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Assessment of colony nests for laying hens in conjunction with the authorization procedure

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ABSTRACT

Pretesting farm animal housing systems that are intended for mass production is a practical way to increase and ensure product quality of livestock systems. The Swiss authorization procedure requires new systems and equipment for farm animals to show improvement with regard to animal welfare. For this purpose, a method to test hen colony nests was developed and subsequently applied to assess the suitability of five commercial nests. First, we developed a "minimal nest" that fulfilled the minimum requirements of the Swiss Animal Welfare Regulations (development phase). In a second step consisting of five experiments, the minimal nest was offered in combination with one of the commercial nests (assessment phase). For approval, the hens' preference for the commercial nest must be better than or equal to their preference for the minimal nest. The experiments were carried out with eight (development phase) or nine (assessment phase) groups of 20 hens and spanned the 18th to the 26th (development phase) or 28th (assessment phase) week of age. The numbers of eggs in the nests and on the floor were registered daily, and the behaviour and positions of the hens were recorded during the last two weeks of the experiments. The hens significantly preferred an open litter box to a minimal nest with an open front side (59% vs. 36% of the eggs, $p < 0.03$), while the minimal nest in which the front side was covered by a plastic curtain was significantly more attractive than the open litter box (86% vs. 12% of the eggs, $p < 0.001$). One of the commercial nests was significantly preferred over the minimal nest with the plastic curtain (78% vs. 17% of the eggs, $p < 0.01$), whereas two of the commercial nests were significantly less favored than the minimal nest (39% vs. 58% of the eggs, $p < 0.01$; 16% vs. 82% of the eggs, $p < 0.002$). No significant differences were found for the other two commercial nests (42% vs. 56% of the eggs, $p = 0.33$; 29% vs. 65% of the eggs, $p = 0.17$). Behavioural and positional data for the laying hens are also presented. Seclusion seems to be an essential factor for hens searching for a nest site. With regard to the authorization procedure, the commercial nests that were preferred or rated similarly to the minimal nest may become definitively authorized, whereas the disliked nests are likely to be disapproved. Investigations on commercial farms are needed for final assessment.

1. Introduction

Consumer demands regarding animal welfare play an important role in animal production (Wechsler, 2005). It is now widely accepted that animals have behavioural needs (Duncan, 1998). Housing systems and management should meet these needs to safeguard the welfare of the animals. Therefore, according to the Swiss Animal Welfare Act (2005) and the Swiss Animal Protection Regulations (2008) livestock housing equipment, in our case commercial laying nests, should be subjected to an authorization procedure (Wechsler, 2005) to assess their suitability with

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respect to animal needs. That means that companies carrying out housing systems and equipment of farm animals must request authorization to sell their products. The Swiss Federal Veterinary Office has received several authorization requests from manufacturers of housing equipment for new laying nests and needs to investigate if the nests are in accordance with the requirements of the Swiss Animal Welfare Legislation before providing said authorization.

Aviary systems for laying hens are generally equipped with colony nests. These nests are arranged in rows 60–100 cm above the ground either on the walls of the stable or integrated into the aviary system itself. The hens reach the nest using a perch or a grid in front of the nest. These usually automated rollaway nests are 50–60 cm wide and 100–150 cm long, with the laying area covered by mats of rubber pimples or astroturf pieces measuring 0.5–0.9 m^2. Commercial laying nests are closed on the top and on three sides. On the front side, there is a plastic curtain with an entrance hole 15–25 cm wide. According to the Swiss Animal Protection Regulations (2008), 1 m^2 of nest floor suffices for a maximum of 100 laying hens.

The aims of the study were to develop an appropriate method to assess if the nests are in accordance with the requirements of the Swiss Animal Welfare Legislation and to subsequently test five commercial nests. The idea was to create a reference nest, which was accepted by the hens for egg-laying but would barely fulfill the minimal requirements of the Swiss Animal Protection Regulations (2008). We call it "minimal nest". The approval of a commercial laying nest seeking authorization required that it be accepted by the hens to a greater or equal degree as the "minimal nest" when offering it in combination with one of the commercial nests in a preference test. Knowledge of the preferences of an animal often gives valuable information about what conditions are likely to result in better welfare, but direct measurements of the state of the animal must also be used in attempts to assess welfare and improve it (Broom, 1991). Therefore we additionally recorded the behaviour of the animals. Hens that do not have access to suitable nest-sites may display elaborate sequences of nest-seeking and nest-building behaviour during the hour preceding oviposition, showing signs of apparent frustration. Behaviours suggestive of frustration in laying hens include excessive locomotion or exploring activities (Cooper and Appleby, 1996). On the basis of the results shown by Cooper and Appleby (1996), we formulated the hypothesis that hens staying close to a suitable nest may perform less excessive exploring activities than hens staying close to a less attractive nest during the hour preceding oviposition. As the hens have to cross the landing platform of the nest to enter the nest, it is not desirable that they use the platform for performing other behaviour than nest-exploring activity and thus block the entrance of other hens. One must be aware of the fact that preference tests sometimes confuse familiarity with preference. As the animals in our study had neither experience with the "minimal nest" nor with one of the commercial nests before the test situation we can exclude this false confusion.

Now then the first step of this study was to develop the "minimal nest" (development phase). As parts of the floor of Swiss housing systems for laying hens are covered with litter and as laying hens like to lay their eggs on a manipulable substrate like litter (Appleby and McRae, 1986), suitable nests have to be more attractive than an open litter area to minimize floor eggs. Therefore we tested the "minimal nest" against an open litter box in a preference test. To be usable for the approval of the commercial nests the "minimal nest" had on the one hand to be preferred to the litter by the hens and on the other hand it has to fulfill the minimal requirements of the Swiss Animal Protection Regulations (2008). The requirements of the Animal Protection Regulations are the following: "protected and suitable individual or group nests with a floor covered by litter or soft material such as mats of synthetic grass or rubber". Creating the "minimal nest", additional nest attributes that enhance attractiveness (Appleby et al., 1988; Appleby and Smith, 1991; Duncan and Kite, 1989), such as visual cover at the nest front, grappable or manipulable floor structure and nest floors with a low-pitched slope, would preferably be omitted.

The second step of the study was to investigate the attractiveness of five commercial laying nests in comparison with the developed "minimal nest" by means of a preference test. Approval of a commercial laying nest required that it be accepted by the hens to a greater or equal degree as the "minimal nest". A given nest was deemed more attractive than another when more eggs were laid in it, when the rate of nest visits to the laid eggs was lower, when more animals stayed in the nest and when the animals showed less nest-exploring activity (Cooper and Appleby, 1996; Meijsser and Hughes, 1989; Sherwin and Nicol, 1993; Struelens et al., 2005; Zupan et al., 2008) in the proximity of the nest and less standing or walking with head down, resting and sitting or standing with grooming on the landing platform of the nest. If a nest turned out to be less attractive than the "minimal nest," it was classified as not fulfilling the requirements of the Animal Protection Regulations, and the request for authorization should be rejected.

2. Methods

2.1. Animals and housing

The experiments were carried out with non-beak-trimmed white laying hens of a commercial hybrid strain (LSL). They were obtained at one day old and litter-reared in groups of 160–180 animals with access to perches but without access to nests.

At the age of 17 weeks, birds were assigned randomly into eight or nine groups of 20 hens each. All animals were housed in identical test pens measuring 3 m × 4 m × 2.5 m (length × width × height, Fig. 1). Test pens were arranged in three rows of three pens each. The front side of each pen was made of spruce laths covered with wire mesh, and plywood walls separated the pens. A net was attached above the pens, preventing birds from escaping through the top. Pens contained a feeder (35 cm in diameter) and eight nipple drinkers with cups installed beneath a perch (4 m in length, 60 cm in height). Each test pen had two nest site areas separated from the rest of the pen by a partition made of wire mesh. The partition contained two openings

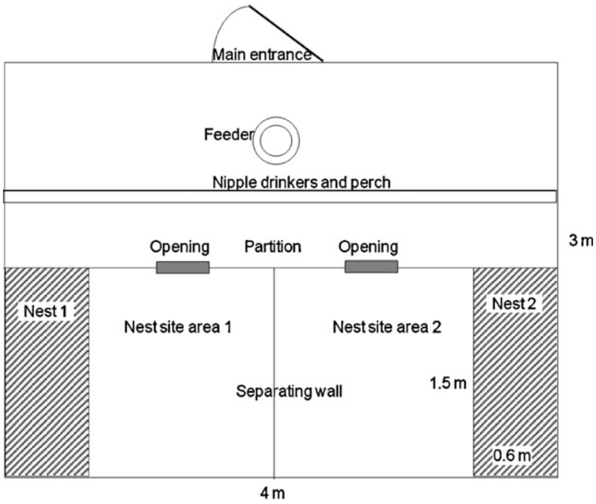

Fig. 1. Test pen viewed from above.

(35 cm × 35 cm) that allowed the animals to enter the nest site areas. A plywood wall separated the nest site areas. The position of the two different nests in the two rear corners (left or right) was randomly and equally distributed over all pens (Fig. 1). The average daily temperature in the poultry house was 16 °C (min. 9 °C, max. 26 °C), and artificial lighting produced a mean light intensity of approximately 11 lx at the height of the animals. At the nest entrance we measured 5 lx on average and at the back of the nest we measured 3 lx. According to the standard lighting schedule in commercial egg production, day length was successively increased from 8 h in the 18th week of age to 14 h at the 22nd week of age, with a twilight period of 15 min at the beginning and end of each light period.

2.2. Procedure

The study consisted of two parts: the initial development phase for the "minimal nest", followed by the assessment phase for the commercial nests. The development phase involved two experiments and the assessment phase five experiments. The experimental design was comparable in all experiments. In each experiment, two different nest types were offered in a preference test situation.

After each experiment, the animals were sold to conventional egg producers and replaced by young hens for the next experiment.

2.2.1. Development phase

In the development phase, the "minimal nest" type that barely fulfilled the minimal requirements of the regulations and was preferred by the hens to an open litter box (OB) was developed testing different prototypes of "minimal nests" against an OB in a preference test. The floor of the "minimal nests" inclined in such a way that the eggs rolled away (floor slope: 10%). A tunnel in front of the "minimal nests" served for egg collection. The "minimal nests" were positioned 70 cm over the floor and could be reached from a landing platform measuring 22 cm. According to the regulations nests have to be "protected...and...with a floor covered by...soft material". There is no further definition of the term "protected" and "soft material" in the regulations. In the first experiment, starting with a nest characterised by minimal protection and minimal softness on the nest floor, we offered "minimal nest 1″ (MN1) equipped with a roof and walls at three sides, but with the front side left open and with a thin plastic mat without structure on the nest floor. In the second experiment, the front side (30 cm high) of "minimal nest 2″ (MN2) was covered by a plastic curtain with an opening (30 cm × 20 cm) in the middle. Characteristics of the "minimal nests" are given in Table 1. The open litter box made of wood had a 16 cm border and a landing platform identical to the MN1 and MN2 and was littered with wood shavings to a height of 5 cm and positioned 70 cm over the floor.

Identical test conditions were set up in nine test pens for each of the two experiments (Table 2). The floors of the test pens were equipped with plastic slats, so the hens only had access to litter material in the OB.

2.2.2. Assessment phase

In the assessment phase, five commercial rollaway nests were examined (CN1–CN5, Table 1). Each commercial nest type was individually presented in combination with MN2 or MN3 (Table 1). MN3 only differed from MN2 in size. We had to readjust the size of the "minimal nest" MN2 according to the size of the new, larger commercial nests. Hence, CN1, CN3 and CN4 were tested against MN2, and CN2 and CN5 were tested in half of the pens against MN2 and in the other half of the pens against MN3. The experiments assessing CN2, CN3 and CN5 were repeated eight times, whereas the experiments with CN1 and CN4 were

Table 1
Nest characteristics (OB: open litter box; MN: minimal nest; CN: commercial nest).

Nest type	Floor space (cm)	Floor slope	Floor cover	Floor form	Material of side walls	Configuration of landing platform
OB	40 × 80	No slope	Wood shavings	Not divided	Plywood	Plastic slats
MN1/2	40 × 80	10% toward back	Flat plastic mat	Not divided	Plywood	Plastic slats
MN3	50 × 120	10% toward back	Flat plastic mat	Not divided	Plywood	Plastic slats
CN1	49 × 110	10% toward front	Mat of rubber pimples	Not divided	Plywood	Plastic slats
CN2	57 × 110	11/11% toward centre	Mat of rubber pimples	Divided in two	Plywood	Metal grid
CN3	46 × 119	18.5% toward back	Astroturf mat	Not divided	Hard plastic	Plastic slats
CN4	44 × 120	9% toward back	Astroturf mat	Not divided	Sheet metal	Metal grid
CN5	60 × 144	10/15% toward centre	Astroturf mat	Divided in two	Plywood	Metal grid

Table 2
Overview of the experiments (OB: open litter box; MN: minimal nest; CN: commercial nest).

Phase	Experiment	Nest comparison	Pens	Video recording	Pen floor
Development	1	MN1 vs. OB	9	No	Plastic slats
	2	MN2 vs. OB	9	No	Plastic slats
Assessment	3	MN2 vs. CN1	4	Yes	Wood shavings
	4	MN2/3 vs. CN2	8	Yes	Wood shavings
	5	MN2 vs. CN3	8	Yes	Wood shavings
	6	MN2 vs. CN4	4	Yes	Wood shavings
	7	MN2/3 vs. CN5	8	Yes	Wood shavings

repeated only four times because of time pressure (CN1) and an intense infestation of red mites (CN4). The commercial nests were positioned 70–80 cm over the floor, according to their positions under commercial conditions. The minimal nests were adjusted to the same height as the commercial nests in each experiment. The floor of the test pens was littered with wood shavings.

2.3. Data collection

For the development and assessment phases, the numbers of eggs laid in the two nests and on the floor were registered daily from the beginning of the egg-laying stage until the age of 26 or 28 weeks. In the assessment phase, the behaviour and positions of the hens at the 27th/28th week of age were videotaped in the nest site area (Fig. 1) once per pen from the 2nd to the 5th h after the light was switched on, when most of the hens were in the nests. Behaviour was classified into two categories: (1) *nest-exploring activity*: standing or walking with head up (the head was held above the level of the highest point of the shoulder); (2) *all the rest of behaviour: standing or walking with head down* (the head was held below the level of the highest point of the shoulder), *resting and sitting or standing with grooming*; and positions were classified into three categories: (a) *on the floor in the nest site area*; (b) *in the nest* (the number of hens in the nest was deduced from the nest entries and exits; behaviour was not recorded inside the nest); (c) *on the landing platform in front of the nest*. The number of nest entries was counted continuously during the 4 h of video recording. The number of animals showing certain behaviours at each position was recorded every 10 min by means of scan sampling. Animals were not individually distinguished.

2.4. Statistical analysis

Data were averaged at pen level, and their residuals were checked for normality. The ratios of eggs laid in the two nests were compared using a repeated measurement ANOVA in NCSS, version 07.1.8 (Hintze, 2006), from the 21st week of age onwards when the mean laying performance was 40% or more in each experiment. The number of nest entries per egg and the number of animals showing certain behaviours at each position in the two nest site areas were analyzed with the Wilcoxon signed ranks test for matched pairs (Siegel and Castellan, 1988). The matched pairs were the two offered nest-sites in each pen. The nest floor area of the minimal nests (MN2 and MN3) had no significant effect on the different parameters recorded. Therefore, data from the CN2 and CN5 preference experiments were pooled together for statistical analysis. The results are presented as the mean ± SEM. Statistical significance refers to $p < 0.05$, and all reported p-values are two-sided.

3. Results

3.1. Laying performance

The hens started laying at 18–19 weeks of age. At 21 weeks, laying performance in all seven experimental regimes averaged 52% (min. 40%, max. 63%); at week 25, it reached an average of 96% (min. 93%, max. 98%). The mean ratio of floor eggs to total eggs ranged between 1.2% and 6.0% for each experiment.

3.2. Development phase

In the first experiment of the development phase, the hens laid significantly more eggs in the OB than in MN1, which had an open front side, while in the second experiment, a significantly higher percentage of eggs was found in MN2, which had the front side covered by a plastic curtain, compared to the OB (Table 3).

3.3. Assessment phase

As can be seen in Table 3, the hens significantly preferred CN1 to MN2 as an egg-laying site. In contrast, there were significantly fewer eggs found in CN2 and a tendency for fewer eggs in CN5 relative to MN2/3. If one outlier pen, where the animals showed a side preference, was excluded, the hens laid significantly fewer eggs in CN5 than in MN2/3. No difference was found between the numbers of eggs laid in CN3 and MN2.

No significant differences in the number of nest visits per egg were found between CN2, CN3, CN4 or CN5 and MN2/3 (Table 4). The hens tended to show fewer nest visits per egg in CN1 than in MN2 (Table 4). With the outlier pen excluded, the hens performed significantly more nest visits per egg in CN5 than in MN2/3.

More hens appeared to stay in CN1 relative to MN2, and the percentage of animals showing nest-exploring activity on the floor tended to be lower in CN1 than in MN2 (Table 5). Compared to MN2/3, a significantly lower percentage of animals showed all the rest of behaviour, that means standing or walking with head down, resting and

Table 3
Comparison of the mean percentage of eggs laid per day from the 21st to the 28th week of age (OB: open litter box; MN: minimal nest; CN: commercial nest).

Nest types	Mean ± SEM	Mean ± SEM	*N*	*p*-Value	*F*-ratio
MN1: OB	36.2% ± 1.04	58.7% ± 1.04	9	$p < 0.03$	7.48
MN2: OB	86.0% ± 1.03	12.4% ± 1.03	9	$p < 0.001$	41.52
MN2: CN1	16.9% ± 0.96	78.1% ± 0.96	4	$p < 0.01$	39.90
MN2/3: CN2	58.2% ± 0.66	38.6% ± 0.66	8	$p < 0.01$	13.63
MN2: CN3	55.6% ± 0.95	41.8% ± 0.95	8	$p = 0.33$	1.09
MN2: CN4	65.0% ± 1.33	29.0% ± 1.33	4	$p = 0.17$	3.17
MN2/3: CN5	72.7% ± 1.45	26.1% ± 1.45	8	$p = 0.06$	4.78
Excluding outlier pen	82.2% ± 0.86	16.3% ± 0.86	7	$p < 0.002$	31.89

Table 4
Mean number of nest visits per egg from the 2nd to the 5th h on one day in the 27th or 28th week of age (MN: minimal nest; CN: commercial nest).

Nest types	Mean ± SEM	Mean ± SEM	*N*	*p*-Value	*Z*-value
MN2: CN1	26.95 ± 6.15	2.20 ± 0.44	4	$p = 0.07$	1.83
MN2/3: CN2	7.00 ± 2.37	8.18 ± 2.20	8	$p = 0.26$	1.12
MN2: CN3	4.65 ± 1.49	5.18 ± 1.42	8	$p = 0.67$	0.42
MN2: CN4	6.00 ± 2.20	17.53 ± 6.68	4	$p = 0.47$	0.73
MN2/3: CN5	32.15 ± 25.15	40.29 ± 11.38	8	$p = 0.26$	1.12
Excluding outlier pen	7.17 ± 3.40	45.06 ± 11.93	7	$p = 0.04$	2.03

sitting or standing with grooming, on the landing platform of CN3, whereas a significantly higher percentage showed these behaviours on the landing platform of CN5 (Table 5). In the other experiments, no differences were found in the number or percentage of animals showing nest-exploring activity or all the rest of behaviour (Table 5).

4. Discussion

4.1. Laying performance

The hens started to lay at the expected age and showed a laying performance comparable to that of LSL hybrid strains under commercial housing conditions (Abrahamsson and Tauson, 1998; Lohmann Tierzucht GmbH, 2010). The mean ratio of floor eggs to total eggs per experiment peaked at 6% between the 21st and the 28th week of age, which is also in accordance with floor egg ratios under commercial conditions (Cooper and Appleby, 1996). The percentage of floor eggs normally declines with increasing time spent in laying conditions. Cooper and Appleby (1996) reported a decrease in floor eggs from 25% in the 22nd week of age to 5% in the 27th week. From 27th week onwards, the percentage drops to 1% to 2% (Häne, 1999). On the homepage of the Canadians Poultry Consultants Ltd. Cox (2011) reports that in modern broiler breeder birds like in the Ross bird,

Table 5
Mean number of animals per scan in the nest, percentage of animals showing nest-exploring activity on the floor in the nest site area and percentage of animals showing all the rest of behaviour, that means standing or walking with head down, resting and sitting or standing with grooming on the landing platform of the nest in the two nest-sites per pen in the assessment phase (MN: minimal nest; CN: commercial nest).

Nest types	Mean ± SEM	Mean ± SEM	*N*	*p*-Value	*Z*-value
Number of animals in the nest per scan					
MN2: CN1	0.79 ± 0.27	3.54 ± 0.67	4	$p = 0.07$	1.83
MN2/3: CN2	2.86 ± 0.37	2.87 ± 0.43	8	$p = 0.94$	0.07
MN2: CN3	2.00 ± 0.23	2.28 ± 0.27	8	$p = 0.78$	0.28
MN2: CN4	2.65 ± 0.59	1.59 ± 0.40	4	$p = 0.47$	0.73
MN2/3: CN5	2.79 ± 0.19	2.41 ± 0.68	8	$p = 0.57$	0.56
Percentage of animals showing nest-exploring activity on the floor					
MN2: CN1	60 ± 2.2	42 ± 9	4	$p = 0.07$	1.83
MN2/3: CN2	59 ± 5.8	49 ± 6	8	$p = 0.29$	1.05
MN2: CN3	56 ± 5.4	52 ± 6	8	$p = 0.58$	0.56
MN2: CN4	65 ± 6.6	51 ± 7	4	$p = 0.14$	1.46
MN2/3: CN5	60 ± 6.1	52 ± 8	8	$p = 0.40$	0.84
Percentage of animals showing all the rest of behaviour on the landing platform					
MN2: CN1	7 ± 7	15 ± 4	4	$p = 0.27$	1.09
MN2/3: CN2	7 ± 4	22 ± 7	8	$p = 0.18$	1.35
MN2: CN3	18 ± 6	9 ± 3	8	$p = 0.05$	1.96
MN2: CN4	13 ± 3	16 ± 6	4	$p = 0.27$	1.10
MN2/3: CN5	8 ± 3	32 ± 8	8	$p = 0.05$	1.96

for example, there are differences in the expected number of floor eggs among the various strains. The Ross 308 can be expected to produce 3–6% floor eggs, the 508, 5–7%, and the 708, 1–2%.

4.2. Development phase

With respect to the number of eggs found in the two nests, the majority of hens preferred the OB over MN1, which had an open front side. The floor of the OB was horizontal, littered with wood shavings and equipped with a 16 cm high rim, while MN1 had an inclined wooden floor covered with a thin plastic mat and was equipped with a roof and three side walls made of plywood. Because of the seclusion from potential birds of prey, we expected that hens would prefer a nest with a top cover to a nest without a roof (Kruschwitz et al., 2008; Struelens et al., 2005; Zupan et al., 2008). From an evolutionary perspective, a laying site with a horizontal surface is advantageous relative to a laying site with an inclined floor, as a flat surface prevents the eggs from rolling away and becoming lost to the hen. Appleby and McRae (1986), Appleby et al. (1988) and Huber et al. (1985) showed that hens prefer nests with manipulable floor structures. Although the litter box was open at the top, it seemed to be more attractive than MN1. As the hens did not prefer the MN1 to an open litter box, we built another "minimal nest" (MN2) with a plastic curtain at the front side. Offering MN2 with the OB, the hens preferred this nest to the OB, despite the horizontal floor and wood shavings of the latter. Accordingly, hiding places as well as a manipulable substrate and horizontal surface are important parameters related to nest preference; a small change in one parameter can outweigh a preference for another. Appleby and Smith (1991), Keeling (2004), Kruschwitz et al. (2008), Struelens et al. (2005) and Zupan et al. (2008) also showed that hens prefer closed nests to open nests. Likewise, nesting sites of wild chickens are usually hidden and well protected (Duncan et al., 1978). According to our investigation, the term "protected" in the Swiss Animal Protection Regulations (2008) should be interpreted as "with a roof, three walls and a front side with a curtain" to assure that the nest is accepted by the hens. The seclusion of a potential nest site seems to be an especially critical parameter for hens seeking a place to lay their eggs (Appleby and Smith, 1991). As MN2 fulfilled the minimal requirements of the Swiss Animal Protection Regulations (2008) and was preferred by the hens to the OB, it was used as reference nest in the assessment phase.

4.3. Assessment phase

In the assessment phase, only one commercial nest, CN1, was explicitly preferred by the hens as a laying site compared to the minimal nest. Regarding the number of collected eggs, no difference was found in two preference tests (CN3:MN2 and CN4:MN2). In the other three commercial nests, one (CN2) was significantly less preferred as a laying place, and one (CN5) tended to be less attractive than MN2/3. If one outlier pen was excluded in the preference test for CN5, the difference in the number of eggs between the commercial nest and the minimal nest became significant with a sample size of seven. In the outlier pen, 93% of the eggs were laid in CN5, with only 7% of the eggs found in MN3, in contrast to the other seven pens, where 50%, 80%, 83%, 90%, 92% and 98% of the eggs were laid in MN2/3. In the outlier pen, we assume a side preference of the hens, because several animals rested on a small ledge above the roof of the minimal nest instead of resting on the perches. This behaviour may have contributed to the bias in the data from this pen. Judging by the number of eggs collected daily at each nest, two commercial nests could be authorized (CN1 and CN3), though two nests (CN2 and CN5) made the request for authorization unlikely to be fulfilled. Regarding CN4, further study is recommended for final assessment.

Beside the "number of eggs" parameter, the "nest visits per egg" parameter was also used to assess the hens' acceptance of the commercial nests. In general, the more nest visits a hen performs per egg-laying act, the greater disturbance experienced by the other hens in the nest. We therefore predicted that the number of eggs laid in a given nest would show a negative correlation with the number of visits per egg. This expectation was confirmed in the CN1 experiment, where more eggs were found in CN1 and a trend toward fewer nest visits per egg was observed relative to MN2. However, the sample size in this analysis was only four, so the difference in the number of nest visits per egg might reach significance with a higher sample size. Excluding one outlier pen, the number of nest visits per egg was also higher in CN5, where fewer eggs were laid relative to MN2. In the analyses of the other three commercial nests, no difference in the number of nest visits per egg was found, perhaps because of the relatively small sample sizes of eight, and four pens in CN2, CN3 and CN4, respectively.

Considering the fact that, more eggs, more animals per scan but less visits per egg were observed in CN1 compared to MN2, we conclude that hens staid longer in CN1 than in MN2. It is plausible that the more eggs laid into a given nest, the more hens need to stay there for egg-laying. Since fewer nest visits per egg were performed in CN1 than in MN2, we conclude that the more comfortable the animals feel inside a nest, the longer they stay in the nest when they lay the egg and the less often they enter the nest per laid egg. However, in the other preference tests, this assumption could not be confirmed by statistical analysis.

Hens may show more nest-exploring activity in the nest site area of an unattractive nest than near an attractive nest because lower confidence in the choice of the nest may stimulate the animal to more actively examine the area (Cooper and Appleby, 1996; Cooper and Albentosa, 2003; Olsson and Keeling, 2000; Sherwin and Nicol, 1993; Yue and Duncan, 2003; Zimmerman et al., 2000). However, no significant differences in the percentage of animals showing nest-exploring activity on the floor were detected in any of the preference tests, with the exception of CN1, where the hens showed a non-significant trend toward greater nest-exploring activity than in the MN2 nest area. We therefore conclude that the floor in the nest area was scarcely distinguished from the floor of the rest of the pen with respect to hen behaviour.

Only one of the five commercial nest landing platforms seemed to be more attractive as a place for performing other behaviour than nest-exploring activity than the landing platform of the minimal nest. The entrance of this commercial nest seemed to be blocked for hens, which are willing to enter the nest.

4.4. Nest attributes

We can only speculate about the reasons for acceptance or rejection of a nest because the nests differ in numerous attributes. All of the commercial nests were equipped with a mat of rubber pins or astroturf, which are classified as grappable structures and therefore considered attractive to the hens (Appleby and McRae, 1986; Appleby et al., 1988; Huber et al., 1985), in contrast to the thin hard plastic mat of the MNs. One could argue that astroturf is too stiff and pointed and might be unpleasant for the hens to sit on. Another crucial attribute might be the material of the walls. While the walls of the MNs and CN1 were made of plywood, the walls of CN3 were made of hard plastic, and the walls of CN2, CN4 and CN5 were made of sheet metal. Hens might feel more comfortable surrounded by wood and plastic because of its higher capacity for insulation. CN2 and CN5 have a divided floor, which might be less attractive than a one-piece floor because the extra edges might be unpleasant for the hens to sit on or pose a risk of falling from one part of the floor to the other. The slightly inclined landing platform of CN4 might have contributed to its rejection as a laying site as compared to MN2, which had a horizontally fixed landing platform. Therefore, this kind of preference test, where two nests differing in more than one attribute are offered, permits only speculation on which nest characteristics are crucial for their acceptance by the hens. Further studies investigating different nest attributes individually are needed to assess and discriminate their individual importance with respect to the attractiveness of a given nest.

4.5. Conclusions

In summary, this method of first developing a reference prototype for farm animal equipment that minimally fulfils the requirements of the Animal Protection Regulations and then presenting it together with a commercial option in a preference test will be useful for improving the quality of farm animal housing systems with respect to animal welfare. The parameters "number of eggs per nest" and "number of nest visits per egg" are considered effective in the evaluation of the preferred nest in a preference test with laying hens. Taking into account the few differences noted in behaviour and positions of the animals around the CNs and MNs, these parameters seem to be less helpful for evaluating the attractiveness of a nest. To optimize the nest assessment protocol, laying behaviour inside the nest might also be taken into account. In our study, no video cameras were installed inside the nests, so the behaviour of the animals could not be observed in this area. Further studies investigating the assessment parameters are needed to discriminate their individual importance with respect to the decision of authorisation of a given nest. For final assessment, further investigations under commercial conditions are recommended because group sizes are much larger in these conditions, which may influence the laying behaviour of the hens.

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