Northern fowl mite infestation affects the nocturnal behavior of laying hens

Leonie Jacobsa,*, Giuseppe Vezzolib, Bonne Beerdac, Joy A. Menchd

a Department of Animal & Poultry Sciences, Virginia Tech, 175 West Campus Drive, Blacksburg, VA 24061, USA
b College of the Desert, School of Mathematics and Sciences, 43-500 Monterey Avenue, Palm Desert, CA 92260, USA
c Department of Animal Sciences, Behavioural Ecology Group, Wageningen University & Research, De Elt 1, 6708 WD Wageningen, the Netherlands
d Department of Animal Science and Center for Animal Welfare, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

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Sleep
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A B S T R A C T
The northern fowl mite (Ornithonyssus sylviarum) is a key pest for caged laying hens. High infestation levels can lead to anemia, reduced production, and mortality. Although the mites cause skin irritation and thus likely affect resting behavior, the nocturnal behavior of infested chickens has not yet been studied. We investigated the impact of mite infestation on nighttime behavior of 16 beak-trimmed White Leghorn hens. The hens were housed individually in wire cages. They were experimentally inoculated with approximately 35 mites at 25 weeks of age and observed for the behaviors dozing, sleeping, preening and being active. Continuous observations were made from video recordings taken from 22:00 h until 06:00 h for two consecutive nights at pre-infestation week 0 and post-infestation weeks 3, 5 and 7. Mite infestation levels were measured weekly on an 8-point scale (0 (no mites) to 7 (≥10,000 mites per hen)). For statistical analyses with linear mixed models, mite infestation levels were categorized as score 0 (no mites), 3–4 (51–500), 5 (501–1000) and 6–7 (> 1000). Higher levels of mite infestation were associated with hens spending less time dozing (P < 0.001) and more time preening (P < 0.001) and being active (P = 0.012). Dozing decreased from 90% of the observed time for mite score 0 to 76% for score 6–7. Preening increased from 2% (score 0) to 9% (6–7) and time spent being active increased from 1% (0) to 7% (6–7). Mite infestation increased the number of uninterrupted bouts of all behaviors (P ≤ 0.020), especially dozing and preening (P < 0.001), suggesting restlessness in the mite-infested hens due to irritation and itching. The mite-infested hens’ increased nocturnal activity, including preening, as well as the fragmentation of behavioral activities together with decreased dozing, indicate disturbed resting behavior and suggest a reduction in the welfare of mite-infested hens.

1. Introduction

Laying hens (Gallus gallus domesticus) are vulnerable to several ectoparasites, including the poultry red mite (Dermanyssus gallinae), the chicken body louse (Menacanthus stramineus), and the northern fowl mite (Ornithonyssus sylviarum). The latter is considered a key pest of laying hens in North America, Brazil, China, and Australia (reviewed by Murillo and Mullens, 2017). The global incidence of infestation is unknown, but one study found northern fowl mite infestations on 25 out of 26 conventional cage farms in Southern California (Mullens et al., 2004), illustrating the endemic nature of these ectoparasites.

Infestation with northern fowl mites can originate from equipment, people, pullets and wild birds such as starlings and house sparrows (Axtell and Arends, 1990; Szabó et al., 2002). Northern fowl mites complete their life cycle on the skin of their hosts and these hemaphagous ectoparasites can cause up to 6% blood loss per day (DeLoach and DeVaney, 1981). All life stages (from egg to adult, with adults being 0.6 mm in size; Murillo and Mullens, 2017), along with their cast skins and excrement, can be present in the feathers, mainly near the vent (Vezzoli et al., 2015a). Northern fowl mites may live up to several weeks in the environment, increasing the risk of re-infestation (Mullens et al., 2009). Northern fowl mite infestation triggers the host’s immune response, indicated by mite-specific antibody production (Minniffield et al., 1993) or skin inflammation (Owen et al., 2009). Skin conditions and the associated itchiness make it likely that hens will show more grooming-related behaviors, both during the day and at night, as was observed when hens were infested with red mites (Kilpinen et al., 2005). Vezzoli et al. (2015b) did not find increased daytime preening in hens infested with northern fowl mites, but did find that more of the preening was directed at the vent area. Whether northern fowl mites cause more nocturnal preening is so far undetermined, though this could have serious welfare consequences if resting is disrupted.

* Corresponding author at: 175 West Campus Drive, Blacksburg, VA 24061, USA.
E-mail address: jacobsbl@vt.edu (L. Jacobs).

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Physiologically, sleep restores homeostasis of the brain after a period of wakefulness, with sleep deprivation leading to reduced alertness and performance (Boerema et al., 2003). Studies have associated sleep or rest with energy conservation (Malleau et al., 2007), tissue restoration and growth (Adam, 1980; Takahashi et al., 1968), retention of learning, and better adaptation to stimuli (McGrath and Cohen, 1978). A disruption in sleep due to mite infestation could therefore affect welfare. Relatively little is known about laying hen nocturnal behavior in relation to northern fowl mite infestation. Here, we investigated whether northern fowl mite infestations in laying hens affect dozing, sleeping, preening and activity during the dark period. We hypothesized that nocturnal resting behaviors would decrease with increasing mite infestation levels while activity and preening would increase. Because this was the first time nocturnal laying hen behavior was recorded in relation to northern fowl mite infestation, we were also interested in whether the behavioral repertoire of hens changed throughout the night and was influenced by mite infestation levels. We hypothesized that mites could impact behavior differently at different times at night.

2. Materials and methods

The Institutional Animal Care and Use Committee (IACUC) of the University of California, Davis, approved this experiment.

2.1. Animals and housing

Sixteen birds were randomly selected from a flock of CV20/W36 beak-trimmed pullets and moved to another facility. The pullets were raised in grower cages until being transferred to their experimental cages (wire cages measuring 94 * 46 * 46 cm) in two experimental rooms at 18 weeks of age, where they were kept individually but in visual, auditory and olfactory contact with other hens. Each cage contained a 32 * 32 cm plastic tray (Akro Mills SOR12500A34) that was either empty or filled with 1.2 kg sand (Sakrete Natural Play Sand, Dixon, California), cleaned and refilled daily. The behavioral observations reported in the current study made up part of a larger study looking at the effects of providing sand and AstroTurf substrates for dustbathing in cages on mite infestation levels (Vezzoli et al., 2015a). Since that study showed that mite levels were unaffected by the provision of either the dustbathing substrates, substrate effects are not further considered in the present paper.

The banks of cages had automatic water cups and a feed trough with ad libitum access to commercial layer mash. The lighting regime was 16L:8D and the temperature was kept at 21 ± 1°C. The experiment began when the hens were 25 weeks of age. It was necessary to experimentally infest all the sixteen hens, because we wanted to investigate how the time course of infestation affected the behaviors of interest in both this study and the larger study (Vezzoli et al., 2015a). Each hen (n = 16) was first determined to be mite-free, and then inoculated with approximately 35 northern fowl mites on her abdomen (for more details of the inoculation procedure see Vezzoli et al., 2015a). Mites were collected from a naturally infested flock at the UC Davis Avian Science Facility, which was separate from the facilities used in the current study.

2.2. Measurements

Hens were removed from their cages and scored visually for intensity of mite infestation during weeks 0 (before inoculation = control), 3, 5 and 7 of infestation (respectively 25, 28, 30, and 32 weeks of age). Hens thus served as their own controls. The feathers immediately anterior to the vent (an area approximately 8 cm long and 6 cm across) were sorted through from anterior to posterior to observe the skin. The estimated number of mites was recorded by one observer (GV). The number of mites were then categorized similarly as described by Mullens et al. (2010) and Vezzoli et al. (2015b), using a scoring system with a logarithmic scale: 0 = no mites seen; 1 = 1 to 10; 2 = 11 to 50; 3 = 51 to 100; 4 = 101 to 500; 5 = 501 to 1000; 6 > 1000 but < 10,000; and 7 ≥ 10,000 (Arthur and Axtell, 1983). It was not feasible to accurately count thousands of mites, yet for an estimate in numbers, we back-transformed average categorical scores to obtain average mite numbers per bird.

Video recordings were made with infrared cameras (SVB-70IR48, Veilux, Inc., TX, USA) mounted in front of every cage at a 50–70 cm distance. Cameras recorded for eight hours per night, from 22:00 h until 06:00 h. Recordings were made in 2011 on two consecutive nights per week resulting in 1024 h of video of which 717 h were analyzed. Technical errors resulted in missing video footage, yet all available recordings were analyzed. Five observers coded nocturnal behavior using GeoVision software (GeoVision V-Series Surveillance System, Vision Systems Inc., Irvine CA). An experienced observer (LJ) developed the method. The other four observers were first trained to identify the different behaviors by studying the description (Table 1) and viewing examples. Then, these observers viewed videos together with the experienced observer, learning to identify behaviors in longer sequences of video. Observers knew the observation week (0 = control; 3, 5 and 7), but were unaware of the mite infestation levels per bird. Inter-observer reliability was established for the four inexperienced observers based on the behavior of one hen during one night, with hourly durations of four behaviors being expressed as percentages of the observation time (32 records per observer). There was significant agreement based on the ranking of hourly behavior scores (Kendall's coefficient of concordance, adjusted for ties: 0.82; n = 32; P < 0.001). Behavior (Table 1) was observed for the full eight-hour nocturnal period using focal sampling and continuous recording. The start and end times of each of the four behaviors were recorded.

2.3. Statistics and analysis

Data were analyzed by means of linear mixed models using Restricted Maximum Likelihood (REML) in GenStat 14.1 (Oxford, UK, 2011). Mite numbers were expressed originally on an 8-point scale, but some categories either did not occur (categories 1 and 2) or occurred only rarely, i.e. category 3 with 4 cases and category 7 with 2 cases. Thus, we pooled the two rare categories with the adjoining ones, merging mite category 3 with 4 (labelled as 3) and 7 with 6 (labelled as 7). Mite scores (64 records on 16 hens) were analyzed for the effect of week (0 = control without mites, 3, 5 and 7). The fixed effect of week was included as a discrete factor and the random components of the model included room (n = 2) and individual hens (n = 16). Behavioral data were expressed both as durations (percentages of the observation
time) and number of bouts of uninterrupted performance. Duration scores were analyzed per night (118 records) and per clock hour (824 records). Bout numbers were analyzed only per night as bouts often spanned multiple hours. Behavior scores were analyzed for the fixed effect of mite infestation (0, 3, 5 and 7). Hourly behavior scores were analyzed as described, but with the additional fixed effect of clock hour (22, 23, 0, 1, 2, 3, 4, 5) and the 2-way interaction between mite infestation and clock hour. Fixed effects were all categorical and the random components of the model were again room and individual hen. Contrasts were considered significant when differences between two predicted means exceeded two times the standard error of difference for that contrast and statistical outcomes are presented as predicted means ± SE. Statistical analyses were also performed on transformed data, using the logit for percentages and the natural logarithm for means. Statistical outcomes are presented as predicted contrasts and statistical outcomes were all categorical and the random effects were signifi cant, except for one interaction effect (clock hour * mite infestation for dozing), although the P-values for the analysis of the transformed data were typically smaller, thus more significant. Since this further evaluation did not lead to meaningful information other than what was evident from the significant main effects, here we present the statistical outcomes only for the untransformed data.

3. Results

3.1. Nocturnal laying hen behavior

Hens spent most of their night time dozing (81.4 ± 2.2%), followed by sleeping (7.3 ± 1.9), being active (4.2 ± 2.1), and preening (7.0 ± 1.9). Dozing bouts occurred with the highest frequency (22.1 ± 1.9 bouts per night), followed by preening (19.3 ± 1.7), being active (12.8 ± 6.8) and sleeping (4.4 ± 0.8). The proportions of time hens performed specific behaviors differed according to clock hour (P < 0.001 for all behaviors; Table 2). Proportions of time the hens were active or preening were highest in the evening from 22:00 to 23:00 h and in the early morning from 04:00 to 06:00 h. Noteworthy is the balance of dozing and sleeping, with the latter becoming increasingly prominent with the passing of time. Peaks in behavior occurred from 22:00 to 23:00 h for activity (9.1 ± 2.5%), from 05:00 to 06:00 h for preening (11.6 ± 1.0%), from 1:00 to 2:00 h for dozing (84.9 ± 3.0%) and from 05:00 to 06:00 h for sleeping (11.8 ± 2.5%; Table 2).

3.2. Mite infestation

Mite infestation peaked at week 5, with the number of mites differing per week of infestation (P < 0.001). We found significant pairwise differences between week 0 and week 3, 5 and 7, and between week 3 and 5 (P < 0.05). Predicted mean mite numbers (± SE) were 0 in week 0, 1421 ± 656 in week 3 (score 4.6 on the original 8-point scale of 0–7), 3000 ± 656 in week 5 (score 5.4), and 1889 ± 643 in week 7 (score 4.6).

3.3. Mite infestation impact on behavior

Mite infestation levels had a significant impact on behavior over the entire night (22:00 to 06:00 h), both when expressed as percentages of the observation time and as bout frequencies (see Table 3), with only the percentage of time sleeping unaffected. Increased infestation levels were associated with hens being more active and dozing less and preening more, with the strongest effects at the two highest levels of mite infestation. The number of bouts of uninterrupted behavior increased with increasing levels of mite infestation for all four behaviors (Table 3). There was a significant 2-way interaction effect of mite infestation and clock hour on preening (P = 0.007), although comparisons of means between hours did not reveal particular times during which effects of mite infestation differed from what was described previously.

4. Discussion

Mite infestation caused a change in nocturnal behavior of laying hens in our study. Results showed that mites affected all four observed behavioral categories, with hens being more active during the night, preening more and dozing less than prior to infestation. Shortened bouts of uninterrupted behavior indicated behavioral fragmentation, which supports the notion that mites cause irritation and restlessness. Hens carrying mites are likely to experience impaired welfare, for example due to itching-related stress and sleep disruption.

Mite infestations peaked at week 5 after experimental inoculation, which was later than the peaks in weeks 3 and 4 reported by Owen et al. (2009, 2008), possibly due to the different initial inoculation numbers (35 versus 50 mites per hen respectively). Mullens et al. (2010) inoculated birds with 20 mites and found mite scores during weeks 3–6 that were comparable to our findings.

Time spent performing any of the four behaviors differed depending on the time of night. Generally, hens spent most time dozing (predicted mean 81% of the observation time), with low levels from 04:00 till 06:00 h when the time spent sleeping was highest. Sleeping (7%) increased as the night progressed. This pattern was somewhat similar to findings of Blokhuis (1984), who observed fewer birds dozing (decrease from approximately 65% to 60%), and more birds sleeping as the night progressed (20 to 35%). Preening (7%) and activity other than preening (4%) occurred most during the first (22:00 to 23:00 h) and final two observation hours (04:00 to 06:00 h).

Mite infestation did not have a clock-hour specific impact on the hens’ time budget during the night. The one significant interaction effect of clock hour and level of mite infestation on preening did not reveal patterns other than the main effects of mite infestation. Mites increased preening duration and frequency even at the lowest levels of infestation (51–500 mites per hen), with a further increment at higher levels of infestation. Furthermore, mite infestation levels of more than 500 mites per hen caused increased activity and reduced dozing. Mites stimulated preening and disrupted resting, and in addition increased the number of uninterrupted bouts of behavior. High mite loads can lead to anemia in hens, which in humans was associated with fatigue as a symptom (Sobrero et al., 2001), suggesting a greater need for resting.

<table>
<thead>
<tr>
<th>Behavior (%)</th>
<th>Time of night (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>22:00</td>
</tr>
<tr>
<td>Doze</td>
<td>81.1 ± 0.9a</td>
</tr>
<tr>
<td>Sleep</td>
<td>2.2 ± 2.4a</td>
</tr>
</tbody>
</table>

Table 2

Predicted mean (± SE, n = 16 hens) percentages of the observation time for behaviors (first column) shown per hour between 22:00 h and 06:00 h. Means within a row with no common superscripts differ significantly (P < 0.05), with clock hour effects being significant for all behaviors (P < 0.001, n = 824 records).
when anemic. However, further study is needed to confirm this in birds. Mite infestation thus resulted in fragmentation of the hens’ behavior. This applied to all behaviors, with strong effects already seen at the lowest level of mite infestation for dozing and preening. Multiple short dozing/sleeping bouts are likely to have a negative impact on hen welfare. The study of sleep disruption in humans showed that subjects needed uninterrupted sleeping bouts of ≥10 min for sleep to be restorative, otherwise there was reduced performance in a series of tests (Bonnet, 1986). Similarly, Blokhuis (1983) theorized that rest is important for poultry production performance, with disruption in circadian sleep-wake cycles negatively affecting protein synthesis, which is important for yolk and albumen formation (Moran, 1987). Hens may have compensated during the day for the rest fragmentation we saw at night. This was not assessed in our current study, but Boerema et al. (2003) found that chickens that were sleep-deprived during the day spent more time in deep sleep states at night. The reduced dozing duration and increased behavioral fragmentation suggest a reduction in resting quality due to mite infestation.

Hens regularly interrupt resting to preen during the dark period. Blokhuis (1984) found that approximately 5–10% chickens were preening at any given time during the night. As mite infestation levels increased more nocturnal preening was shown by hens in our study, which is similar to the findings with red mites (Kilpinen et al., 2005). The increase in preening and other active behavior likely results from itchiness caused by mites. Furthermore, mite-induced anemia itself could have induced itchiness. Although this has not been shown in birds, in humans iron deficiency anemia was associated with generalized itchiness (Polat et al., 2008; Patel and Yosipovitch, 2013). Self-preening is an important behavior of birds in relation to their control of ectoparasite infestation (reviewed by Clayton et al., 2010), although itchiness or irritation are not mentioned in the review as a potential trigger. In humans itchiness can cause suffering, reduce quality of life, and affect mental health (reviewed by Misery et al., 2018). Itchiness induced by prolonged infestation may therefore represent a serious welfare concern. Yet, welfare problems associated with (prolonged) itchiness are understudied and this topic is rarely—if ever— included in production animal welfare research and assessments, and warrants further research.

**Declarations of interest**

None.

**Acknowledgements**

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**References**


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**Table 3**

Predicted means (± SE, n = 16 hens) for behaviors shown between 22:00 h and 06:00 h with p-values for mite infestation. Values represent percentages of the observation time or number of uninterrupted bouts per 8–hour period. Means within a row with no common superscripts differ signiﬁcantly (P < 0.05, n = 118 records).

<table>
<thead>
<tr>
<th>Behavior</th>
<th>P-value</th>
<th>Mite scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 (no mites)</td>
</tr>
<tr>
<td>Active (%)</td>
<td>0.012</td>
<td>1.3 ± 2.3a</td>
</tr>
<tr>
<td>Preen (%)</td>
<td>&lt; 0.001</td>
<td>2.1 ± 1.6a</td>
</tr>
<tr>
<td>Doze (%)</td>
<td>&lt; 0.001</td>
<td>90.0 ± 2.8a</td>
</tr>
<tr>
<td>Sleep (%)</td>
<td>0.262</td>
<td>6.6 ± 2.3</td>
</tr>
<tr>
<td>Active (bouts)</td>
<td>0.010</td>
<td>7.8 ± 7.1a</td>
</tr>
<tr>
<td>Preen (bouts)</td>
<td>&lt; 0.001</td>
<td>8.0 ± 2.2a</td>
</tr>
<tr>
<td>Doze (bouts)</td>
<td>&lt; 0.001</td>
<td>10.4 ± 2.5a</td>
</tr>
<tr>
<td>Sleep (bouts)</td>
<td>0.020</td>
<td>2.2 ± 1.1a</td>
</tr>
</tbody>
</table>

**5. Conclusion**

Mite infestation affected nocturnal laying hen behavior, resulting in behavioral fragmentation and disruption of resting in addition to the previously reported biological impact. The increased time spent preening and number of preening bouts suggest that the hens experienced irritation and itchiness, which could be associated with a negative emotional state. This means there is a direct welfare impairment due to mites, regardless of whether or not hens are rest-deprived. Itchiness has received little to no attention in production animal welfare research and assessments, and warrants further research.


