Relationships between beak condition, preening behavior and ectoparasite infestation levels in laying hens

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ABSTRACT The effects of beak condition on ectoparasite populations and preening in laying hens were investigated. Beak-trimmed and beak-intact caged Hy-Line W-36 hens were infested with either chicken body lice or northern fowl mites using a 2 × 2 factorial design with 4 replicate cages (each containing 2 hens)/treatment: 1) **BTL** (beak-trimmed lice-infested); 2) **BTM** (beak-trimmed mite-infested); 3) **BIL** (beak-intact lice-infested); and 4) **BIM** (beak-intact mite-infested). Mite scores and lice numbers were estimated weekly. Hens were video recorded the wk before infestation and at wk 6 and 9 post-infestation. Time spent preening on 6 body areas and in total were analyzed using a repeated measures ANOVA. There was a wk × beak condition interaction for lice loads, with BTL harboring approximately 17 times more lice than BIL from wk 7 to 10 post-infestation ($P < 0.0001$). Beak condition affected mite loads ($P < 0.0001$), with BTM having a higher mite score (3.8 ± 0.26) than BIM (1.4 ± 0.26). At peak infestation, BTL spent more total time preening ($P = 0.02$, s ± SE: 232.1 ± 37.6) than prior to infestation (33.9 ± 37.6) and directed their preening behavior towards the vent. In contrast, BIL (73.9 ± 37.6), BTM (9.4 ± 1.6), and BIM (8.6 ± 1.6) did not increase total time spent preening over pre-infestation levels (103.6 ± 37.6, 5.8 ± 1.6, 6.7 ± 1.6 respectively), although BTM did redirect their preening behavior toward the vent. This study confirmed previous studies showing that an intact beak is important for reducing ectoparasite infestations. Preening behavior increased in response to lice infestation, but only in beak-trimmed hens; preening behavior and louse load were correlated at peak infestation. In contrast, mite infestation did not lead to increased preening, and there was no correlation between preening and mite load. However, both lice- and mite-infested hens directed preening behavior predominantly towards the vent where these parasites are typically found.

Key words: hen, preening, lice, mites, beak-trimming

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INTRODUCTION

Grooming behavior maintains the body surface (Rowell, 1961; Clayton, 1991; Spruijt et al., 1992) and can be motivated by both internal (e.g., hormones, neuropeptides, opioids) and external (e.g., moisture, dirt, parasites) stimuli (Lefebvre and Joly, 1982; Delius, 1988; Spruijt et al., 1992). On average across species, birds spend approximately 9% of their daily time budget grooming, with preening being the predominant component of that grooming behavior (Clayton, 1991; Cotgreave and Clayton, 1994; Bush et al., 2012).

Clayton (1991) suggested that preening behavior reflects coevolution between hosts and parasites. Cantarero et al. (2013) found decreased grooming activity in pied flycatchers when the level of ectoparasites in their nests was reduced by heat treatment, which suggests a relationship between grooming activity and ectoparasites. However, data on the time infested wild birds spend preening are scarce and sometimes contradictory. For example, rock doves infested with chewing lice do not groom more than uninfested birds (Clayton, 1990), but pigeons infested with hippoboscid flies do (Waite et al., 2012). Differences between studies could be due to a variety of factors, including the types of ectoparasites. The majority of host-ectoparasite behavior studies have been conducted with feather-dwelling lice, which are relatively slow-moving and thus may have different effects on grooming behavior than other more mobile parasites (Waite et al., 2012). They may also be less physiologically damaging or irritating than ectoparasites that feed on blood or skin.

Similar to wild birds, the relationship between time spent preening and ectoparasite load in chickens has received relatively little investigation. Chickens have been reported to spend 13% of their daily time budget

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preening (Dawkins, 1989). An older study (Brown, 1974) showed that there was a positive correlation between the number of lice and "grooming acts," defined as preening, scratching with the foot, pecking the foot, feather settling, and bill wiping in domestic chickens. Kilpinen et al. (2005) is the only other study that evaluated this relationship, and they showed that laying hens infested with the poultry red mite (*Dermanyssus gallinae*) performed more preening bouts than uninfested hens, although they did not spend more time preening.

The 2 most important chicken ectoparasites in North America (Axtell and Arends, 1990; Ruff, 1999; Mullens et al., 2010; Sparagano et al., 2014) are the chicken body louse (*Menacanthus stramineus*) and the northern fowl mite (*Ornithonyssus sylviarum*). Because of the different nature and size of these ectoparasites, they could have different effects on preening. According to Delius (1988), preening is stimulated by local stimuli (e.g., dirt, parasites) on the skin and feathers. It is therefore possible that the size of this local stimulus is important and that lice, which are larger than mites, might elicit different preening responses. The body areas on which these different parasites are found could also influence the sites to which preening activity is directed. Northern fowl mites are found predominately on the vent, whereas chicken body lice are found under the wings, on the back, and on the vent-abdomen area (Trivedi et al., 1991; Mullens et al., 2010; and Chen et al., 2011). In addition, chicken body lice are extremely mobile parasites, which could also affect local stimulation and thus preening responses. To our knowledge, there have been no studies comparing the effect of different ectoparasites on the time spent preening or the parts of the body to which preening behavior is directed in birds.

While information about the relationship between time spent preening and ectoparasite load is scarce, the effect of preening on ectoparasite load is well known. Many studies have shown that preening is the most common and efficient strategy for defending against ectoparasites (Cotgreave and Clayton, 1994; Koop et al., 2012; Cantarero et al., 2013), and the beak therefore plays a crucial role in the effectiveness of this defense. Beak-impaired wild rock doves and wild rock pigeons have significantly more feather lice and parasitic flies than birds with non-impaired beaks (Clayton, 1991; Bush and Malenke, 2008; Waite et al., 2012, 2014). In their comparative study on the diversity of chewing lice among 52 species of birds, Clayton and Walther (2001) showed that beak morphology played a critical role in controlling parasites and hypothesized that the shear force between the upper beak and lower beak during preening damages the ectoparasites. They suggested that the evolution of beak morphology is related not only to feeding but also to preening. These results were supported by Clayton et al. (2005), where the authors found that trimming the upper beak of pigeons significantly increased the number of lice. Studies with chickens (Brown, 1972; Matthysse et al., 1974; Mullens et al., 2010; Chen et al., 2011) showed that both beak-trimmed chicks and adult birds had more chicken body lice and northern fowl mites than beak-intact birds.

The aims of the study were to evaluate the effects of beak condition on the course of infestation of northern fowl mites or chicken body lice, as well as the effects of ectoparasite infestation on preening behavior. It was hypothesized that: 1) hens infested with either lice or mites would spend more time preening than they did prior to infestation and would redirect their preening activity to the body area(s) on which the ectoparasites are known to be found; 2) beak-trimmed (**BT**) hens would have more mites and lice and thus spend more time preening than beak-intact (**BI**) hens; and 3) lice would elicit different preening activity than mites.

MATERIALS AND METHODS

Animal Housing and Ectoparasite Treatments

Hy-Line W-36 White Leghorn pullets (N = 32, 18-week-old) were purchased from a commercial farm. Half of the pullets had been beak-trimmed using a hot-blade trimmer at the farm at 10 days of age, while the other half were beak-intact. Although beak lengths were not measured in the experimental pullets, Mullens et al. (2010) showed in a previous study that beak length variation among trimmed hens from this same farm was not a significant factor in the development of mite or louse populations. All trimmed hens did lack the curved upper mandible tip that is critical for ectoparasite removal (Clayton et al., 2005). None of the beak-intact birds used in the experiment had serious beak asymmetry or beak damage.

At 21 wk of age, 16 hens were experimentally infested with 40 mixed adult/juvenile chicken body lice and 16 were infested with 20 mixed adult/juvenile northern fowl mites according to the methods described in Mullens et al. (2010). Lice reproduce more slowly than mites, so adding a larger number of lice initially helps to ensure that they become established and that their population dynamics better match those of the mites. Hens were randomly assigned to each ectoparasite treatment in a 2 × 2 factorial design, with 8 hens/treatment: 1) BTL (beak-trimmed lice-infested); 2) BTM (beak-trimmed mite-infested); 3) BIL (beak-intact lice-infested); and 4) BIM (beak-intact mite-infested). The chicken body lice and the northern fowl mites were collected from source hens housed at the University of California, Riverside.

The hens from each ectoparasite treatment were kept in 2 adjacent poultry houses at University of California, Riverside. Each house contained only a single parasite treatment in order to prevent cross-contamination of hens by different ectoparasites. The 2 screened houses were 3.8 m wide and 5.8 m long and designed to mimic open-sided commercial layer housing. They had roof sprinklers for cooling, natural light during the day, and were kept on a 16L:8D light cycle using supplemental lighting early and late in the photoperiod. The houses were virtually identical in design, air flow, and directional orientation, so environmental conditions within the houses during moderate weather conditions (e.g., during the end of September through the beginning of December when this experiment was conducted) were comparable. Although in-house environmental conditions were not monitored, the air temperatures in Riverside (wunderground.com) on the days and at the time video recordings were made (see below) were similar throughout the study: 28.9°C pre-infestation; 28.9°C wk 6; and 25.0°C wk 9. Riverside is in a semi-arid zone in California, and relative humidities during the days the hens were video recorded were (wunderground.com): 64% pre-infestation; 49% wk 6; and 25% wk 9.

Each house had 25.4 cm × 30.5 cm suspended wire cages, 8 of which were used to hold the experimental hens, with each of those cages containing 2 hens with the same beak condition. Each cage bank consisted of 4 cages, with hens semi-randomly allocated to the cages such that a cage with trimmed hens was next to a cage with untrimmed hens. The ectoparasite species (either mites or lice) in a particular house could travel easily to contiguous cages, but the cage banks were suspended from separate roof supports, impeding ectoparasite movement between them. So that the hens could be individually identified for parasite counts, 1 hen in each cage was leg banded. The cages were provided with automatic water cups and a feed trough that was filled every morning with commercial laying hen feed (Kruse Perfection Brand, Ontario CA; 17% crude protein and 1062 Kcal/kg).

Weekly from wk 1 through 10 post-infestation each hen was removed from her cage and the number of ectoparasites present was visually estimated. Since the same individuals performed these evaluations, lice and mites were counted on different days for biosecurity reasons; the counts took place on days that the hens were not being video recorded (see below) so as not to alter their behavior. Lice numbers were estimated 2 days before video recording: 1) on the vent, by sorting the feathers from anterior to posterior; 2) under each wing; and 3) on the chest in the anterior keel area (Mullens et al., 2010). All adult and nymphal instar lice were counted. Data from the 3 regions for each hen were then summed for analysis. Mites were counted one day before video recording using the scoring system of Mullens et al. (2000). The feathers of the lower abdomen in a circle approximately 8 cm in diameter anterior to the vent were sorted and the mites were scored as: 0 = no mites, 1 = 1 to 10, 2 = 11 to 50, 3 = 51 to 100, 4 = 101 to 500, 5 = 501 to 1000, 6 = 1001 to 10,000 and 7 ≥ 10,000 mites. The generation interval of northern fowl mites (approximately 5 to 7 d) and chicken body lice (approximately 2.5 wk) is such that there should not have been an appreciable change in the populations of either during the period from counting to video recording. Housing and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of California, Riverside (Protocol AUP #A-0309019-3).

Behavioral Observations

To allow individual identification during observations, one hen within each cage was marked on her back and chest with green marker prior to video recording. Cameras (SONY Handycam, DCR-TRVZ80, Sony Corp., Tokyo, Japan) mounted centrally in front of 2 adjacent cages were used to film the hens. Each cage was filmed in real-time for 2 30-min sessions in the afternoons from 13:05 to 13:35 h and from 14:05 to 14:35 h. These times were selected to avoid the behavioral disturbance of the morning feeding (about 09:00 h) and oviposition (typically 08:00 to 12:00 h). In addition, observations of approximately 100 hours of videos of uninfested hens as part of another study (Vezzoli, unpublished data) showed that about 12 to 13% of each hour during the day was spent preening, and that preening was evenly distributed throughout the day with little evidence of diurnal peaks or troughs. Video recording took place one d before infestation and one d at wk 6 and 9 post-infestation, which were the anticipated peaks of infestation for mites and lice, respectively (Mullens et al., 2010). Each hen served as her own control. The videos were analyzed using the EthoLog 2.2 software program (Ottoni, 2000), which allows transcription of real-time observations. The first and the last 5 min of each session were not coded because personnel were in the house switching on and off the video cameras. The total time spent preening and the body areas to which the preening was directed (back, neck-chest, internal wing, external wing, vent, and uropygial gland area) were analyzed. These body areas were selected based on a preliminary review of the videos: 2 h of video were randomly selected from the pre-infestation and peak-infestation periods, and 60 min segments were coded continuously. Since mutual grooming and severe feather pecking were never observed, and there was only a single incidence of gentle feather pecking, these behaviors were considered to occur too infrequently to be included in the ethogram. A numerical code was used to identify hens, cages, houses, and the time of video recording. Thus, the single observer (Giuseppe Vezzoli) who coded the videos was blind to the ectoparasite treatments and to the week of infestation.

Statistical Analysis

A general linear mixed model repeated measures procedure was used to analyze the mite scores and lice numbers separately. Week and beak condition were the fixed effects. Since examination of the ectoparasite data showed that within-cage infestation levels were more similar than between-cage levels, cage rather than individual hen was used as the experimental unit (random effect). Week was the repeated measure in the model. The wk × beak interaction was included in the model. The mite scores and the lice numbers from the 2 hens in each cage were averaged for analysis. To avoid floor effects, wk 0 for mite scores and lice numbers was not included in the analysis. Similarly, for lice wk 1 was not included because lice were not seen on the hens at that time.

For the behavioral data 2 analyses were conducted. First, to determine whether lice and mites had an effect on preening behavior, preening by hens prior to infestation (wk 0) was compared to preening during the wk of video recording that was nearest to the peak of infestation (wk 6 for mites and wk 9 for lice). Hereafter, wk 6 for mites and wk 9 for lice will be referred as the peak of infestation. The model used to analyze the behavioral data was the same as that used for the ectoparasite populations. Cage was again the experimental unit, and therefore the behavioral data from the 2 hens in each cage were averaged, and the data from the 2 daily observation sessions were then summed. The time spent preening on each body area and the total time spent preening (calculated as the sum of the time spent preening on each body area), were the dependent variables. Second, to determine whether lice and mites had different effects on preening behavior, the time mite- and lice-infested hens spent preening at their respective peaks of infestation were compared. A general linear mixed model with ectoparasite treatment and beak condition as fixed effects was used. The model included the ectoparasite treatment × beak condition interaction. As for the first analysis cage was considered as the experimental unit, therefore the data were averaged.

All data were analyzed using the Statistical Analysis System (SAS, V9.3). The Shapiro-Wilk test was used to assess normality, and homogeneity of variance was assessed via graphical evaluation of the residuals. The assumptions of ANOVA were not met for the following variables: 1) lice numbers; 2) time spent preening on the neck-chest area, and internal wings for the comparison of lice-infested hens prior to and post-infestation; 3) time spent preening on all body areas for the comparison of mite-infested hens prior to and post-infestation, with the exception of the time spent preening on the external wings; and 4) time spent preening on the internal wings, on the uropygial area, and total time spent preening for the peak infestation comparison. All of these data were square-root transformed except for the lice numbers, which were log (n+1) transformed. Transformed and back-transformed data are reported. The level of statistical significance was set at $P < 0.05$, with $0.05 \leq P < 0.10$ considered as showing a trend towards significance. A Tukey post-hoc test was used when significant differences were found. To determine whether preening at the peak of infestation irrespective of beak condition was related to ectoparasite numbers, mite scores were correlated with the total time spent preening at wk 6 using a Spearman rank correlation, and lice numbers with the total time spent preening at wk 9 using a Pearson correlation.

RESULTS

Lice

Lice Numbers Lice numbers are shown in Figure 1. While there were main effects of both beak condition ($F_{1,48} = 42.42$, $P < 0.0001$) and wk ($F_{8,48} = 15.73$, $P < 0.0001$), there was also a significant wk × beak condition interaction ($F_{8,48} = 5.28$, $P < 0.0001$). Lice numbers on BTL continued to increase from wk 5 to 9 post-infestation, while numbers on BIL plateaued during that time. Lice numbers on BTL hens were higher than those on BIL hens from wk 7 to wk 10 post-infestation ($P < 0.0001$). BTL hens had 16.9 times more lice than BIL hens from wk 7 to wk 10; at the peak of infestation BTL hens had 15.5 times more lice than BIL hens.

Preening There was a main effect of wk for the time spent preening on the vent and external wings, and similar trends for the total time spent preening and time spent preening on the neck-chest (Table 1). This pattern of preening was, however, strongly affected by beak condition (Figure 2). There were wk × beak condition interactions for the total time spent preening ($F_{1,6} = 9.79$, $P = 0.02$) and for the time spent preening on all body areas except the internal wings and uropygial gland: back ($F_{1,6} = 6.97$, $P = 0.04$), external wing ($F_{1,6} = 8.54$, $P = 0.03$), neck-chest ($F_{1,6} = 7.33$, $P = 0.04$), and vent ($F_{1,6} = 19.49$, $P = 0.005$). Post-hoc tests revealed that BTL hens at wk 9 spent more time preening in total (s ± SE: 232.1 ± 37.6) than they did at wk 0 (33.9 ± 37.6). They also spent more time preening on their backs, external wings, and neck-chest than they did at wk 0 (Figure 3). At wk 9, BTL hens not only spent more time preening on the vent area (s ± SE: 111.0 ± 13.6) than they did prior to infestation (11.9 ± 13.6), but also more time than BIL hens spent preening their vent area at wk 9 (27.3 ± 13.6).

There were no main effects of wk or beak condition on preening on the remaining body areas. There was a significant positive correlation between the total time spent preening and lice numbers at wk 9 post-infestation (n = 8; r = 0.91 $P = 0.002$).

Mites

Mite Score There was a main effect of beak condition ($F_{1,54} = 44.05$, $P < 0.0001$) and wk ($F_{9,54} = 5.84$, $P < 0.0001$) for mite score, with the mite score of BTM hens being higher (mite score ± SE: 3.8 ± 0.26) than that of BIM hens (1.4 ± 0.26) and with mite score increasing post-infestation. The mite score at wk 5 was higher (3.5 ± 0.27) than during the preceding wk and not statistically different than the mite scores at

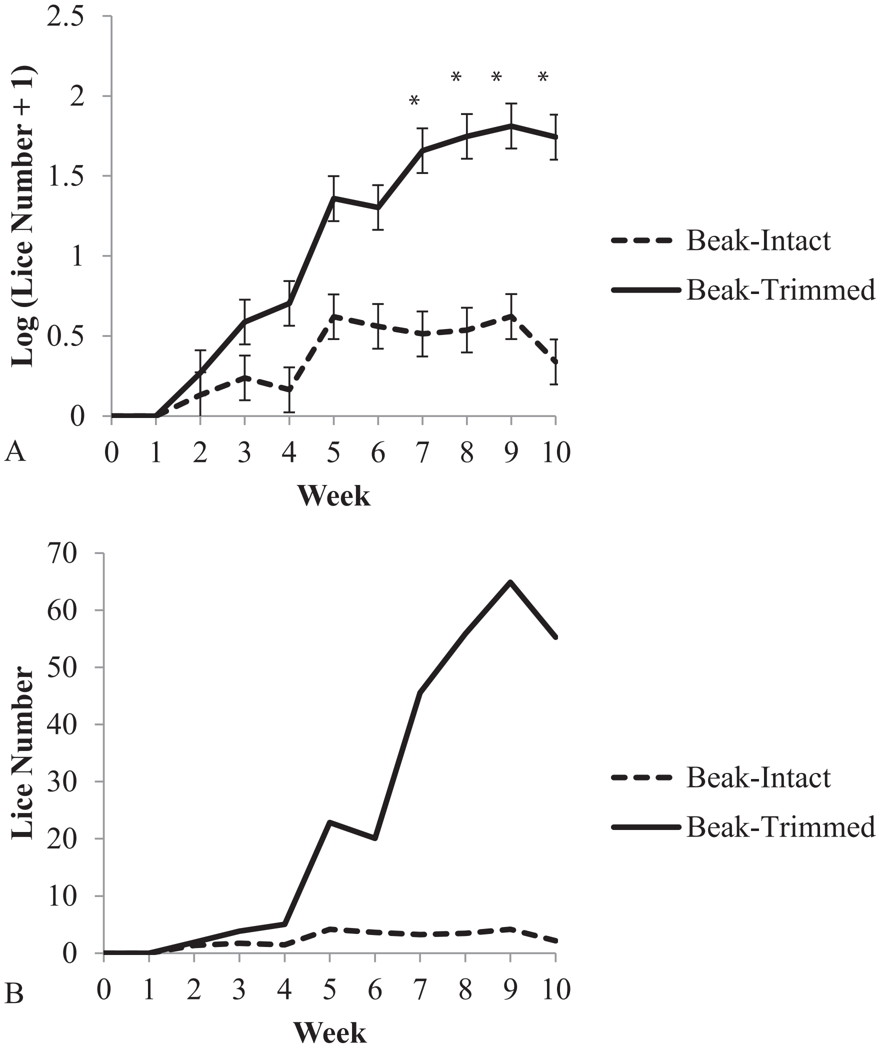

Figure 1. Log transformed means ± SE of lice numbers (A) and back-transformed means of the lice numbers in beak-trimmed and beak-intact hens. Stars (*) indicate significant differences between beak-intact and beak-trimmed hens during particular weeks at $P < 0.05$.

Table 1. Main effects of week on the time spent preening by lice-infested hens. Means and standard errors are reported for both significant effects and trends.

	Time spent preening (s)			
Week	Vent	External Wings	Neck-chest	Total time spent preening (s)
0	28.1[a] ± 9.6	2.0[a] ± 2	3.5 ± 0.8	68.7 ± 26.6
9	69.1[b] ± 9.6	10.7[b] ± 2	6.3 ± 0.8	152.9 ± 26.6
Test Statistic	$F_{1,6} = 9.74$	$F_{1,6} = 7.84$	$F_{1,6} = 5.42$	$F_{1,6} = 5.34$
P-value	0.02	0.03	0.06	0.06

[a,b]Different superscripts in a column indicate a significant difference ($P < 0.05$).

wk 6, 7, 8, 9, and 10 (3.2, 2.7, 2.8, 2.8, and 2.8, respectively). However, there was also a trend for an interaction between wk and beak condition ($F_{9,54} = 1.99$, $P = 0.058$; Figure 4). This shows that mite scores on BTM hens tended to be higher than those on BIM hens from wk 3 until wk 10 post-infestation. On average the mite score of BTM hens from wk 3 to wk 10 was 4.2 while for BTM hens it was 1.5. At the peak of infestation BTM had a score of 4.7 and BIM a score of 1.8.

Preening There was a wk effect on the time spent preening on the vent ($F_{1,6} = 11.76$, $P = 0.01$) and the uropygial area ($F_{1,6} = 8.34$, $P = 0.03$), with hens at wk 6 spending more time preening on those areas than they did at wk 0 prior to infestation (Figure 5). There were no wk, beak condition, or wk × beak condition effects for the time spent preening on the back, internal wings, external wings, neck-chest area, or in total (Figure 6). There was no wk × beak condition interaction for preening on the vent. There was no correlation between mite score at the peak of infestation and the total time spent preening (n = 8; r = 0.13 $P = 0.76$).

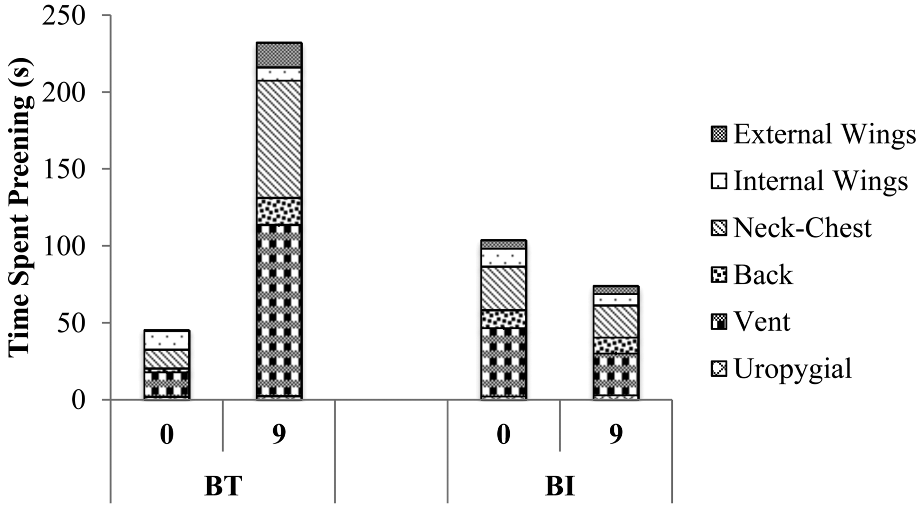

Figure 2. Mean time spent preening on different body areas by beak-trimmed (BT) and beak-intact (BI) lice-infested hens prior to infestation (wk 0) and at the respective peak of infestation (wk 9).

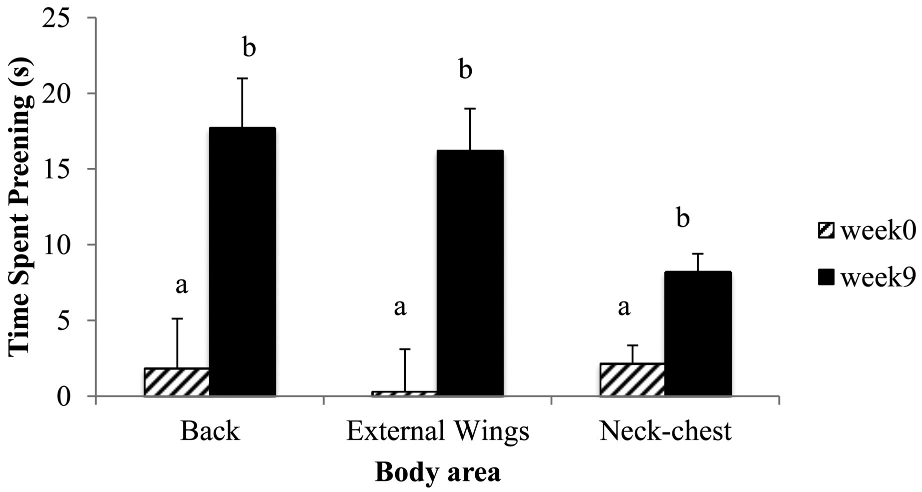

Figure 3. Week × beak condition interaction effects on the time spent preening on different body areas in beak-trimmed lice-infested hens. Means ± SE are reported. Letters indicate significant differences between weeks for each body area at $P < 0.05$. Back-transformed means for the neck-chest area are 4.6 at wk 0 and 67.2 at wk 9.

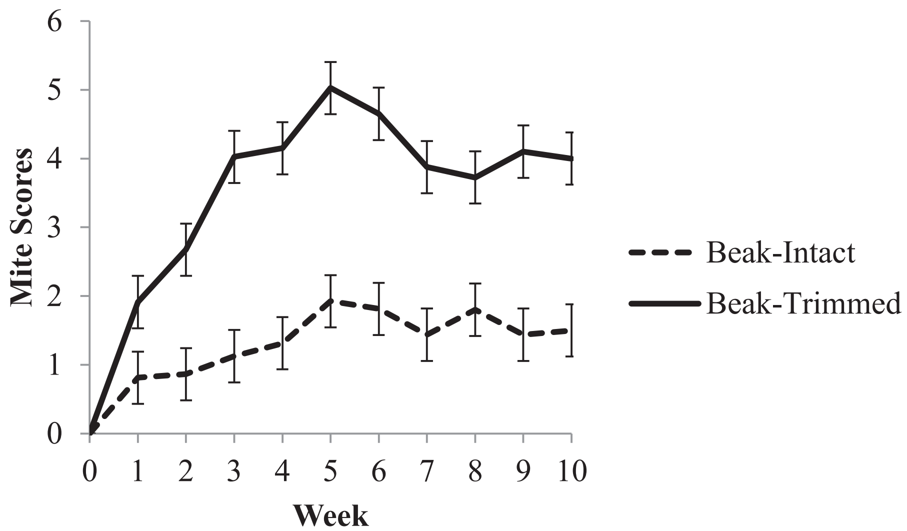

Figure 4. Trend for the beak condition × ectoparasite treatment interaction for mite score on beak-trimmed and beak-intact hens. Means (mite score) ± SE are reported.

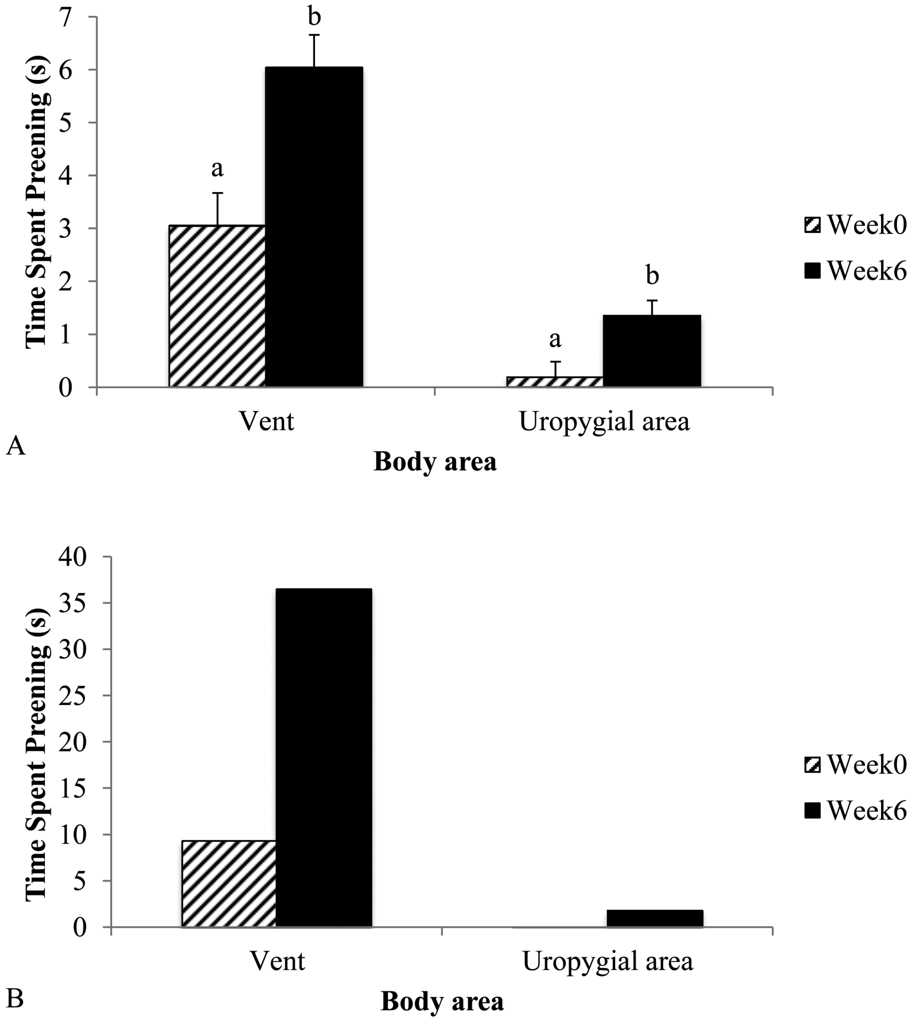

Figure 5. Effect of week on the time spent preening on the vent and on the uropygial area in mite-infested hens. Transformed means ± SE are reported in (A) and back-transformed means are reported in (B). Letters indicate significant differences between weeks for the vent and uropygial areas at $P < 0.05$.

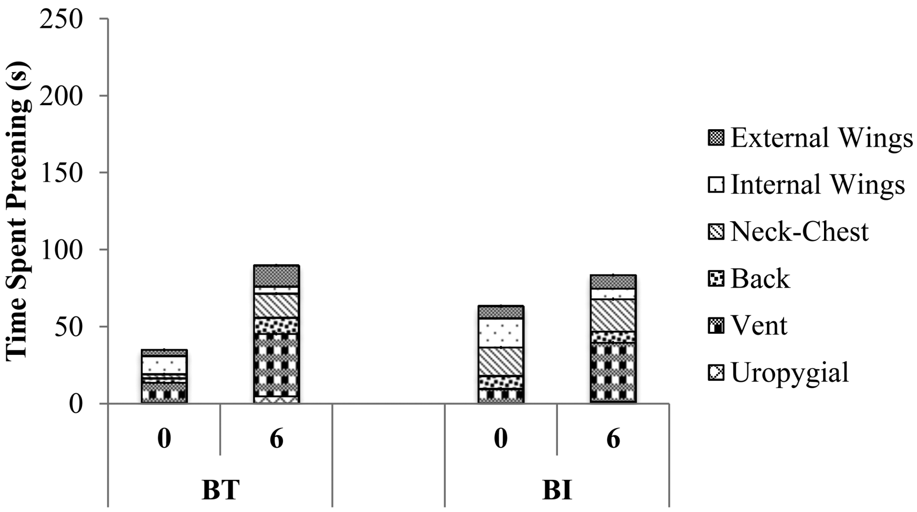

Figure 6. Mean time spent preening on different body areas by beak-trimmed (BT) and beak-intact (BI) mite-infested hens prior to infestation (wk 0) and at the respective peak of infestation (wk 6).

Table 2. Main effects of beak condition on the time spent preening for the peak infestation comparison of lice- and mite-infested hens. Means and standard errors for the time spent preening on the vent and external wings are reported for both significant effects and trends. Back-transformed and transformed means and standard errors for the total time spent preening are also reported, in parentheses.

	Time spent preening (s)		
Beak condition	Vent	External Wings	Total time spent preening (s)
Beak-Trimmed (BT)	75.8[a] ± 10.2	14.9 ± 2.7	146.4[a] (12.1 ± 1.03)
Beak-Intact (BI)	32.8[b] ± 10.2	7.0 ± 2.7	72.3[b] (8.5 ± 1.03)
Test Statistic	$F_{1,12} = 8.86$	$F_{1,12} = 4.37$	$F_{1,12} = 5.96$
P-value	0.01	0.058	0.03

[a,b]Different superscripts in a column indicate a significant difference ($P < 0.05$).

Table 3. Main effects of ectoparasite treatment on the time spent preening for the peak infestation comparison of lice- and mite-infested hens. Means and standard errors are reported for the trends on the vent and neck-chest area.

	Time spent preening (s)	
Ectoparasite treatment	Vent	Neck-chest
Lice	69.2 ± 10.2	6.4 ± 0.9
Mites	39.5 ± 10.2	3.7 ± 0.9
Test Statistic	$F_{1,12} = 4.23$	$F_{1,12} = 4.60$
P-value	$P = 0.06$	$P = 0.053$

Peak Infestation Comparison of Lice and Mites

Table 2 shows the main effects of beak condition on the time spent preening. There was a significant difference for the total time spent preening, with BT hens spending more time preening than BI hens; there was also a trend for BT hens to spend more time preening on the external wing. There was a trend for ectoparasite treatment to affect preening on the neck-chest, with lice-infested hens tending to spend more time preening on the neck-chest area than mite-infested hens (Table 3).

Although there were main effects of beak condition and ectoparasite treatment on the time spent preening on the vent, they were affected by the beak condition × ectoparasite interaction ($F_{1,12} = 7.96$, $P = 0.02$). BTL hens spent more time preening (s ± SE: 111.0 ± 14.4) than BTM (40.6 ± 14.4), BIM (38.3 ± 14.4), and BIL (27.3 ± 14.4) hens. There was a trend toward significance ($F_{1,12} = 3.75$, $P = 0.08$) for the ectoparasite treatment × beak condition interaction on the total time spent preening, with BTL hens tending to spend more time preening [back-transformed means and transformed means s ± SE in parenthesis: 219.0 (14.8 ± 1.5)] than BTM [88.4 (9.4 ± 1.5)], BIL [72.3 (8.5 ± 1.5)], and BIM [74.0 (8.6 ± 1.5)]. There were no effects of ectoparasite treatment, beak condition, or ectoparasite treatment × beak condition on the time spent preening on the internal wing, on the back or on the uropygial area. There were also no effects of ectoparasite treatment on the total time spent preening.

DISCUSSION

These data confirm the beak trimming effects reported by Mullens et al. (2010) and Chen et al. (2011). In the current study, it was found that BT hens harbored more mites than BI hens. Although the beak condition × wk interaction was not significant for mite score, BTM hens tended to have mite scores approximately 2 to 3 times those of BIM hens from wk 3 to 10 post-infestation and at peak infestation, a numerical score difference that indicates a tenfold difference in mite loads. It was also found that BTL hens had approximately 17 times more lice from wk 7 to 10 post-infestation than BIL hens, and 16 times more lice at the peak of infestation. Mullens et al. (2010) found that, by wk 11 post-infestation, beak-trimmed hens had approximately 15 times more lice and 10 times more mites than beak-intact hens. Chen et al. (2011) also showed that, at the respective peaks of infestation, beak-trimmed hens had approximately 5 to 8 times more lice than beak-intact hens and a mite score of 5 (501 to 1000 mites) as compared to 2 (10 to 50 mites) for beak-intact hens. Diverse factors can affect the magnitude of infestation found in different studies, for example the temperature and the humidity of the rooms in which the hens are housed. However all of the studies indicate that beak-trimmed hens are unable to efficiently remove ectoparasites, possibly because their upper beaks are not long enough to generate shear forces on the tip of the lower beak that are sufficient to pick off, damage, or kill ectoparasites (Clayton et al., 2005; Clayton et al., 2010).

This is the first experiment systematically evaluating the relationship between preening and ectoparasite infestation in chickens. BIL hens showed no change in preening behavior after infestation, whereas BTL spent more time preening on the back, on the vent, on the neck-chest area, and in total than they did prior to infestation. All these body regions are inhabited by *Menacanthus stramineus*, especially the vent (Trivedi et al., 1991; Mullens et al., 2010). These findings suggest that BTL hens may be irritated by the lice that they cannot remove, producing an increase in the amount of time spent preening. While it would have been interesting to correlate the areas preened with actual louse count on those areas, this was impossible for logistical reasons. Lice are mobile parasites that move rapidly when disturbed, as they are during counting. While the louse counts took place 2 days before filming, the relatively long generation interval of lice (about 2.5 wk) means that total populations should not increase significantly in 2 d, although the population densities in specific body areas could have changed.

There was also a positive correlation between the total time spent preening and the number of lice. The BIL data show a similar pattern as found by Clayton (1990), who found that rock doves infested with chewing lice did not increase the time spent preening compared to uninfested controls. Clayton (1990) noted that the rock dove chewing louse lives on the host's feathers, but suggested that the chicken body louse, which lives mainly on the skin, could cause dermatitis and itchiness that could affect preening. Dermatitis and itchiness could result from the chicken body louse chewing on the skin and pinfeathers to obtain serum and blood (Crutchfield and Hixon, 1943). Although plumage condition was not scored, the hens were carefully examined when the ectoparasites were counted and all hens were well-feathered throughout the study, irrespective of infestation type or beak treatment.

According to Mullens et al. (2010), lice on beak-trimmed hens are found predominantly on the vent. The current study showed that BTL hens mainly directed their preening behavior towards the vent, spending approximately 48% of their time preening on the vent at the peak of infestation compared to the 35% they spent prior to infestation. Mullens et al. (2010) also found that the few lice that are present on beak-intact hens are predominantly under the wings, but in the current study there was no increase in the time spent preening on the internal wings in BIL hens, perhaps due to the extremely low infestation rate.

The interaction between beak condition and lice numbers is an important finding of this study. BIL hens were able to more efficiently control their lice populations than BTL hens without increasing their daily preening activity. This could be energetically advantageous, since preening has both direct and indirect energetic costs. In thick-billed murres for example, the metabolic rate associated with preening is 15 to 27% of the daily metabolic rate (Croll and McLaren, 1993). The indirect costs are related to the need to redirect behaviors like foraging and feeding towards preening (Cotgreave and Clayton, 1994; Simon et al., 2005). BIL hens thus can use their metabolic energy for these kinds of important behaviors while still controlling their ectoparasite populations via normal rates of preening behavior.

Contrary to what was found for BTL hens, neither BIM nor BTM showed an increase in the total time spent preening and there was no correlation between the total time spent preening and mite scores. However, mite-infested hens at the peak of infestation did spend more time preening on the vent and on the uropygial area than they did prior to infestation. These findings are in agreement with Kilpinen et al. (2005) who did not see an increase in the total amount of time spent preening by hens infested with red mites, although red mites differ from northern fowl mites in that that they only infest the host at night. Møller (1991) also found that tropical fowl mite (*Ornithonyssus bursa)* infestation did not affect the percentage of time spent in "preening activity" (preening and scratching) by adult swallows.

Mite infested hens in this study redirected their preening activity toward the vent, the body area on which the northern fowl mite mainly lives (Chen et al., 2011). Similar to BTL hens, BTM hens increased the time spent preening on the vent, from 36% prior to infestation to 45% at the peak of infestation. The increase in time spent preening on the vent might have been related to skin irritation in this area due to infestation (Owen et al., 2009). However, this cannot be confirmed since the level of irritation was not scored in the current study, nor are there empirical studies of whether preening behavior is triggered by skin irritation in birds.

It is interesting to note that another body area where an increase in time spent preening was observed was the uropygial area, raising the possibility that one function of the preen oil exudate is to reduce mites. Perhaps preen oil has chemical constituents that can increase mite mortality, or cause suffocation of the parasites by occluding the spherical pores (spiracles) or cuticles through which the mites breathe (Clayton et al., 2010). Alternatively, the gland area is close to the vent and hens may have experienced mites crawling or feeding there.

This study compared for the first time the effects of northern fowl mite and chicken body louse loads at their respective peaks of infestation on the time spent preening. Mullens et al. (2010) estimated that at the peak of infestation 30% of the lice on beak-trimmed hens were located under the wings and 45 to 50% were located in the vent area. In contrast, mite infestations are almost exclusively on the vent (Chen et al., 2011). These results showed that BTL hens spent more time preening on the vent than BTM, whereas no differences were seen between BIL and BIM hens. However, these results require confirmation in future studies since the lice and mite infested hens were located in 2 different poultry houses. Although these houses were comparable in design and were located adjacent to one another, it is possible that the results could have been influenced by conditions within each house at the wk of peak infestation.

Overall, this study confirms that an intact beak is important for combating both types of ectoparasites in chickens. It was demonstrated for the first time that, at their respective peaks of infestation, lice affected the total time spent preening by beak-trimmed hens while mites had no effect on total time spent preening for either beak-trimmed or beak-intact hens. However, BTM did direct their preening activity towards the areas where mites are known to be found. The 2 ectoparasite treatments in this study were in open-sided poultry houses and the hens were thus exposed to ambient fluctuations in temperature and humidity. It would be interesting in future studies to systematically evaluate the effects of controlled variations in temperature and humidity on preening behavior in response to ectoparasite infestation, for example during more extreme environmental conditions when the hens have competing behavioral motivations related to thermoregulation (e.g., to minimize exposure of skin during cold weather in order to conserve body heat).

The results of this experiment highlight the importance of an intact beak for controlling northern fowl mite and chicken body lice populations. Given that hens are typically beak-trimmed to reduce damage due to feather pecking and cannibalism, this suggests that developing effective strategies to reduce the need for trimming could also help to reduce ectoparasite populations on commercial laying hen farms, and minimize the need to employ chemical control methods. Finding alternative strategies might be particularly important for organic egg producers, who are restricted in their use of chemical products for ectoparasite control.

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