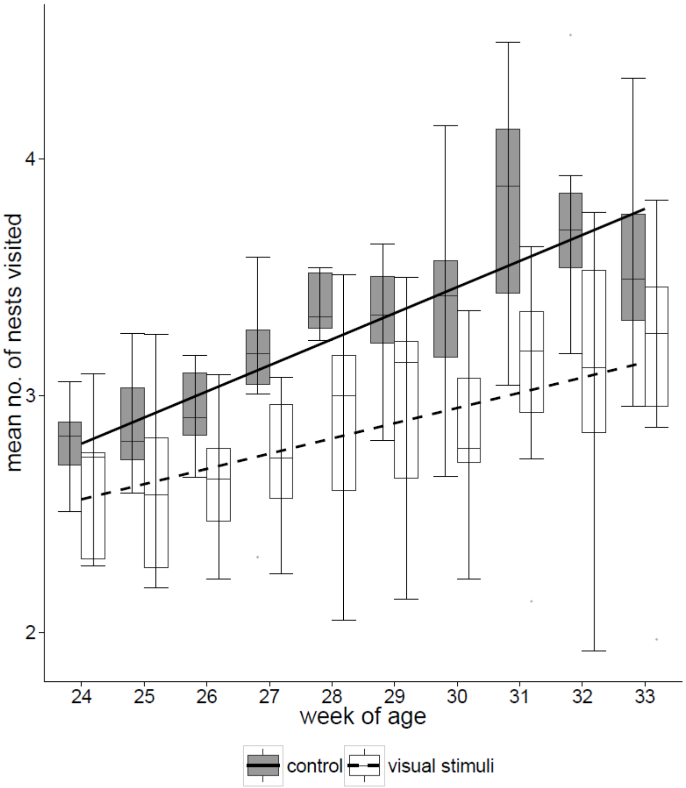

Fig. 5. Mean number of different nests visited per week of age. The solid line represents the model estimates for the control treatment and the dashed line represents the model estimates for the symbols treatment. Boxplots: boxes represent 1st and 3rd quartile, the lines in the boxes are the medians and whiskers extend to most extreme data points (within 1.5 × interquartile range).

Nest visits according to nest occupancy status

The final model for proportion of nest visits included the interactions between occupancy status and entrance, and occupancy status and week of age (Table 2). The proportion of nest visits to occupied nests increased with age while the proportion of nest visits to unoccupied nests decreased (Fig. 6). The entrance change resulted in similar effects (Table 3b). However, the proportion of visits to all occupied and all unoccupied nests remained stable throughout the experiment.

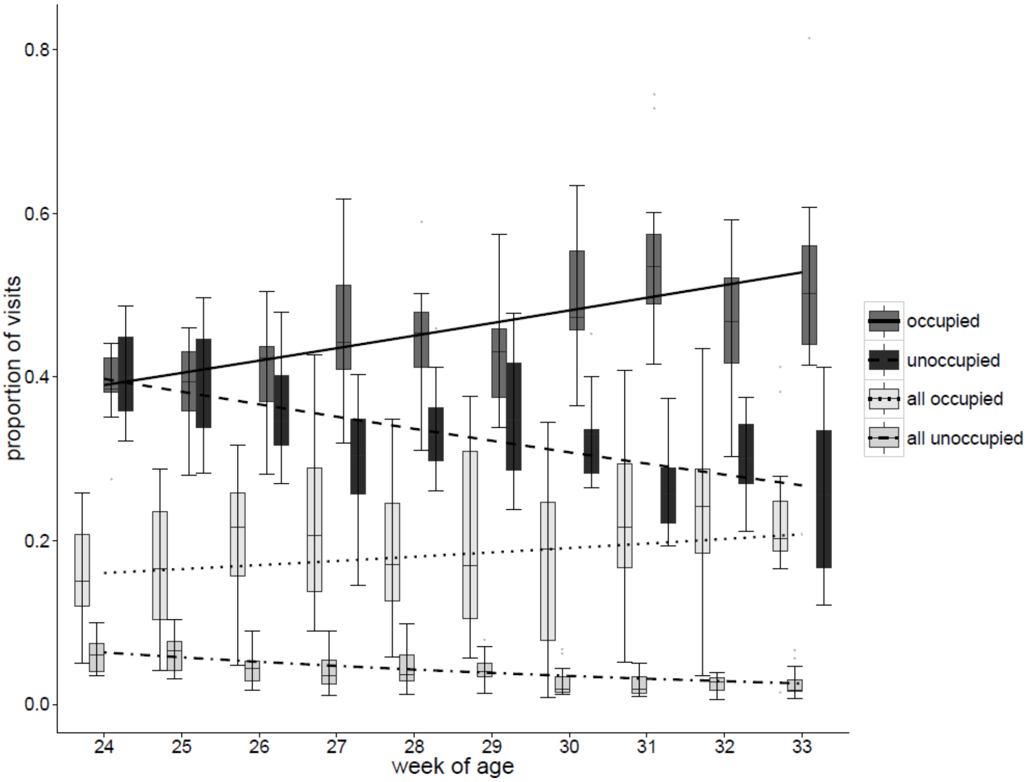

Fig. 6. Mean proportion of visits according to nest occupancy status and week of age, lines are model estimates. Boxplots: boxes represent 1st and 3rd quartile, the lines in the boxes are the medians and whiskers extend to most extreme data points (within 1.5 × interquartile range).

Discussion

We hypothesized that nest curtain appearance would affect nest choice in laying hens. As expected, the birds with access to the nests with symbols and colours visited fewer different nests before choosing where to lay their eggs, than the hens with access to identical nests. However, we found no other evidence that hens selected nests based on the visual stimuli; nest position, nest occupancy, and age of the hens had greater effects.

Given that the hens in the visual stimuli treatment visited fewer different nests than the hens in the control treatment, we conclude that hens used nest curtain design as a guiding factor when choosing nests. Although the difference in the number of nests visited was small (an average difference of 0.5 nests between the treatments), this represents almost a 15 % difference, which is likely relevant given that the conditions were very relaxed compared with commercial settings (six times more nest surface area available per hen). However, regardless of treatment, the majority of hens were inconsistent in the nests that they visited over time and in the location of egg-laying, and they did not make fewer nest visits per egg in the visual stimuli treatment. This was likely due to the fact that once inside the nests, they all looked identical and thus provided similar attractiveness. When designing the study, similarity of nest interior was chosen so that there was only one factor (outer nest curtain appearance) that differed between treatments.

Independent of treatment group, the effect of nest position on the proportion of eggs per nest was most pronounced during early egg-laying (weeks 18 to 21 of age) when hens laid the majority of eggs in both corner nests but favoring the nests closest to the entrance. Similar to the current findings, Clausen and Riber (2012) reported that hens in small experimental pens laid a disproportionate amount of eggs in corner nest boxes and similar results have been reported in commercial settings (Lentfer et al., 2013; Niebuhr, 2007). Corner nests may seem more isolated and more enclosed than other nests especially if they are adjacent to a wall. However, it is difficult to explain the preference for nests closest to the entrance, as caretakers were never present nor active in the barn during the period of egg-laying. In our experiment, the hens did not adjust their egg-laying location after the entrance change, suggesting that the entrance side is relevant in early lay or that once decided, preference for a nest location persists. Riber and Nielsen (2013) reported that changing the position of a nest (corner vs. not a corner) and whether the nest was isolated from other nests resulted in some hens continuing to use the same nest location regardless of nest type, while other hens changed nest location to keep laying in the most isolated nest. However, due to the low consistency of individual hens visiting the same nests over time, it is unlikely that in our study some hens were nest location conservative or nest type conservative as in Sherwin and Nicol (1994) and Riber and Nielson (2013). In addition, consistency of nest use, i.e., stability of egg proportions per nest over time, was very low. Given that the effect of nest position was so strong at the beginning of lay in small groups of hens, it is not surprising that many eggs are laid in corner nests when thousands of hens have access to only two corner nests.

The proportion of eggs laid per nest was negatively correlated to the number of visits per egg recorded, a finding consistent with results reported by Buchwalder and Fröhlich (2011) and Ringgenberg et al. (2014) where hens performed more nest visits per egg in least preferred nests.

But unlike predicted, hens in the visual stimuli pens did not perform fewer nest visits per egg; therefore whether nests were identical or not had no influence on this part of pre-laying behaviour. The number of nest visits per egg increased with age as did the variance of the data; we previously reported a similar increase in number of nest visits per egg (Ringgenberg et al., 2014). These results are somewhat unexpected as experienced hens should be more accustomed to egg-laying and therefore nest choice should happen faster. This was suggested by Zupan et al. (2008) although egg-laying was only observed until the 20th egg laid by each hen. We suggest that younger hens may be more focused on the process of egg-laying itself, and with increasing age they may focus more on housing and social factors, a shift that might require more nest visits to explore and identify a preferred nest. Lentfer et al. (2013), examining a commercial aviary, reported an increase in nest inspections with age (from 25 to 43 weeks of age) of least preferred nests, although the number of visits per nest was not examined.

Based on the suggestion that heterogeneous nests may result in less gregarious nesting (Appleby and McRae, 1986), Clausen and Riber (2012) attempted to diversify nest types with the specific aim to reduce gregarious nesting. Hens had access to group-nests with different characteristics (yellow walls, black plastic flaps, and standard nest) and their nest choice was compared with that of hens having access to three identical standard nests. Unexpectedly, they found an increase in gregarious nesting in the groups with the diverse nests which they reasoned was likely due to the hens developing individual preferences for the same nest. In contrast, we found no evidence that the differences in the nest curtain design affected gregarious or solitary nesting. These differences in results may have been due to the use of rollaway nests in our study whereas in Clausen and Riber (2012), eggs remained in the nests and may have acted as a factor in nest attractiveness.

Regardless of nest curtain design, the nest occupancy status had a marked effect on the proportion of nest visits, especially when the hens had a choice between occupied and unoccupied nests: by week 33 of age, almost 50 % of all visits were made to occupied nests. A possible reason in line with the concept of changing interests mentioned above is that as a hen ages and becomes more experienced with egg-laying and more accustomed to the environment, social factors may become more important in nest selection. Although the current study was confounded by effects of entrance change and week of age, we found that entrance change was associated with the increase in gregarious nest visits. This association may provide support for the hypothesis that gregarious nesting is an anti-predator response in laying hens (Riber, 2012b), as it may have been stressful that the caretaker suddenly entered the pens through the opposite side from the one the hens were accustomed. However, in another experiment, Riber (2010) found opposite results than in the current study with more solitary nesting compared with gregarious nesting starting at 26 weeks of age and an increase in solitary nesting with time. This difference could be explained by the sudden change in the environment (i.e., changing position of nest boxes and entrance side) which only occurred in our experiment. Alternatively, we could explain the differences between the studies by the different hybrids, as it is known that brown laying hybrids show different pre-laying behaviour than white hybrids (i.e. egg-laying in brown hybrids is generally less synchronized than in white hybrids) (Icken et al., 2012).

Other factors may also result in an increase in gregarious nesting such as shared nest preference (Clausen and Riber, 2012), more conspicuous nests due to other hens (Appleby and McRae, 1986) or an increase in synchrony of egg-laying. However, anecdotal evidence from Lentfer et al. (2013) suggests that the timing of oviposition becomes less synchronized with age. Similarly, Cronin and Barnett (2008) reported a decrease in synchrony of egg-laying with age in hens in furnished cages. When comparing the two situations where hens did not have a choice between occupied nests and empty nests, we report low proportions of nest visits similar to Riber (2010). These two situations remained relatively stable over the course of the experiment unlike the other behavioural patterns measured.

In summary, nest curtain appearance had little influence on nest choice in small groups of laying hens, although nest position relative to the entrance, social factors and age were relevant. Our results point to the complex interactions that affect pre-laying behaviour in laying hens in housing systems with group-nests.

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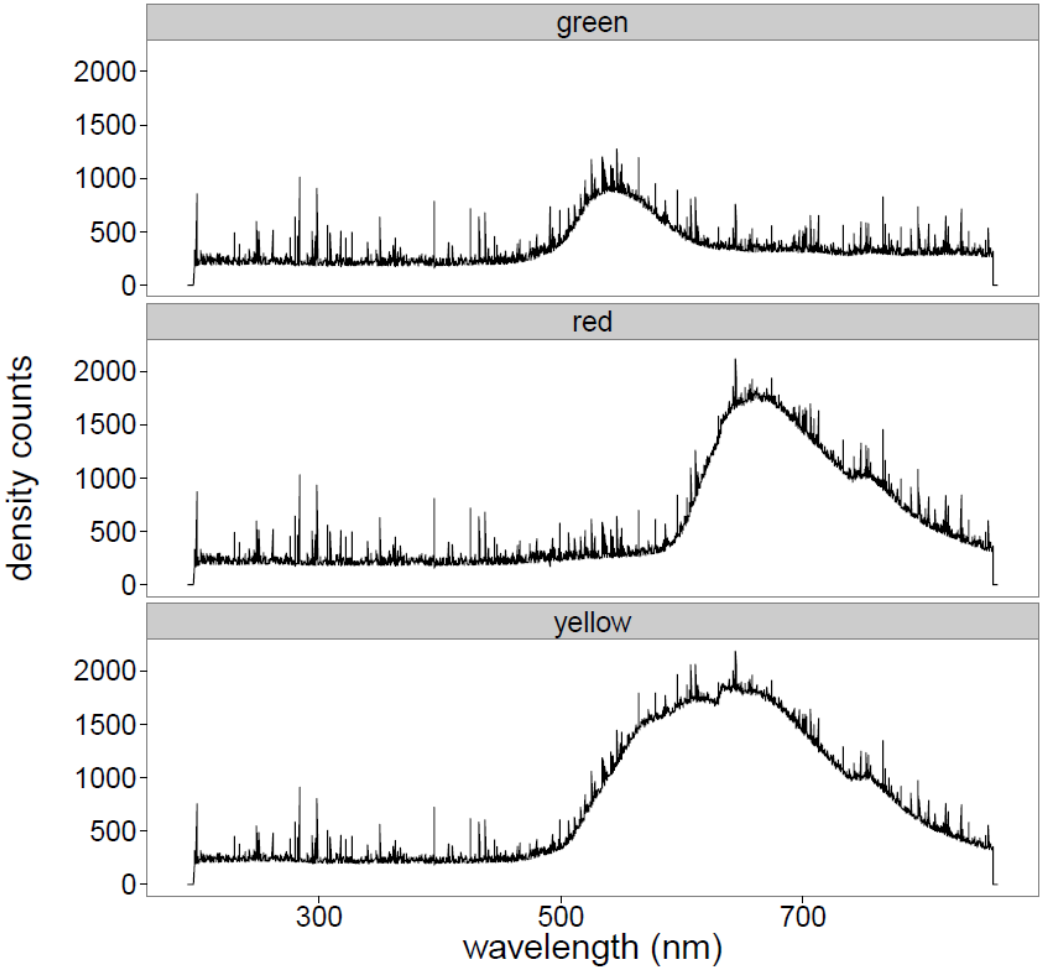
Appendix

Colour reflectance spectrum of the three different coloured curtains under the lighting conditions of the experiment.



Chapter 5

DISCUSSION

General discussion

Domestic hens used for egg production perform pre-laying behaviour almost every single day during their productive life. Given that pre-laying behaviour is a behavioural priority, it is essential to provide hens with an optimal environment – with an attractive nest site – allowing its performance. The aims of this thesis were to evaluate different nest characteristics and their effects on nest preference and pre-laying behaviour. This general discussion focuses on the methodology used in the thesis, its benefits and limitations and future research.

Preference tests

Preference tests involve placing animals in a situation where they can choose between two or more options or environments. We then measure the time that the animals spend in a specific environment or the resources are used and assume they make choices that are best for their welfare (Fraser and Matthews, 1997; Kirkden and Pajor, 2006). Preference tests allow us to objectively assess the animal's preference from their own perspective and can help us understand and improve animal welfare, i.e. by designing nests with specific characteristics that are attractive to laying hens. There are however some limitations in this methodology: previous experiences can affect preference, we can only conclude on the relative preference between the choices offered (not the strength of preference), and it is difficult to account for partial preferences (Duncan, 2005; Fraser and Matthews, 1997).

Buchwalder and Fröhlich (2011) developed a method to test commercial group-nests in terms of the welfare of hens around egg-laying. They used a free choice preference test where hens had access to two group-nests: a commercial nest and a so-called "minimal" nest which just complied with the minimal requirements for a nest in the Swiss Animal Welfare Ordinance. This allowed them to detect major problems with commercial nests if for example hens preferred to lay their eggs in the minimal nest. However, this method did not allow identifying which nest characteristic caused a nest preference. We used a methodology based on Buchwalder and Fröhlich (2011) but focused on comparisons between two nests differing only in one characteristic in order to ask the hens a very specific question.

When it comes to preference tests using nests, the final choice of the hens is the egg-laying location, which is very specific and easy to measure (number of eggs per nest). Although nests are meant to be used solely for egg-laying, some hens may use them for withdrawing, resting or hiding from other hens, we therefore focused on data on nest use during the first 5 h of daylight, which is when the majority of eggs are laid (Johnston and Gous, 2007; Lentfer et al., 2011; Riber, 2010). We also used hens with no prior experience of nests and that began egg-laying in our experiment. It is however very clear that the preferences reported in this thesis are only relative preferences between the nest types offered. But those are the nests that hens also experience in commercial aviaries, therefore the questions asked to the hens were relevant, and we showed that they clearly did notice the differences between the nest types offered.

In neither experiment did we find an absolute preference for a nest: some hens always laid their eggs in the least preferred nests and reasons for this are discussed in Chapter 2. In Chapter 3, we switched the nests in order to test the strength of preference and surprisingly, the hens did not switch their egg-laying location, showing that the strength of preference was not very strong after early lay or that familiarity with the egg-laying location was more important. However early lay is a key point in time during which attractive nests are crucial in order to reduce floor eggs as much as possible (Appleby, 1983). The interpretation of nest preference was also based on observations of pre-laying behaviour which gave us additional information on nest use.

Based on the results from the preference tests in this thesis, very large group-nests used in aviary systems are likely also unattractive to laying hens. We therefore recommend to at the very least keep the original sizes of manufactured group-nests or to use partial side walls. Further experiments should be conducted on-farm to assess how nest size or structured nests affect the prevalence of floor eggs and pre-laying behaviour (for example by measuring the number of visits per egg).

Measures of nest preference

Although the main outcome measure in all three experiments was the number of eggs, the number of visits per egg may be quite informative when it comes to the attractiveness of a nest as argued in Chapter 3. It is a measure that remains relatively consistent across studies for the same nest type. The mean number of visits per egg were of the same magnitude in our experiments as in Buchwalder and Fröhlich (2011) when using the same nest types: > 40 visits per egg in the unaltered nest used in Chapter 2 (nest CN5 in Buchwalder and Fröhlich, 2011), and < 10 visits per egg in the unaltered nest used in Chapter 3 (nest CN1 in Buchwalder and Fröhlich, 2011). These results suggest that these nests differed in attractiveness, perhaps due to the split floor design of the nest used in Chapter 2. Indeed, Buchwalder and Fröhlich (2011) reported a greater preference for the nest we used in Chapter 3 than the nest with the split floor design that we used in Chapter 2. The measure "nest visits per egg" now remains to be tested in large commercial systems to evaluate its consistency across nests in commercial conditions. If hens perform the same magnitude of nest visits per egg in the same nests on different farms, it could then be used to test new commercial group-nests on-farm for the Swiss authorization procedure (Wechsler, 2005) and a benchmark could be set as to the maximum number of visits per egg that is still acceptable in an attractive nest.

Nest characteristics

The goal of the two preference tests was to ask the hens a very specific question: whether they preferred smaller or larger nests and nests with a partition or without a partition. However, nest size is a multi-dimensional concept and involves a difference in the surface area of the walls, the curtains and the floor. Similarly, for the nest partition preference test, the nests differed not only in the presence or absence of a partition; they also differed in the amount of wall surface area available. It is therefore difficult to precisely conclude if the hens made their choice based on the overall concepts of size and the partition or if they preferred nests with increased ratios of wall to floor surface area. It was unfeasible to separate these characteristics while still keeping the basic nest features. These experiments were however important for the Swiss authorization procedure and to give recommendations and feed-back to companies designing and selling group-nests.

In the last experiment, I attempted to use variations in nest curtain appearance to guide hens in their nest selection. Unlike expected, these visual stimuli had very little effect on pre-laying behaviour. The colours and symbols used were likely too subtle or insignificant for the hens to use them as guides for nest selection. Other concepts that could be tested to even out egg distribution in long rows of nests such as changing the inner nest appearance or using different nest types, these may however be less practical to apply on-farm.

Experimental setup

I observed the pre-laying behaviour of small groups of hens with access to rollaway group-nests that were either purchased from aviary manufacturers or custom built to resemble commercial nests. As many of the earlier studies on pre-laying behaviour focused on laying hens housed individually, I wanted to focus on groups of hens in order to study the social factors contributing to nest selection. The experiments were conducted in small groups of 20-30 hens rather than in commercial farms in order to allow the observation of individual hens to gain knowledge about fundamental aspects of pre-laying behavior rather than just nest preference. Although there was approximately six times more nest surface area available per hen than in commercial situations, this allowed the hens to make a nest choice without too much disruption from conspecifics. I also used rollaway group-nests specifically to investigate the attractiveness of commercial group-nests which not only makes the results more relevant to commercial production but also eliminate the confounding factor of "eggs in nest" which are highly attractive to laying hens.

Social status

Given that we studied nest selection in groups of hens, it was only logical to observe the effects of social factors on pre-laying behaviour. I investigated social status based on agonistic interactions in the early morning which is the usual way to measure social rank in laying hens. Aggressive interactions that occurred on nest entry platforms or in the nests were not analysed because they occurred very rarely. It is however questionable if these recorded aggressive interactions, which occurred on the pen floor, and the resulting social status, are also relevant for nest access. Social dominance may not be such as straight-forward concept and an animal that is dominant in one situation is not necessarily dominant in all situations (Banks et al., 1979).

However, our results are in accordance with Freire et al. (1998) who found that subordinate hens in small groups with one nest site were more active before oviposition than dominant hens with social status measured using aggressive interactions.

Gregarious nesting, or nest choice based on nest occupancy status, was also investigated in Chapter 4. This is the first time that this phenomenon was studied in laying hens with access to rollaway nests. Gregarious nesting is described as an unwanted behaviour in Clausen and Riber (2012), due to the risk of injuries from overcrowding. But it has also been observed in hens in semi-natural conditions (Riber, 2012) and may mean that the way in which wild or feral hens isolate themselves for egg-laying is not important in hybrid strains of laying hens when incubation does not occur. Thus, the high density of hens for nest surface area found in commercial systems may not be a problem in terms of animal welfare as hens seem to choose nests that already have hens in them rather than empty nests.

Conclusions

This thesis focused on pre-laying behaviour and nest preference in laying hens. We report that domestic hens of a white hybrid show a preference for smaller group-nests as well as for nests with a partition in the middle. More eggs and fewer nest visits per egg were representative of the preferred nests. These results point to the attractiveness of smaller nest size and added cover provided by the partition in the nest for laying hens. We found some evidence that hens use visual stimuli in the form of colours and symbols on nest front curtains as an aid in nest choice although this did not affect the distribution of eggs among nests nor the consistency of nest selection. In terms of social factors, we found a high occurrence of gregarious nesting which increased with age although its frequency did not differ between groups of hens having access to identical or heterogeneous nests. Therefore, whether or not nests were occupied or not was more important to the hens than nest appearance. We also report that hens of a lower social status show a different pre-laying pattern than higher ranking hens characterized by laying slightly later in the day and performing more nest visits, although final nest choice was not affected.

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Erklärung

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Bachelor Master Dissertation X

Titel der Arbeit:

An investigation of nest characteristics and social factors affecting pre-laying behaviour and nest choice in laying hens

LeiterIn der Arbeit: Prof. Dr. Hanno Würbel

Ich erkläre hiermit, dass ich diese Arbeit selbständig verfasst und keine anderen als die angegebenen Quellen benutzt habe. Alle Stellen, die wörtlich oder sinngemäss aus Quellen entnommen wurden, habe ich als solche gekennzeichnet. Mir ist bekannt, dass andernfalls der Senat gemäss Artikel 36 Absatz 1 Buchstabe r des Gesetzes vom 5. September 1996 über die Universität zum Entzug des auf Grund dieser Arbeit Verliehenen Titels berechtigt ist.

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Publications

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Ringgenberg, N., Fröhlich, E.K.F., Harlander-Matauschek, A., Würbel, H., Roth, B.A. Effects of variation in nest curtain design on pre-laying behaviour of domestic hens. Submitted to *Applied Animal Behaviour Science*

Ringgenberg, N., Fröhlich, E.K.F., Harlander-Matauschek, A., Würbel, H., Roth, B.A. 2014. Does nest size matter to laying hens? *Applied Animal Behaviour Science*, 155, 66-73

Ringgenberg, N., Bergeron, R., Meunier-Salaün, M.C., Devillers, N., 2012. Impact of social stress during gestation and environmental enrichment during lactation on the maternal behaviour of sows. *Applied Animal Behaviour Science*, 136, 126-135

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Ringgenberg, N., Bergeron, R., Devillers, N. 2010. Validation of accelerometers to automatically record sow postures and stepping behaviour. Proceedings of the 44th Congress of the International Society for Applied Ethology (ISAE), Swedish University of Agricultural Sciences, Uppsala, Sweden (oral presentation)

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