Beak condition and cage density determine abundance and spatial distribution of northern fowl mites, *Ornithonyssus sylviarum*, and chicken body lice, *Menacanthus stramineus*, on caged laying hens[1]

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ABSTRACT Adult White Leghorn hens (Hy-Line strain W-36) were inoculated with either northern fowl mites or chicken body lice, and the ectoparasite populations were monitored over periods of 9 to 16 wk. Two beak conditions (beak trimmed or beak intact) and 2 housing densities (1 or 2 hens per 25 × 31 cm suspended wire cage) were tested. Populations of both ectoparasites were at least 10 times lower on beak-intact hens compared with populations on beak-trimmed hens. Cage density did not influence mite numbers, but higher numbers of lice (2 to 3 times) developed on hens held at the higher cage density. Louse distribution on the body and louse population age structure were also influenced by host beak condition. Beak-intact hens had a higher proportion of lice under the wings, whereas beak-trimmed hens had the majority of lice on the lower abdomen. Louse populations on beak-trimmed hens also comprised relatively more immature stages than populations found on beak-intact hens. The effects are likely related to decreased grooming efficiency by beak-trimmed hens and, in the case of lice, the higher host density. The high mite and louse populations on most commercial caged laying hens are probably a direct result of beak trimming. However, selection of more docile breeds that can be held without trimming may allow the hens themselves to reduce ectoparasites below economically damaging levels. This could benefit producers, animal welfare advocates, and human health by reducing 1) costs of beak trimming, 2) pesticide treatment costs (including human and bird chemical exposure concerns), and 3) objections to beak trimming from the animal welfare community.

Key words: grooming, welfare, northern fowl mite, chicken body louse, ectoparasite

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INTRODUCTION

Interactions between wild birds and ectoparasites have received a substantial amount of research attention by ecologists (Loye and Zuk, 1991; Clayton and Moore, 1997; Clayton et al., 2010). For example, areas of interest include the influence of host morphology and behavior on ectoparasite numbers and distribution (Murray, 1990; Moyer et al., 2002; Clayton et al., 2005; Bush and Malenke, 2008), host immunity interactions (Møller and Rózsa, 2005; Heylen and Matthysen, 2008), interactions between host sociality, dispersal, and ectoparasite population biology (Møller et al., 2004; Whiteman and Parker, 2004), aspects of host-parasite coevolution (Rózsa, 1993; Clayton et al., 1999, 2003; Johnson and Clayton, 2004), and parasite effects on host ornamentation-sexual selection and host fitness parameters (Hamilton and Zuk, 1982; Lehmann, 1993).

Agricultural researchers are also interested in ectoparasites, primarily because of their effects on production in livestock and poultry systems, or as vectors of disease agents to those animals and birds. In the United States, the blood-feeding northern fowl mite, *Ornithonyssus sylviarum*, is the most common and economically damaging ectoparasite on caged laying hens and on both chickens and turkeys used for breeding; it can also be a human pest for workers who handle hens or eggs from infested flocks (DeVaney, 1978; Axtell and Arends, 1990). Most studies have concluded that mites do affect production (Mullens et al., 2009). Much of the direct damage (e.g., effects on egg numbers, feed conversion efficiency, or BW) is thought to be due to the energy or resource demands of the host immune response (Owen et al., 2008, 2009; Mullens et al., 2009). Although domestic chickens are host to several louse species, one of the most widespread and severe pests of poultry worldwide is the chicken body louse,

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Menacanthus stramineus (DeVaney, 1976; Trivedi et al., 1991; Permin et al., 2002). Economic impact studies have shown that infestation by *M. stramineus* can reduce egg production and BW gain (DeVaney, 1976; Price and Graham, 1997). Despite their economic importance, interactions between the domestic chicken and *O. sylviarum* and *M. stramineus* have not been well characterized. Ectoparasites of domestic chickens have been controlled using insecticides for more than 50 yr, quite effectively reducing the incentive for agricultural researchers to study them. Chemical control, however, is increasingly difficult because of the very limited arsenal of registered pesticides and the development of resistance (Mullens et al., 2004). As a result, there is a pressing need to explore alternative methods of ectoparasite control.

Very little is known about how the host beak condition affects *M. stramineus* or other lice on chickens. Kartman (1949) amputated half of the upper beak of very young (8-wk-old) chickens and observed minor increases in lice (relative to beak-intact birds), which were not presented in detail or analyzed statistically. Brown (1972) infested rather small numbers of chicks (1 or 31 d of age) with lice and destructively sampled them 33 to 39 d later. She observed large increases in *M. stramineus* on beak-trimmed chicks (half of the upper mandible removed by clippers) relative to untrimmed chicks. Although agricultural researchers such as DeVaney (1976) have documented that commercial-type, beak-trimmed hens develop large louse populations, no comparisons have been done with beak-intact hens. The effect of beak condition on the louse loads of chickens has never been rigorously examined with adequate numbers of birds of production age or under conditions representative of production agriculture. Similarly, only one small set of observations suggests that beak condition might affect northern fowl mites (Matthysse et al., 1974).

This lack of data is particularly pertinent now. Animal welfare concerns are changing the way agricultural animals are maintained, but even defining welfare is hardly an easy task (Rushen, 2003). In caged laying hens, key welfare discussions involve cage design, cage density, beak trimming, molting, and methods of transport and handling (Mench, 1992; Hester, 2005; Tauson, 2005). Several of these factors would be expected to affect ectoparasite numbers on poultry. The present study was conducted to examine the effect of beak condition and cage density on both *O. sylviarum* and *M. stramineus* under commercial-type conditions.

MATERIALS AND METHODS

Hen Housing and Experimental Array

All aspects of hen housing, care, and experimental manipulation were approved according to Animal Use Protocol A-0608023, University of California, Riverside. White Leghorn hens (*Gallus gallus*), Hy-Line strain W-36 (now CV-20), were used for the tests. The relatively docile W-36 strain is a very popular bird for commercial egg production, but may be held without beak trimming (Craig et al., 1992). For the present trials, a commercial cooperator trimmed the hens, but also held back part of the same cohort with intact beaks. All the pullets were housed under identical conditions until transport to the University of California, Riverside Agricultural Operations Property at 18 wk of age, a typical age for stocking in commercial egg houses (Bell and Weaver, 2002). There the birds were placed into suspended wire cages above a concrete floor, typical for the industry in California. Banks of cages had automatic water cups and a feed trough with ad libitum access to commercial lay mash, and eggs were collected into a rollout rack. Houses were screened to prevent access by wild birds, rodents, and mosquitoes. The houses were also equipped with roof sprinklers and internal misters; the lighting regimen was 16L:8D, also typical for the industry.

The birds were arranged in banks of 4 cages each. Each cage was 25.4 cm wide × 30.5 cm deep × 30.5 cm tall (774 cm^2 of floor space). The 2 experimental factors were beak condition (intact or trimmed) and cage density (1 or 2 hens per cage), constituting 4 treatments (2 × 2 factorial) that were randomly allocated to 1 of the 4 cages in each bank (randomized complete block design). The higher cage density used was approximately the industry standard (387 cm^2/hen) in California caged layer systems and was somewhat less than the recommended space per hen in the United States adopted by the United Egg Producers (465 cm^2/hen; United Egg Producers, 2010). In cages with 2 hens, 1 hen was marked with leg bands to distinguish it as the monitored hen, and paired hens (cage mates) were always the same beak condition.

Two test houses were used for the northern fowl mite tests. Within each mite test house, there were 9 hens for each of 4 hen treatments (single and trimmed, single and intact, double and trimmed, double and intact). Two test houses were also used for the louse tests. One house had 9 hens per treatment; the other house used 4 hens per treatment.

Ectoparasite Treatments and Monitoring

For each hen, the lengths of the upper and lower mandibles were measured using a millimeter ruler from the front of the nares along the beak axis to an imaginary line perpendicular to the tip. This provided an estimate of the severity of the trim and also of beak asymmetry for each individual. As soon as the hens had been assigned to the cages, all hens were inoculated with adult *O. sylviarum* or *M. stramineus* from separately maintained source hens. Twenty mites or 5 lice were placed on the abdominal skin of each hen. Once per week, the hens were removed from their cages and scored visually for relative intensity of the mite or louse infestation, which is the method of choice for longitudinal studies (Clayton and Drown, 2001).

For mites, the feathers and skin immediately anterior to the vent (an area approximately 8 cm long and 6 cm across) were examined by sorting through the feathers from anterior to posterior. The estimated number of mites was recorded according to the scoring system of Arthur and Axtell (1983) and Mullens et al. (2000), augmented by dividing scores 1 to 5 into the upper quartile, mid range, and lower quartile (Owen et al., 2009). The basic system was as follows: 0 = no mites seen; 1 = 1 to 10; 2 = 11 to 50; 3 = 51 to 100; 4 = 101 to 500; 5 = 501 to 1,000; 6 = >1,000 but <10,000; and 7 = >10,000. The mite scores essentially served a similar variance-stabilizing function as $\log_{10}$-transformation, and were increasingly conservative at higher levels but were highly correlated with actual numbers of mites derived from removal and examination of vent feathers (Mullens et al. 2000; Owen et al., 2009).

For lice, 3 body regions of approximately 40 cm^2 (8 × 5 cm) were examined. An area under each wing was relatively free of feathers, so the skin was easy to see once the wings were lifted. The lower abdomen was examined as described above for mites. On the anterior keel, 3 quick feather partings were made and the number of lice was estimated. These regions were far enough apart that the likelihood of "herding" the rapidly running lice from one region to another was reduced. Notes were made on impressions of relative numbers of nymphs vs. adults, but the visual estimate (count) recorded all stages. Two people consistently did the scoring for 1 house each infested with lice (B.A.M., B.L.C.) or mites (B.A.M., J.P.O.). Hens were scored weekly for both ectoparasites from wk 1 to 11 postinfestation for mite house 1 and both louse houses, with another score at 16 wk postinfestation for lice. Mite house 2 was scored through wk 9.

The visual louse estimates at 10 wk postinfestation were compared with absolute louse numbers extracted from hens, as well as their population age structure, by body washing as follows. A separate group of hens was infested, held similarly, and visually scored (as above) by B.A.M. After scoring, the hens were killed, and the bodies were placed into tightly sealed plastic bags and frozen (−20°C). Later, each hen was thawed overnight and placed into a large dishpan with 6 L of water plus 6 mL of dishwashing detergent. The hen was steadily washed (by ruffling the feathers by hand) for a timed period of 5 min. The hen was removed, and the wash water plus detergent was strained through a 60 mesh sieve, backwashing the lice into a glass container using 70% ethanol. This procedure was repeated twice more. The 3 consecutive washings were counted separately by pouring them into a 9-cm-diameter Petri dish divided (scored on the underside) into 21 pie-shaped sections. On hens with relatively low numbers of lice per washing (<100 lice), all lice were counted and categorized as nymphs or adults. For washings containing more than 100 lice, a portion (33%, every third section) of the lice were counted, and this number was multiplied by 3 to yield the total louse count for that bird.

Statistical Analysis

A repeated-measures ANOVA was used on the visual mite scores or on the louse count data after transformation to $\log_{10}$ (n + 1) to stabilize the variance (version 14, Minitab Inc., State College, PA). This 2 × 2 factorial design estimated the effect of the main factors (hen density and beak condition) and their interactions on ectoparasite populations over time on individual hens examined repeatedly (random factor). Analysis of variance was conducted on the proportional distribution of lice in each of the 3 sampled body regions (under wing, anterior keel, vent) to distinguish whether this might vary with beak condition (beak-intact vs. beak-trimmed hens). The proportional data were transformed using the arcsine of the square root of the proportion before analysis. Correlation analysis was used to determine whether the ratio of the upper to lower beak length (a measure of asymmetry and relative length of upper and lower mandibles) was related to numbers of mites or lice on beak-trimmed hens. We used $\alpha = 0.05$ throughout.

RESULTS

Mite Experiments

Experimental houses were analyzed separately for the mite experiments because the 2 individuals doing the visual scoring judged the mite populations (especially high scores of 5 to 7) somewhat differently, and mite scores in house 2 were thus higher than those in house 1 ($F = 13.47$; df = 1, 681; $P < 0.001$). The results, however, were consistent (Figure 1). Beak-trimmed hens, held separately or in pairs, supported significantly more mites in house 1 ($F = 51.70$; df = 1, 395; $P < 0.001$) and house 2 ($F = 47.88$; df = 1, 323; $P < 0.001$); more than 10 times the number of mites were documented on beak-trimmed hens. The beak effect, in fact, was evident as early as wk 1. Some beak-intact hens showed signs of skin bruising at the bases of the vent feathers (Figure 2), and such bruising was never seen in beak-trimmed hens.

Cage density did not affect mite numbers overall, and there were no significant interactions with beak condition. The interaction approached statistical significance in house 1 ($P = 0.09$) but was not evident in house 2.

The correlations (by hen) between average mite scores and the ratio of top to bottom beak length in the trimmed hens were slightly negative, but these were not significant in house 1 (r = −0.07) or house 2 (r = −0.22). The variation among the hens in top:bottom beak length ratios was large, ranging from 0.55 to 1.4. In general, the top mandible was equal to or shorter than the bottom one in trimmed hens, but their relative lengths did not affect the number of ectoparasites.

Louse Experiments

Numbers of lice did not differ for the 2 louse houses, which were pooled for analysis (Figure 3). As was true for mites, the beak-trimming effect was highly significant ($F = 91.86$; df = 1, 610; $P < 0.001$). By wk 11, the visually estimated louse numbers were approximately 10 to 15 times higher on beak-trimmed hens relative to their counterparts in either single- or multiple-hen cages.

Cage density also affected louse numbers, however. Although less pronounced than the beak effect, significantly higher numbers of lice were still found on hens held together in cages vs. hens held singly ($F = 25.24$; df = 1, 610; $P < 0.001$). The effect was consistent with beak condition; that is, there was no beak × density interaction. By wk 11, hens held together harbored approximately 2 to 3 times the numbers of lice seen on hens held singly. As was true for mites, the ratio of top:bottom beak length was not significantly correlated with louse densities in the trimmed hens ($r = -0.01$).

The distribution of lice among sampled body regions differed between beak-trimmed and beak-intact hens (Figure 4). Numbers of visible lice were very low early after infestation. Nevertheless, through wk 4, lice were seen almost exclusively only under the wings of beak-intact birds. In contrast, approximately half of the lice on beak-trimmed hens were observed in the vent area (lower abdomen) during wk 3 and 4. Relatively few lice were seen on the anterior keel vs. the other body regions for the duration of the trial. Beyond wk 5, the louse numbers were sufficient for statistical analysis. Examining the weeks separately, proportionally more lice were found in the vent region of the beak-trimmed hens in each of the 8 weekly sampling periods. Likewise, proportionally more lice were found under the wings of the beak-intact hens for all but wk 5.

Louse-infested hens were washed to remove the entire louse population for study. Based on a logarithmic decay curve fit to the consecutive washing data, an estimated 99.8% of lice were removed in 3 washing cycles, with 86.5% in the first wash ($y = 17352e^{-1.9992x}$, $r^2 = 0.998$). With unequal variances, a Mann-Whitney U-test was applied to the median lice per hen, and beak-trimmed hens had far more lice on their bodies ($P < 0.01$). The beak-trimmed hens had an average (±SE) of 3,069 ± 437 lice, whereas the beak-intact hens had an average of 352 ± 84 lice (Figure 5).

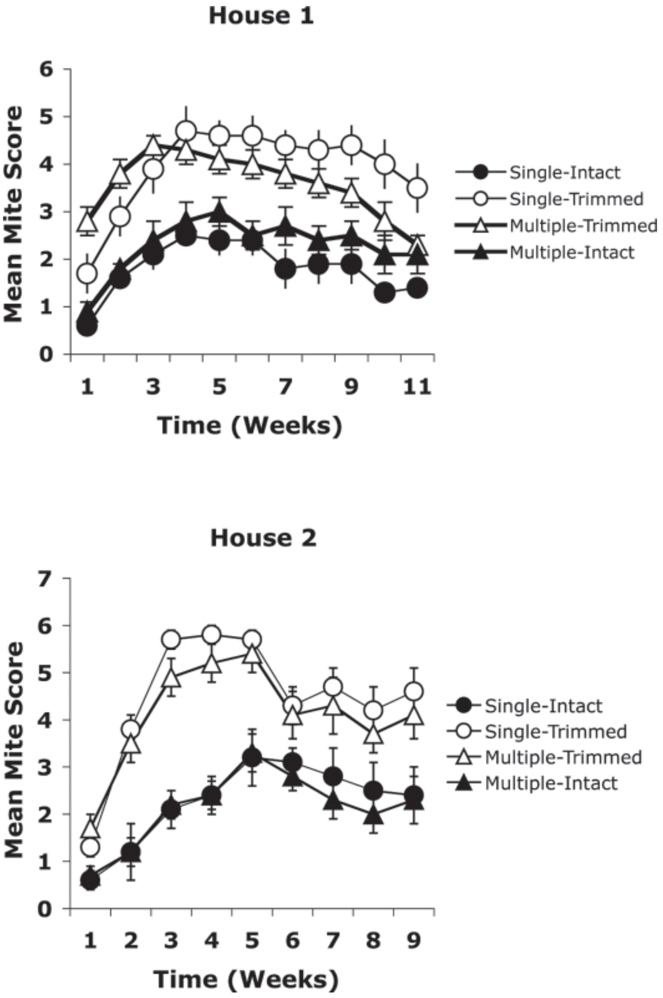
Notably, the louse age structure was very different on beak-trimmed vs. beak-intact hosts. Lice on beak-intact hens were 19% adults, whereas lice on beak-trimmed hens (with far higher numbers of lice overall) consisted of only 7% adults (Figure 5), indicating that host beak condition significantly affected louse population age structure ($P < 0.01$).

DISCUSSION

Beak Condition and Cage Density Effects on Ectoparasites

Our study shows, for the first time in commercial poultry, that beak trimming has a large effect on ectoparasite densities. This extends and confirms earlier, very preliminary observations (Kartman, 1949) and a small study on chicks (Brown, 1972) indicating that having an intact beak reduces chicken body louse numbers. The fact that an intact beak also dramatically reduces numbers of northern fowl mites confirms the earlier suggestions of Matthysse et al. (1974). An intact beak frequently reduced ectoparasite densities (both lice and mites) by 10-fold or greater in our studies.

The mechanism of ectoparasite reduction is almost certainly through enhanced grooming efficiency by beak-intact chickens. Excellent, extensive experimental work


House 1

House 2

Figure 1. Northern fowl mite population densities over time on hens that were held singly or in pairs in cages (single vs. multiple) and either beak trimmed or beak intact (trimmed vs. intact). The basic scoring system was as follows: 0 = no mites seen; 1 = 1 to 10; 2 = 11 to 50; 3 = 51 to 100; 4 = 101 to 500; 5 = 501 to 1,000; 6 = >1,000 but <10,000; and 7 = >10,000.

Figure 2. Skin bruising at the base of vent region feathers in a beak-intact hen inoculated with northern fowl mites. Color version available in the online PDF.

has been done with wild birds, particularly concerning louse infestations, showing how critical an intact beak is to ectoparasite reduction through grooming (Clayton et al., 2010). Specifically, the scoop-shaped upper mandible tip edge overhang and resulting shear forces during preening are critical; even shaving the edges from the upper beak resulted in increased louse numbers on pigeons (Clayton et al., 2005). Among 52 Peruvian bird species, those with more of an upper mandible overhang tended to harbor lower numbers of a wide variety of louse species (Clayton and Walther, 2001). In wild scrub jays, natural variation in bill morphology seemed to be reflected in louse numbers, with more lice on birds with naturally more pointed (as opposed to scoop-shaped) bills (Moyer et al., 2002).

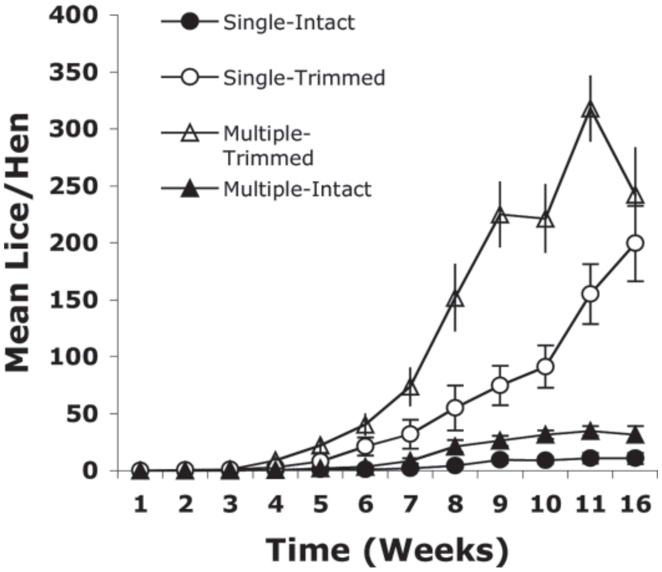


Figure 3. Chicken body louse populations over time on hens that were held singly or in pairs in cages (single vs. multiple) and either beak trimmed or beak intact (trimmed vs. intact).

In the present experiments, we could not detect any graded responses of ectoparasite populations based on beak asymmetry, despite quite a bit of variation among the hens, suggesting the effect was present if the hens were trimmed at all. In our experiments, we could observe infested hens (lice and mites) working through the feathers by using their beaks, and the skin bruising at the feather bases in mite-infested hens was probably a result of preening and pulling individual or small groups of feathers. We have experimented with mites on beak-trimmed, commercial caged laying hens for many years and have never seen this type of bruising commonly until the present experiments with beak-intact birds. This implies that beak-intact birds were better able to grab and pull the feathers vigorously in the vent region, the specific area mites favor on hens (Lemke et al., 1988; Axtell and Arends, 1990; Hogsette et al., 1991). Interestingly, the bruising was mainly in beak-intact, mite-infested hens, rather than in louse-infested ones, perhaps implying a greater degree of irritation caused by mites that appeared even quite early after infestation (within the first week).

That host grooming (intact beak) causes ectoparasite population reduction is further supported by the change in spatial distribution of lice on the body and louse age structure. Grooming activities can affect body locations used by ectoparasites, which then may occupy less accessible areas (Marshall, 1981; Murray, 1987, 1990). Although lice were much less abundant on beak-intact hosts overall, they were relatively much more common under the wings, which may be harder to groom compared with the lower abdomen. Brown (1972) also noted that early louse stages were most common under the wings. In our studies, louse occupation of the lower abdomen, including considerable louse oviposition on abdominal feathers, was extensive only in beak-trimmed hens. The lower abdomen was preferred by *M. stramineus*, as also noted by Trivedi et al. (1991). We have not found examples of ectoparasite age structure changes resulting from host grooming in the literature. The beak-intact hens perhaps were better able to groom off the egg masses that were attached to the feather bases. This could contribute to the far more adult-biased age structure seen in beak-intact hens (19% adult lice in beak-intact hens vs. 7% adults in beak-trimmed hens). Additionally, or perhaps alternatively, a beak-intact hen might be better able to kill lice at younger (and possibly slower or more accessible) life stages.

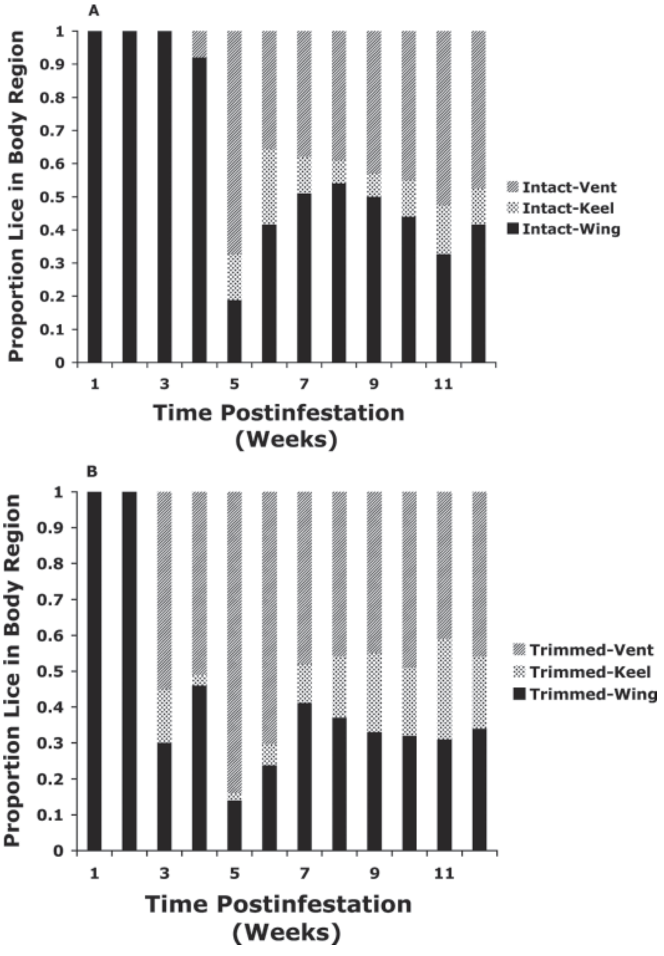


Figure 4. Proportional presence of body lice in 3 different body regions (anterior to vent, under wings, and anterior portion of keel) of hens with intact beaks (top) or trimmed beaks (bottom).

Earlier work exclusively with beak-trimmed, caged hens showed experimentally that more northern fowl mites were found on hens held singly in cages, as opposed to hens in denser groups (Hall et al., 1978; Arthur and Axtell, 1983; Mullens et al., 2000). This pattern was not statistically significant in the present studies, although there was a suggestion of it in beak-trimmed hens, particularly in house 1 (Figure 1), as evident in the density × beak interaction term ($P < 0.09$). In contrast, the density effects in our study were obvious in numbers of lice on both beak-trimmed and beak-intact hens. The trend for lice, however, is opposite that seen in the literature for mites (Hall et al., 1978; Arthur and Axtell, 1983; Mullens et al., 2000). Far more lice (2- to 3-fold) were seen on hens held together in cages compared with lice on hens held singly. We hypothesize this was due to space limitations or the inability to reach certain critical body regions, such as the lower abdomen.

Broader Implications for Poultry Welfare and Pest Control

The most common method for beak trimming of chickens is to cauterize the distal half of the beak (both upper and lower mandibles) by using a hot blade trimmer at approximately 7 to 10 d of age, although supplemental or later trims may be done (Glatz, 2000). In caged laying hens, beak trimming is ubiquitous because it reduces cannibalism and feed waste, despite its debatable welfare consequences (Hester, 2005; Tauson, 2005). Consequently, development of docile breeds that can be held without beak trimming has been mentioned as a breeding goal for many years (Hughes and Gentle, 1995; Hester, 2005). Based on the relatively robust literature on beak-mediated interactions between lice and wild birds (Clayton et al., 2005) and the very limited data on beak condition and lice or mites on chickens (Kartman, 1949; Brown, 1972; Matthysse et al., 1974), one would predict that beak-intact hens might reduce louse or mite densities, as we have shown.

Animal welfare concerns are changing how agricultural animals, including poultry, are housed and handled (Aggrey, 2010; Cheng, 2010), and beak trimming is among the key poultry welfare concerns (Glatz, 2000; Hester, 2005; Henderson et al., 2009). The relationship between beak condition and ectoparasite control in commercial poultry seems to have been overlooked among poultry scientists, although the effects in wild bird-louse interactions are well known among basic ectoparasite ecologists (Clayton et al., 2010). The high ectoparasite numbers commonly encountered in commercial poultry settings are likely a direct result of beak trimming.

We are currently examining several years' worth of experimental data on production parameters because they may vary with ectoparasite loads and beak condition [B. A. Mullens, J. Conklin (Department of Entomology, University of California, Riverside), D. R. Kuney (University of California Cooperative Extension), J. A. Mench (Department of Animal Science, University of California, Davis), G. Vezzoli (Department of Animal Science, University of California, Davis), N. O'Sullivan (Hy-Line International, Dallas Center, IA), unpublished data]. Nevertheless, it is clear that beak-intact hens can very substantially reduce their own ectoparasite numbers, perhaps below the level of economic damage. At the least, worker irritation caused by mites in a flock should decline drastically in beak-intact flocks because off-host mite numbers generally are obvious only at high mite densities (Mullens et al., 2000).

As breeders progress in developing docile hen strains that still retain desirable production characteristics, the use of beak-intact hens in commercial production has a suite of intriguing possibilities. First, it is conceivable that hens can reduce their own ectoparasites to a level that no longer requires chemical control. The situation with poultry ectoparasite control via pesticides is dire. In California, for example, the 2 currently registered traditional pesticides are tetrachlorvinphos/dichlorvos (RaVap, KMG Chemicals Inc., Houston, TX) and permethrin. Mite populations are extensively resistant to both materials (Mullens et al., 2004), and permethrin resistance has rendered the chemical completely useless for at least half of the mite populations in our region. Further, human health and environmental concerns are bringing into question the reregistration of several compounds, particularly the traditional organophosphate (e.g., RaVap) and carbamate (e.g., carbaryl) insecticide classes. In the United States, pesticides registered before 1984 are subject to comprehensive review and reregistration under the Federal Insecticide, Fungicide, and Rodenticide Act of 1988, as amended by the Food Quality Protection Act of 1996 (Duggan et al. 2003).

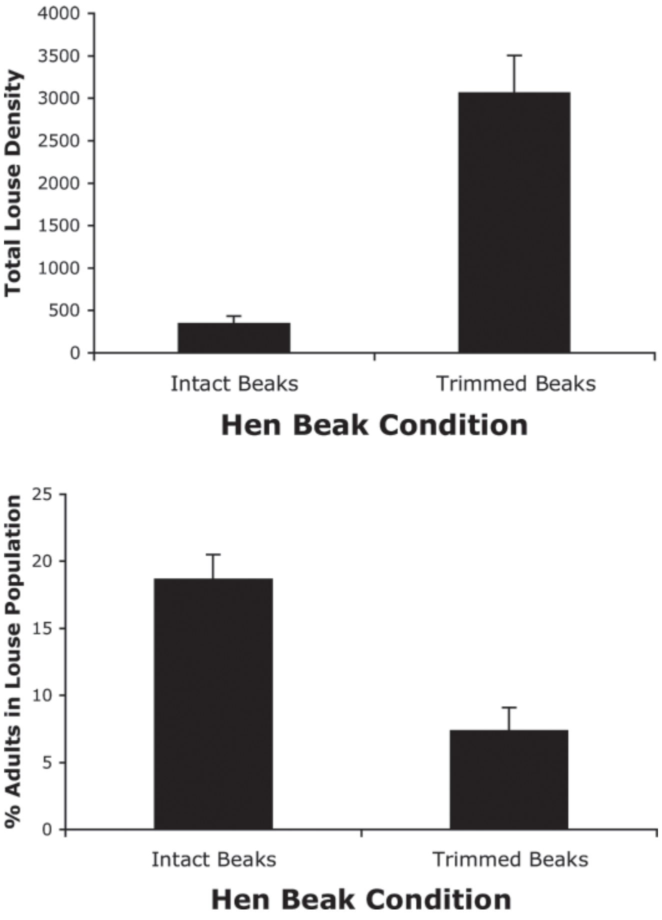


Figure 5. Numbers of chicken body lice (mean ± SE) recovered from beak-intact and beak-trimmed hens (top) and the proportion of those lice that were adults (bottom).

The second potential major benefit is to alleviate animal welfare concerns related to beak trimming. As discussed, it is likely that the degree of trimming, or the trimming method, matters little for ectoparasite control through grooming. This study shows another benefit to using beak-intact hens for producers and breeders to consider: ectoparasite control. It could provide a win-win scenario, yielding free ectoparasite control and the reduction or elimination of pesticide costs and exposure concerns and helping with perceptions of the industry by animal welfare advocates.

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