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Studies of Northern Fowl Mite Host-Parasite Interactions and Evaluation of Novel Control Strategies in Poultry

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Entomology

by

Amy Christine Murillo

June 2016

Dissertation Committee
Dr. Bradley A. Mullens, Chairperson
Dr. Alec C. Gerry
Dr. Timothy D. Paine

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The Dissertation of Amy Christine Murillo is approved:

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University of California, Riverside

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DEDICATION

To Kelly & Vader –

Oh the places we'll go

ABSTRACT OF THE DISSERTATION

Studies of Northern Fowl Mite Host-Parasite Interactions and Evaluation of Novel Control Strategies in Poultry

by

Amy Christine Murillo

Doctor of Philosophy, Graduate Program in Entomology
University of California, Riverside, June 2016
Dr. Bradley A. Mullens, Chairperson

Northern fowl mites (*Ornithonyssus sylviarum*) are the most common and economically important ectoparasites in US laying hen flocks. Mites are permanent ectoparasites and cause damage to birds via blood feeding. Once in a flock, they can be very difficult to eradicate from a property. Mite control has traditionally relied on chemical sprays directed at high pressure underneath the birds and into the vent region where mites live; these applications are most effective when birds are held in battery cages. However, in recent years animal welfare concerns and consumer demand has affected how laying hens are housed. The transition to furnished-cage or cage-free production systems will require novel control techniques to achieve ectoparasite control. I investigated possibilities for ectoparasite control that would be effective in alternative poultry housing.

Breeding for parasite resistance is an attractive control method. Chickens develop resistance to the northern fowl mite (NFM) and this is under at least some genetic control. I tested whether the haplotype conferring NFM resistance had any effect on chicken resting metabolism (energy expenditure), physiology, or economic performance. Bird

haplotype did not affect hen metabolism or production parameters. NFM infestations negatively affected economic performance.

Dustboxes have been shown to effectively decrease mite populations in cage-free flock when insecticidal dusts are used in combination with sand. I tested whether dustboxes with sand and diatomaceous earth could keep mites below economic thresholds before mite levels became damaging. When dustboxes were present in a flock, mite levels were on average kept below economic threshold regardless of percent of the flock that dustbathed.

Sulfur dust is effective for NFM control in very low doses, though it is not universally approved for organic use. I tested whether sulfur deployed in a dust bag would be effective in caged (non-organic) flocks and if the placement of the bag affected efficacy. Hanging dust bags offered the best NFM control.

Backyard chicken flocks are a growing trend in this country, and nothing is currently known about ectoparasite diversity on these birds. I surveyed 100 birds on 20 properties in southern California. Greater diversity was found on backyard flocks than on commercial birds.

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INTRODUCTION

The research conducted in this dissertation is a direct reflection of recent changes to the chicken egg industry. Increased awareness of consumers about animal welfare has led to a very rapid shift in how US egg producers house chickens. While perhaps not obvious to the average consumer, these changes will have entomological consequences, as discussed below.

Animal Welfare and Housing

Animal welfare concerns and increased consumer awareness are influencing poultry husbandry practices. Legislation in multiple states, including California, is limiting the use of battery cages and forcing producers to move toward alternative poultry housing. Until recently, ca. 95% of egg layers (*Gallus gallus domesticus*) in this country were housed in these conventional battery cages, barren wire cages that are suspended off the ground (Lay et al. 2011). There has been a substantial increase in the use of cage-free or organic facilities for egg production over the last decade, with the number of cage-free layers (organic and conventional) increasing by 148 percent to 24.36 million birds (Oberholtzer et al. 2006, Alonzo 2016), and it is predicted that over the next decade at least 40% of all layers will be in cage-free housing (Egg Industry Feb 2016). In 2015, all layer operations in California were required to provide more space per bird, causing many producers to shut down, move out of state, or switch to enriched cages or cage-free operations (Proposition 2, Nov. 2008). Adapting to these legislative and consumer-driven

demands is a huge challenge for animal agriculture (Mench et al. 2011, Swanson et al. 2011).

At the beginning of 2016 numerous domestic and global restaurant and retail companies made public pledges to transition to selling or sourcing cage-free eggs. Timelines for the switch ranged from within the year to within the next two decades. The number of cage-free eggs will need to more than double in size in the next year alone to meet demand from these pledges (Watt Egg Industry 2016).

One aspect driving consumer demand of enriched-cage or cage-free eggs is the perceived increased health and well-being of poultry in these systems. However animal welfare encompasses many aspects of animal health and behavior, which can be complex and difficult to optimize (e.g. Lay et al. 2011, Sossidou et al. 2011). Specifically, putting birds into new environments, especially cage-free ones, increases the risk of ectoparasite infestation. Enriched-cages offer off-host harborages absent in battery cages, and cage-free chickens have increased opportunities to acquire ectoparasites because of exposure to feces, contaminated soil, and wild birds or other animals (Lay et al, 2011). In addition, organic production is increasing in popularity and demand, which will further limit treatment options for ectoparasites. California currently leads the nation in organic egg production, which is required to be cage-free (http://www.agmrc.org).

European Precedent

This dramatic shift in egg layer housing currently underway in the US has probably been influenced by events in the European Union. From 1999 to 2012, Europe phased out battery cages for alternative housing practices, including enriched cages and

cage-free systems for improved animal welfare (Anonymous 1999). Simultaneously, many pesticides became unavailable and pesticide resistance in mite populations increased (Sparagano et al. 2009, 2014). Subsequently the poultry red mite, *Dermanyssus gallinae* (De Geer), has become a devastating problem in European poultry, costing producers in some countries millions of dollars per year (Mul et al. 2009, Sparagano et al. 2014). Prevalence rates are up to 100% of laying flocks in some countries (Sparagano et al. 2014). Enriched cages and cage-free houses have created favorable conditions for the poultry red mite, which live off-host, as the added complexity of the environment creates mite harborages (Sparagano et al. 2009). Europeans are currently seeking alternative methods for mite control, though *Dermanyssus* remains a serious threat to worldwide layer production (Sparagano et al. 2009, 2014). The poultry red mite emergency was triggered by the welfare-based housing changes and caught the European authorities by surprise. The US is following in Europe's footsteps, but has the opportunity to avoid making the same mistakes that have cost the EU millions of dollars in mite control and lost revenue.

Northern Fowl Mite

The northern fowl mite, *Ornithonyssus sylviarum* (Canestrini & Fanzago) is the most common and damaging ectoparasite of poultry in the United States, including California (Axtell and Arends 1990, Hinkle and Hickle 1999, Mullens et al. 2009). The northern fowl mite (NFM) is cosmopolitan in temperate regions and is found on over 70 wild and domestic bird species (Knee and Proctor 2007). The NFM was first recorded in

the US in Maryland in 1917 as "a slight variety of *Liponyssus bursa* Berlese [tropical fowl mite]" (Wood 1920). The "feather mite," as NFM was first known, was possibly introduced by English sparrows as the mites were found early on in sparrow nests (Wood 1920). Sparrows frequently nest under house eaves and therefore can be near poultry (Wood 1920).

Ornithonyssus sylviarum closely resemble the tropical fowl mite (*O. bursa*) in life history and appearance; however. *O. bursa* is relatively restricted to subtropical and tropical regions. The key character separating the two species is the last pair of setae of the dorsal shield, which are off the shield in *O. sylviarum* (Fig. i). *Ornithonyssus* can be distinguished from *Dermanyssus* by the claw-like chelicerae of NFM compared to whip-like chelicerae of *D. gallinae* (Fig. i).

The NFM is a permanent ectoparasite that resides in the bird's feathers and migrates to the skin surface to feed. Eggs are laid on the feathers. The mite life cycle requires 5-12 days to complete and consists of five life stages; egg, larva, protonymph, deutonymph, and adult (Sikes and Chamberlain 1955). The protonymph and adult stages blood feed. Mites are almost exclusively found on feathers in the vent region (Hogsette et al. 1991) where the deep feather structure and microenvironment are favorable (DeVaney and Beerwinkle, 1980; Halbritter and Mullens, 2011; De La Riva et al., 2015). Wood (1920) described NFM living on the fluffy down feathers lining English sparrow nests; the vent feathers of chickens maintain the fluffy quality as adults and it may be an important characteristic for NFM on-host survival. Though small (adult mites are ca. 600 μm long), in large numbers mites are highly visible especially against white feathers. A

mite infestation will make the feathers look “dirty” because of the collection of mites, cast skins, eggs, and feces. Adult mites can be seen in feathers or running or feeding along or on the skin surface. Mites survive well off-host for periods of at least 1-3 weeks and can infest domestic flocks via wild bird nests or contaminated pullets, people, equipment, or rodents, or carry-over from a previous flock (Kells and Surgeoner 1997, Axtell 1999, Chen and Mullens 2008).

Mites introduced to naïve birds exhibit stereotypical population growth. Mite numbers grow steadily for 4-10 weeks and then decline, with low mite levels generally persisting for the rest of a hen’s life (Matthysse et al. 1974, Mullens et al. 2009). This decline is likely due to bird immune responses (DeVaney and Ziprin 1980, Burg et al. 1988, Minnifield et al. 1993, Owen et al. 2009). In severe cases, NFM numbers can be greater than 100,000 mites per bird (Matthysse et al. 1974). At such high infestation levels mites can cause up to 6% blood loss per day per hen (DeLoach and DeVaney 1981). Mite populations vary among birds of the same age and strain (Matthysse et al. 1974, Mullens 2000), and some of this variation is under genetic control (Owen et al. 2008).

Through their feeding, mites induce skin inflammation, immune activation, irritation, anemia, and even death as a result of exsanguination (DeVaney and Ziprin 1980; Burg et al. 1988; Minnifield et al. 1993; Mullens et al. 2009; Owen et al. 2009). Mite-infested birds exhibit decreased egg production and reduced feed conversion efficiency (Axtell and Arends 1990; Mullens et al. 2009). Mite infestations cause economic losses by reducing egg numbers and weights and feed conversion, all of which

likely are related to mite-induced immune responses (Mullens et al. 2009). Mullens et al. (2009) demonstrated over a 10 week peak-mite period that infestations reduced profits $0.07-0.10 per hen. At that time, the losses were about equal to the expected profit margin. Mites effectively destroyed any profit in that period and on that farm. Mites can also cause irritation to humans. As mite populations grow and become very dense, they are commonly seen on eggs, equipment, and poultry workers (Mullens et al. 2000). Often it is at this point that worker complaints trigger mite treatment (Mullens et al. 2004). By the time workers see mites off-host, populations are well beyond economic thresholds and are more much difficult to control (Mullens et al. 2009).

Other Poultry Ectoparasites

The chicken body louse (*Menacanthus stramineus* Nitzsch) is the most common louse species on poultry (Axtell and Arends 1990). This chewing louse species completes its entire life cycle in 2-3 weeks on the birds (Stockdale and Raun 1965). Eggs are glued to the base of feathers in groups (Axtell and Arends 1990). After hatching, nymphs are generally found under the wings. Adult chicken body lice can be found on the back, breast, and abdomen of the bird (Axtell and Arends 1990, Trivedi et al. 1991, Chen et al. 2011). Both the nymph and adult stages feed on the feathers and blood of hens (Crutchfield and Hixson 1943). Like NFM, louse populations decline somewhat over time, possibly due to host-immune responses (Chen et al. 2011, Martin and Mullens 2012). However these reductions are not as apparent as declines in NFM infestations. While it is fairly safe to attribute NFM declines to host immunity, it is not certain how

much of the louse decline is due to immune responses, or even how dependable such louse declines are, so long-term on-host louse population dynamics require further study. Lice can be transmitted to poultry houses via contaminated wild birds, people, equipment, and possibly rodents, however they disperse poorly and do not survive long off-host (Chen and Mullens 2008). Relative to NFM, *M. stramineus* is much easier to control; leaving a house vacant for even a few days in between flocks can eliminate residual off-host populations. Chicken body louse infestations can cause decreased hen weight, egg production, and feed conversion (DeVaney 1976) however this damage is not as consistent as seen with NFM infestations (DeVaney 1986, Chen et al. 2011).

There are several other species of lice that can infest domestic poultry (Emerson 1956). Comparatively little is known about these species since they are rarely seen infesting commercial flocks, and it is unlikely that they are of major economic importance,with the possible exception of Menoponidae lice (*Menacanthus* spp., *Menopon gallinae*) which can blood feed.

The chicken red mite, *Dermanyssus gallinae*, is not commonly reported as a problem in US commercial caged layer flocks, though it is probably distributed throughout North America. The *Dermanyssus* life cycle, which takes 7-14 d to complete, is very similar to NFM; there are five life stages including egg, larva, protonymph, deutonymph, and adult (Sparagano et al. 2014). However, the protonymph, deutonymph, and adult stages all blood feed. Eggs are laid off-host in cracks and crevices in the environment and all life stages live off-host then travel short distances to the hosts at night to feed. The chicken red mite is able to survive in extreme cases for up to 8 mo

without access to a host (Chauve 1998), which is better off-host survival than is known for *O. sylviarum* (Chen and Mullens 2008). *D. gallinae* has been implicated as a possible reservoir or vector for several poultry pathogens (Sparagano et al. 2014). *Dermanyssus gallinae* is found on poultry and wild birds world-wide, making it a potential threat for US poultry especially as the industry transitions away from battery cages (Chauve 1998, Sparagano et al. 2014).

The sticktight flea, *Echidnophaga gallinacea* (Westwood), is parasitic on chickens and some other birds such as pheasants, rodents, and other mammals (Durden and Hinkle 2009). The adult stages embed themselves into the skin around the eyes, face, and wattles of chickens where they blood feed. The immature stages live in soil, litter, or bedding and feed on organic material and feces (with host blood) from the adult stages; the life cycle is completed in 1-2 months (Suter 1964). Sticktight fleas are not found on commercial caged layers, probably because battery cage facilities prevent the birds from contacting wild animals that may be infested and, in particular, they do not provide appropriate habitat for the immatures. Infestation with sticktight fleas can be quite heavy on chickens, especially young, old, or sickly birds, though economic data on this pest are lacking.

Scaly leg mites, *Knemidocoptes mutans* (Robin & Lanquetin), are permanent ectoparasites in the mite group Astigmata. These mites burrow into the skin under the scales of chicken legs where they feed and reproduce. They are found only when birds are granted ground or roost access (Axtell 1985), so are not generally found in caged layers. Mites cause irritation, inflammation, and a crusty, scabby appearance, and chronic

infestations can cause deformities and lameness (Axtell 1985). It is presumed that scaly leg mites, like other Astigmata such as the scabies mite *Sarcoptes scabiei*, are spread by direct contact among hosts. However little is known regarding how these mites get into chicken flocks and why they are not found infesting caged birds, which could presumably spread the mite within a flock. Perhaps breed-specific immunological differences explain this phenomenon. Scaly leg mites are difficult to confirm because signs of infestation are not absolutely diagnostic and the mites themselves are very small and not visible to the naked eye. Economic impact data are lacking for this parasite.

Current Control Methods

On most US large-scale production farms, northern fowl mites are currently managed with chemical pesticides sprayed directly onto birds at high pressures directed from under hanging battery cages (Mullens et al. 2009). These pesticides must get through the feather layer and wet the vent region where the mites are concentrated. While historically many chemical classes were approved for mite control on laying hens, currently only spinosyn, organophosphate, and pyrethroid chemical classes are available for commercial use. Mite resistance to organophosphates and especially pyrethroids is widespread (Mullens et al. 2004), and non-target toxicity concerns and increasing organic production will likely prevent these materials from continued widespread use. Spinosad has fairly recently been registered for NFM control, but is rather expensive and variably toxic to mites (Sparagano et al. 2014); label rates are barely sufficient to kill 95-99% of NFM using *in vitro* exposures (Mullens unpublished).

Limited pesticide availability is not the only factor affecting mite control. As birds transition away from battery cages to enriched or cage-free housing, it will become increasingly difficult to effectively spray birds with pesticides. Enriched cages are not completely standardized, but they do require a scratch pad and other features such as roosts and nest boxes which would block sprayers from treating all birds. Cage-free birds, especially free-range or pastured, could maybe be treated at night while on perches or in coops, though this would generally be labor intensive and suboptimal. Current chemical control methods are unlikely to work in enriched cage or cage-free settings, and would not even be allowed in organic production systems.

Alternative Control Methods

Northern fowl mites and other permanent on-host dwelling ectoparasites will continue to be an issue in poultry regardless of housing type (Martin and Mullens 2012). However, the current control methods available are unlikely do well in these new systems. Successful management programs will need to integrate multiple control tactics in order to keep ectoparasites from causing economic damage.

Resistance Breeding. Selective breeding for ectoparasite resistance has potential for use as a management tool for reducing ectoparasite burdens. While several studies have demonstrated hen-immune reactions in the form of mite-specific antibodies (DeVaney and Ziprin 1980, Burg et al. 1988, Minnifield et al. 1993). Owen et al. (2008) were the first to explore resistance genes for control of NFM. The major histocompatibility complex (MHC), which has a critical role in adaptive immunity, has

two distinct haplotypes associated with white Hy-line leghorn CV20 (formerly W-36) strain chickens (Owen et al. 2008). The two haplotypes, B15 or B21, occurred in about a 1:1 ratio in the W-36 birds, and still dominate in the newer CV20 strain birds. Birds with the B21 haplotype supported lower NFM numbers at the peak of infestation compared to B15 birds. In a subsequent study resistant (B21) birds experienced skin inflammation great enough to physically prevent NFM protonymphs and adults from successfully acquiring host blood (Owen et al. 2009). These two studies support the potential for resistance breeding or even vaccine development to induce resistant phenotypes. Vaccine development in Europe for *Dermanyssus* has not yet been successful, as least in part because chickens do not seem to develop sufficiently protective resistance to *D. gallinae* (Sparagano et al. 2014).

Grooming. Beak trimming is a common practice in poultry production used to reduce feather-picking, cannibalism, and feed waste (Mench 1992, Chen et al. 2011). The procedure removes the distal 30-50% of the bird's beak. Traditionally a hot blade (up to 750° C) is used to cut and cauterize the beak of birds 7-10 days old (Jendral and Robinson 2004). A new, less labor intensive approach uses infra-red technology to soften the tip of the beak and allow it to erode gradually over a 2 week period (Dennis and Cheng 2010). It has been shown that beak-trimmed hens host significantly higher NFM and CBL numbers than beak-intact birds (Mullens et al. 2010, Chen et al. 2011). While beak-intact birds could therefore modulate their own ectoparasites, docile yet productive strains must be developed and accepted by producers for this practice to become widespread.

Chicken Self-Treatment. An interesting and promising self-treatment strategy is to take advantage of a bird's innate drive to dustbathe. Dustbathing is an innate, complex behavior that has a biological rhythm in chickens, with most birds performing such activities every two days (Olsson and Keeling 2005). This behavior has a feather maintenance function and birds are motivated to dustbathe in fine materials in order to remove excess feather oils (Olsson and Keeling 2005). When performed in very fine texture materials, like diatomaceous earth (DE) or kaolin (clay), ectoparasite suppression can be achieved (Martin and Mullens 2012). DE is an abrasive and sorptive dust that probably causes cuticular damage and desiccation to arthropods (Ebeling 1971). It is a registered organic product (Organic Materials Research Institute, OMRI), has very low mammalian toxicity, but does have inhalation risks. Providing dustbathing opportunities with dusts like DE is an attractive and effective control possibility for cage-free, and especially free-range or pastured poultry systems. However, it would probably be more difficult to use in enriched caged systems.

A self-treatment control option in caged systems could be a slow-release "dust bag," which disperses a low toxicity pesticide. Dust bags filled with insecticides for fly control on cattle have been used for decades (Turner 1965). The animals bump or rub against the bags, which then apply the insecticidal material to the animal. Sulfur has proven to be very effective for poultry ectoparasite control at low doses (Martin and Mullens 2012, Mullens et al. 2012). A dust bag could be hung or clipped in a cage, or near hen resources (nestboxes or food) in cage-free houses.

Economic Threshold. An effective integrated pest management (IPM) program aims to keep pests below an economic threshold, or the point when the cost to treat a pest is less than the cost of damage the pest will cause to a producer. Evidence suggests that mites scores ≤ 3 (≤ 100 mites) per bird are not as economically damaging to egg production (Mullens et al. 2009). Mites are most damaging early in a new flock during first egg production cycle, when young hens are immunologically mite-naïve. Once mites are established on a property they can be very difficult to eliminate and may become a predictable problem in subsequent flocks. Successful poultry IPM will focus on preventative measures, prophylactic control, and keeping mites below economic thresholds.

Backyard Chickens. In addition to the commercial layer changes occurring in this country, backyard or hobby chicken flocks are currently booming in popularity in the US (Elkhorabi et al. 2014). These types of flocks are highly variable in terms of size and husbandry practices, but in general mimic cage-free, free-range, or pastured production compared to traditional caged systems. Thus it is expected that these flocks will also be at risk for ectoparasite infestations. There is a knowledge gap regarding ectoparasite diversity on backyard flocks and control options available to homeowners.

The purpose of this dissertation research is to further our understanding of host-parasite interactions while exploring practical solutions. Poultry production is changing, and the way ectoparasites are managed will have to change as well. Developing effective control methods for caged and cage-free environments, conventional and organic

operations, and commercial and home-use are challenges for the poultry industry and entomologists. The areas of mite-resistance (Chapter 1) and hen self-treatment with dustboxes (Chapter 2) and dust bags (Chapter 3) will be addressed in subsequent chapters. A survey of ectoparasites on backyard chicken flocks was conducted in southern California in order to gain some knowledge of what differences in ectoparasite loads exist in these environments compared to what is historically found in commercial flocks (Chapter 4).

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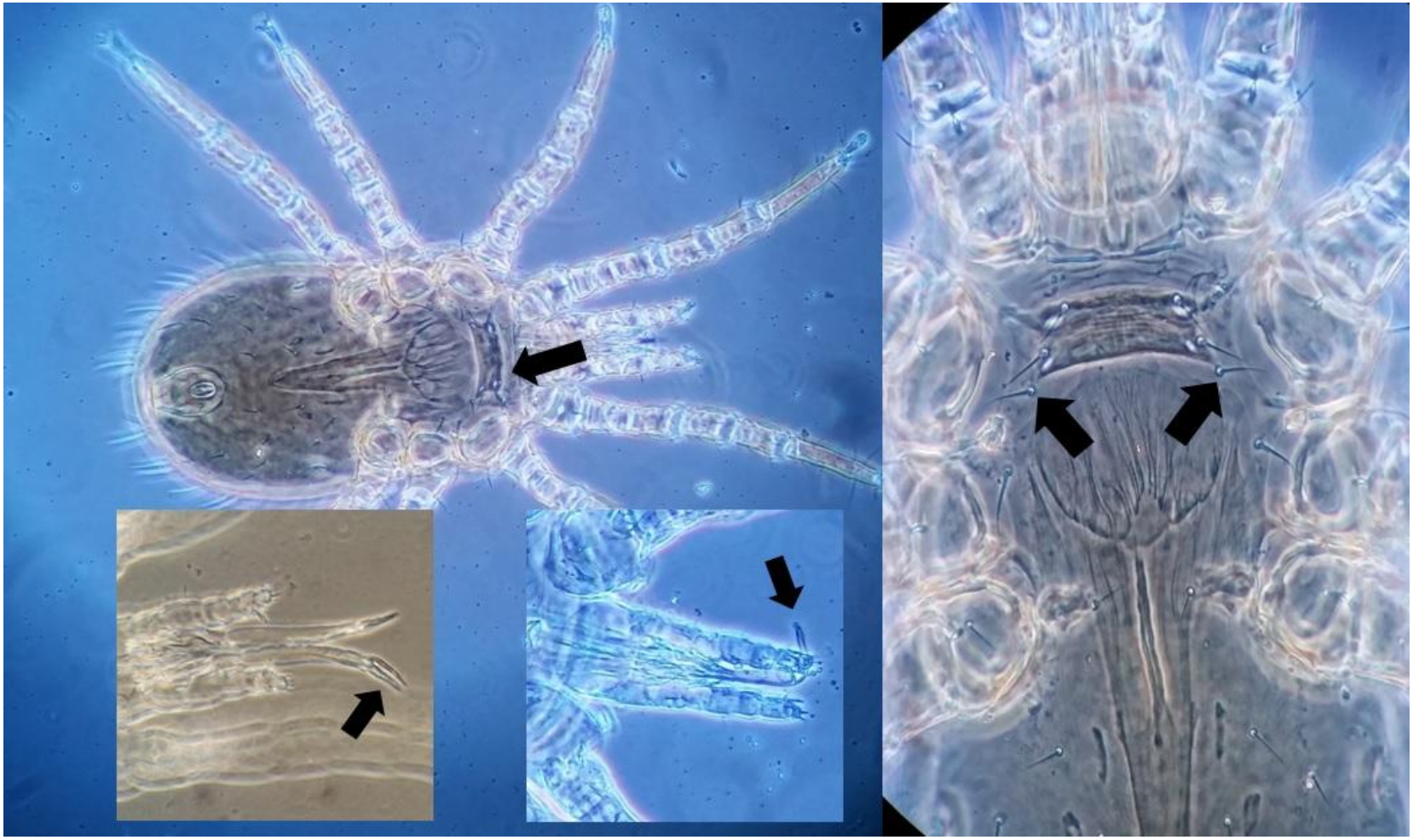


Figure i. Diagnostic character distinguishing common *Ornithonyssus* species. Setae on the sternal shield (arrow left) may have the last pair of setae off the shield (*O. sylviarum,* arrow right) or on the shield (*O. bursa* or *O. bacoti*). The left inset shows the claw-like chelicerae of *Ornithonyssus* compared to the whip-like chelicerae (right inset) of *Dermnayssus.*

CHAPTER 1

Northern fowl mite (*Ornithonyssus sylviarum*) effects on metabolism, body temperatures, skin condition, and egg production as a function of hen MHC haplotype

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ABSTRACT

The northern fowl mite, *Ornithonyssus sylviarum*, is the most damaging ectoparasite on egg-laying hens in the United States. One potential strategy for management is breeding for mite-resistance. Genes of white leghorn chickens linked to the major histocompatibility complex (MHC) were previously identified as conferring more (B21 haplotype) or less (B15 haplotype) mite-resistance. However, immune responses can be energetically costly to the host and affect the economic damage incurred from mite infestations. We tested energy costs (resting metabolic rate) of mite infestations on egg-laying birds of both MHC B-haplotypes. Resting metabolic rates were documented before (pre-) mite infestation, during (mid-) infestation, and after peak (late) mite infestation. Mite scores, economic parameters (egg production, feed consumption) and physiological aspects such as skin inflammation and skin temperature were recorded weekly. Across experiments and different infestation time points, resting metabolic rates generally were not affected by mite infestation or haplotype, although there were instances of lower metabolic rates in infested versus control hens. Skin temperatures were recorded both at the site of mite feeding damage (vent) and under the wing (no mites), which possibly would reflect a systemic fever response. Ambient temperatures modified skin surface temperature, which generally was not affected by mites or haplotype. Feed conversion efficiency was significantly worse (4.9 - 17.0% depending on trial) in birds infested with mites. Overall egg production and average egg weight were not affected significantly, although there was a trend toward reduced egg production (2-8%) by infested hens. The MHC haplotype significantly affected vent skin inflammation. Birds

with the mite-resistant B-21 haplotype showed earlier onset of inflammation, but a reduced overall area of inflammation compared to mite-susceptible B-15 birds. No significant differences in resting energy expenditure related to mite infestation or immune responses were detected. Potential breeding for resistance to mite infestation using these two haplotypes appears to be neutral in terms of impact on hen energy costs or production efficiency, and may be an attractive option for future mite control.

INTRODUCTION

The northern fowl mite (NFM), *Ornithonyssus sylviarum* (Canestrini and Fanzago), is a cosmopolitan parasite found on over 70 wild and domestic bird species (Knee and Proctor 2007). In North America it is the most damaging ectoparasite of domestic poultry (Axtell and Arends 1990, Hinkle and Hickle 1999, Mullens et al. 2009). These mites reside permanently in hen feathers, and adults and protonymphs migrate to the skin surface to blood feed. Only 5-12 days are required to complete the life cycle (Sikes and Chamberlain 1955), leading to high intrinsic population growth rates (Owen et al. 2009). Mites are found almost exclusively on feathers in the vent region of hens (Hogsette et al. 1991), where the deep feather structure and microenvironment are favorable (DeVaney and Beerwinkle 1980, Halbritter and Mullens 2011, De La Riva et al. 2015). Northern fowl mites can invade domestic flocks via wild birds or contaminated people, equipment, or rodents (Kells and Surgeoner 1997), and genetic evidence suggests some layer ranches maintain permanent mite populations from one flock to the next (Owen, unpublished).

Mites introduced to naïve birds exhibit stereotypical population density patterns. Mite numbers grow steadily for 4-10 weeks and then decline to low levels that generally persist for the rest of the bird's life (Matthysse et al. 1974, Mullens et al. 2009, Owen et al. 2009). This decline is due in large part to host immune responses (DeVaney and Ziprin, 1980, Burg et al. 1988, Minnifield et al. 1993, Owen et al. 2009). These vary from bird to bird, even of the same age and breed, because of genetic variation (Matthysse et al., 1974, DeVaney, 1982, Mullens et al. 2000, Owen et al. 2008).

Mite infestations cause skin inflammation and thickening of the epidermis in areas where mites feed. This physically prevents mite protonymphs and then adults from being able to reach blood vessels for feeding (Owen et al. 2009). Mite susceptibility is influenced by the major histocompatibility complex (MHC) (Owen et al. 2008). Two combinations of MHC haplotypes (B2/B15 and B2/B21) are found in CV20 (formerly W36) white leghorn laying chickens. The two heterozygous haplotype pairs occur in roughly a 1:1 ratio among chickens in commercial flocks. Other haplotypes are also found at low frequencies, but those have not been well characterized in terms of mite response. The haplotype B2/B21 is associated with strong skin inflammation in response to NFM and confers more resistance to mites than occurs in birds with B2/B15 haplotypes, which exhibit less inflammation. B21 birds do not completely clear NFM infestations, but the more potent immune response causes the NFM intrinsic rate of increase to fall below replacement level by 16-20 d post-infestation (Owen et al. 2009).

Mite infestations cause economic losses by reducing egg numbers, egg weights, and hen feed conversion efficiency (Mullens et al. 2009). These effects may be related to

mite-induced immune responses. Immune system responses to parasites come at some energetic cost to the host (Sheldon and Verhulst 1996, Norris and Evans, 2000, Sadd and Schmid-Hempel 2008). The host can respond by increasing energy uptake (food consumption) or by allocating energy from other physiological functions such as reproduction (Sheldon and Verhulst 1996, Sadd and Schmid-Hempel 2008). Food is expensive, however, comprising about 70% of a poultry farmer's budget (Bell and Weaver 2002). For egg producers both effects are costly; either egg production is decreased and/or birds are consuming more food.

Innate and adaptive immune responses can benefit hosts exposed to consecutive parasite encounters (Heeb et al. 1998) by minimizing lifetime negative impacts on host fitness. In commercial poultry production settings, host resistance could lessen the need for pesticide sprays or other control methods. In the present study, we determined energy metabolism to help quantify northern fowl mite impacts over time for NFM infested and uninfested hens. In particular, minimal resting metabolic rate was measured, as it is susceptible to manipulation by both pathogens and host immune responses and represents maintenance metabolism exclusive of costs of activity and temperature regulation. We incorporated the primary MHC haplotypes governing hen resistance to NFM (B21 and B15) as an experimental factor relative to NFM population development or altered economic performance.

MATERIALS AND METHODS

Birds. Beak trimmed white leghorn laying hens (Hy-Line CV20 breed; 17-19 weeks old) entering their first egg production cycle were acquired from local poultry facilities and housed at the University of California, Riverside Agricultural Operations property. Procedures were approved under Animal Use Protocols A-20120007E and A-201000026E, University of California, Riverside. Blood samples were collected from fifty hens and sent to Northern Illinois University for MHC haplotype determination. Most birds were B2/B21 or B2/B15 haplotypes and all birds were naïve to NFM. For each trial 16 birds (n = 8 per haplotype) were paired by relative body mass and then randomly separated into treatment (NFM infested) and control (uninfested) groups. During a trial, the haplotype of each hen was unknown to the mite scorer (ACM) to eliminate possible scoring bias. To prevent parasite contamination, control hens were housed on one side of the poultry house while mite infested hens were on the opposite side. Control hens were always fed or handled first. All hens were kept individually in battery cages measuring 31 X 31 x 31 cm in size. Temperatures were moderated by a space heater, window air conditioning unit, and roof sprinklers, but the facilities were designed to mimic southern California commercial production and the environmental variation they experience. A Hobo temperature recorder (Onset Computer Corp., Bourne, MA, U.S.A.) recorded temperatures in the poultry house.

Feeding & Egg Production. Each bird was given its own removable food trough, and known amounts (by weight) of feed (Big Feeder Lay Mash, Star Milling Co., Perris, CA, USA) were provided daily ad libitum. Feed troughs and residual feed were

individually weighed weekly, and the total amount of feed consumed per bird was recorded. Egg production and individual egg weights were recorded daily for each bird. In rare instances where eggs broke, a weekly average weight of the eggs for that bird was substituted.

Mite Density, Temperature, and Inflammation Assessment. Mites were aspirated from source hens using Pasteur pipettes (Martin and Mullens 2012). Each treatment bird received ca. 30 adult mites during week 2, after baseline minimal resting metabolic readings (see below) were complete. A second inoculation at week 3 was conducted the same way to ensure the establishment of mites on treatment birds.

Once a week for the duration of each trial the mite populations on each bird were visually estimated (always by ACM for consistency). These raw numbers were associated with a mite score. The scoring system used is as follows: 0 = no mites, 1 = 1–10, 2 = 11–50, 3 = 51–100, 4 = 101–500, 5 = 501–1000, 6 = 1001–10,000 and 7 = >10,000 (Arthur and Axtell, 1983). This scoring system has been used historically to estimate and describe northern fowl populations over time (Arthur and Axtell 1983, Mullens et al. 2000, Owen et al. 2009, Martin and Mullens 2012).

Air temperature at the time of each assessment was taken at hen cage level in the house. An infrared thermometer (Model 15-007-968, Fisher Scientific, Pittsburgh, PA, U.S.A.) was used to record skin surface temperatures (to the nearest tenth of a degree) in the vent (by parting the feathers about 4 cm anterior to the cloaca) and the bare skin patch under the wing (specifically, the distinct axillary pit). Temperatures were recorded weekly for all hens. On mite infested hens, lesions would often form in the vent, where

almost all mite feeding occurs (Owen et al. 2009). Under wing temperatures were in an area not inhabited by or fed on by mites. The under wing area therefore served as a relative control for localized vent skin inflammation and skin surface temperature effects (versus possible systemic temperature changes, which would be evident under wing). Calipers (Model 06-664-16, Fisher Scientific, Pittsburgh, PA, U.S.A.) were used to estimate the length and width of the visibly inflamed vent skin lesion area (Fig. 1.1). The two measurements were multiplied to yield estimated cm^2 of inflamed skin surface. We did not attempt to measure skin thickness. Photographs were also taken weekly of the vent and under wing skin of each hen to record skin color and visible inflammation. Skin of the vent or under wing was scored as either inflamed (any visible redness) or not (white or normal skin) by one observer who was blinded to the treatments (ACM).

Energy metabolism. On the days metabolism measurements were taken, one mite infested and one control bird (same haplotypes) were transferred in early evening from the poultry house in separate, clear Plexiglas containers (41 cm x 35 cm x 35 cm or 45 cm x 35 cm x 40 cm). Containers had HDPE adapters with plastic tubing for airflow, coated on the inside with a thin layer of sticky material to prevent mite escape. During the brief transportation (about 15 min) from the hen house to the laboratory, battery operated aquarium pumps (Marina 11134, Hagen, Mansfield, MA, U.S.A.) were used to provide air circulation. Hens were transported to the UCR Biology Department, where the chambers were attached to air flow equipment and O_2 analyzers as described below. The two hens were held individually overnight and measured simultaneously.

Metabolism was determined as the rate of oxygen consumption ($\dot{V}O_2$), measured overnight when birds were at rest, using open-circuit respirometry (Chappell et al. 1996). Metabolism chamber temperature was maintained at 26-30 °C, which is thermoneutral for hens (Arieli et al. 1980). Dry air was supplied at 15.0-15.5 liters/min STP by mass flow controllers (Tylan; Torrance, California). Excurrent air from each chamber was subsampled at ~100 ml/min, dried, scrubbed of CO_2, and re-dried (Drierite and soda lime), and routed through one channel of a two-channel analyzer (S-3A; Applied Electrochemistry, Sunnyvale, California). Measurements of O_2 concentration, temperature, and flow rate were recorded every 2.5 seconds for at least 8 hours using Warthog Labhelper software (www.warthog.ucr.edu). Minimal resting metabolic rates (mRMR) were determined by averaging the lowest 10 continuous minutes of $\dot{V}O_2$ when $\dot{V}O_2$ was low and stable between 8pm-3am, reported as ml O_2 /min. We also calculated the average $\dot{V}O_2$ over 6 hours (9 PM – 3 AM).

The following morning, both hens were returned to the poultry house and weighed. The chambers were taken back to the laboratory and all equipment was disinfested by freezing to -15 °C for at least 5 hours to avoid mite cross-contamination. This process was repeated for eight consecutive days to measure all 16 hens.

Each trial was 12 weeks in duration and hens were subjected to basal metabolic readings at three time points during the experiment: 1-2 weeks (pre-infestation; baseline readings), 6-7 weeks (mid-infestation; near peak mite levels), and 11-12 weeks (late infestation; when immune responses are expected to have reduced mite densities; as described above). We expected mRMR to be highest when mite populations were near

peak (6-7 weeks) and remain high compared to baseline at mite decline (11-12 weeks) because of residual immune responses. Three full experimental trials were conducted in Feb-May 2012, Aug-Oct 2012, and Jan-Mar 2013.

Statistics. Statistical analyses were conducted using SAS® software (SAS Institute Inc., Cary, NC, 2010, v. 9.3) and MINITAB (Minitab, Inc. 2005, v. 14). Mixed model repeated-measures were used to analyze the main effects of hen MHC haplotype (B21 or B15) and mite infestation (compared to uninfested birds). Hen was a fixed effect and week was the repeated measure and was included in the model. Mite score was only used as a main effect when examining the effect of bird haplotype. Following a comprehensive initial model, some portions of the data then could be pooled to examine specific variables; however in many cases data were separated by trial and analyzed separately due to variation among trials. The response variables were vent skin temperature, under wing skin temperature, area of vent skin inflammation, mRMR, and average metabolic rates at each time point, weekly feed conversion efficiency (g of feed consumed per g of egg produced), weekly egg production (# eggs per hen), and total weekly egg weight. Tukey's post hoc test was used when significant differences were found.

Vent and under wing skin temperature data were analyzed as above. Ambient temperature was the most significant factor in each trial's model. Interaction plots of temperature*treatment*type did not show consistent effects. Control vent or under wing temperatures were separated in order to create a regression of control hen skin temperatures on air temperature (expected vent or under wing temperatures). In treatment

bird analyses mite scores were included in the mixed model analyses as a main effect. A regression analysis was used to compare observed effects (treatment vent or under wing temperatures) to the control temperature-generated regression in MINITAB.

Non-parametric tests (sign and Mann-Whitney U) were used to assess whether the frequency of treatment hens with positive (visual) inflammation differed between haplotypes for a given week or if the number of consecutive weeks of inflammation for each haplotype differed.

In the metabolism analyses, mRMR and body mass were linearized by $\log_{10}$-transformation (Chappell et al. 1996). A mixed model repeated-measures procedure was used for comparisons of mRMR, with body mass as a covariate. Average metabolic rates were compared in the same was as mRMR. Regression analyses were performed using $\log_{10}$-transformed data.

Regression analyses were performed to determine effect of average mRMR (weeks 6 and 11) and average mite populations (raw numbers transformed $\log_{(n+1)}$) on average egg production (number, weeks 5-11).

RESULTS

Parameters measured in this study are grouped for presentation as follows: 1) mite population trends, 2) physiological responses to mite infestation (temperature and metabolic effects), and 3) responses of production parameters to mite infestation. Weekly means for mite population trends and bird responses to mite infestation, grouped by trial

and haplotype, are presented in full separately (Table 1.1) from weekly means of production factors (Table 1.2).

Mite Population Trends

Mite population trends were similar in cool-weather trials 1 and 3, with populations building for 4-6 weeks and then declining through week 11 (Fig. 1.2). In the warmer trial 2, mite populations grew more slowly and reached their highest levels late in the trial, at weeks 10 and 11. There was no stable mite population peak and decline in trial 2, although there was a slight decline from week 4 to 5, followed by a steady rise in mite densities. Ambient air temperatures at the time of mite scoring (Fig. 1.3) were significantly higher in trial 2 than in trials 1 or 3 ($F = 13.31$; df = 2, 30; $P < 0.0001$). In trial 2, the HOBO temperature recorders logged 192 hours of temperatures above 30 ^{o}C, compared to 1.5 hours in trial 1 and 2.5 hours in trial 3. In mixed models, time (week) had the most influence on mite populations in each trial (trial 1 $F = 32.28$; df = 10, 60; $P < 0.0001$; trial 2 $F = 13.36$; df = 10, 60; $P < 0.0001$; trial 3 $F = 33.04$; df = 10, 60; $P < 0.0001$).

The bird haplotype, resistant B21 versus susceptible B15, did not have a consistent effect on mite scores over time. In trials 1 and 3, there was no relationship between haplotype and mite score (trial 1 $F = 0.25$; df = 1, 6; $P = 0.6342$; trial 3 $F = 0.05$; df = 1, 6; $P = 0.8317$). Mite populations grew steadily and similarly on birds of both haplotypes over the first 4-5 weeks (Fig. 1.4). In comparison, trial 2 mite infestations grew more slowly and were never as high as the other trials, as evident by the mite scores

(Fig. 1.4). The trial 2 absolute peak mite population (raw mean ± SE) was 593 ± 181 versus mite populations of 11,344 ± 3,188 and 5,038 ± 1,248 in trials 3 and 1, respectively. Trial 1 and 3 peak mite levels were observed at week 5 (3-4 weeks post mite inoculation) and the trial 2 peak was observed at week 11. There was a divergence at weeks 4-5 between the two haplotypes in trial 2 (Fig. 1.4). From week 4 onward the B21 hens had on average lower mean scores than the B15 hens, though haplotype was not a significant factor in the repeated measures analysis (F = 2.17; df = 1, 6; P = 0.1910). In trial 2 the mite resistant haplotype (B21) had an overall mite population of 174 ± 37 (raw mean ± SE), while the mite susceptible haplotype (B15) had an average mite population of 380 ± 75. Overall mite levels in trials 1 and 3 were as follows: trial 1 B21 = 2,506 ± 686, trial 1 B15 = 3,506 ± 757; trial 3 B21 = 1,920 ± 364 , trial 3 B15 = 2,199 ± 348. Birds with the resistant (B21) haplotype thus had a slightly lower average mite density than the susceptible (B15) haplotype in each trial, but the differences were not statistically significant.

Physiological Responses

Vent & Under Wing Temperatures. Trials were separated for analysis due to substantial differences in ambient conditions. In control (uninfested) birds, air temperature positively affected vent skin temperature (trial 1 F = 20.98; df = 8, 48; P < 0.0001; trial 2 F = 8.17; df = 5, 30; P < 0.0001; trial 3 F = 28.03; df = 7, 35; P < 0.0001). Overall, vent skin temperature (°C) in the uninfested control hens for all three trials was: B21 = 36.10 ± 0.20, B15 = 36.04 ± 0.22, and B19 = 35.25 ± 0.49 (mean ± SE). In

treatment (infested) birds, vent skin temperature similarly varied with air temperature and week; vent temperatures tended to be higher when air temperatures were higher. The B21 treatment hens had an average vent skin temperature (°C) of 35.55 ± 0.21, while the B15 hens had an average of 35.37 ± 0.21 (mean ± SE). However, mite scores had a significant effect on vent temperature (F = 3.06; df = 7, 82; P = 0.0065). Tukey's test was used to separate the temperature estimate by mite score (Fig. 1.5). Lower vent temperatures, and often larger areas of scabbing, were recorded when a bird had a higher mite score.

Under wing temperatures were regularly about 2-4 ^{o}C higher than temperatures in the vent for the cooler trials 1 and 3, but differences in the warmer trial 2 were about 1-2 ^{o}C (Table 1). Factors influencing under wing temperature varied by trial, although ambient temperatures always influenced under wing skin temperature: trial 1 F = 28.29; df = 8, 13; P < 0.0001; trial 2 F = 8.68; df = 5, 75; P < 0.0001; trial 3 F = 40.22; df = 7, 105; P < 0.0001. There was no indication that mite infestation resulted in elevated under wing temperatures (i.e. a systemic fever response) or that haplotype had any relation to differential body temperature in control or infested birds. In trial 1 under wing temperatures were on average 38.06 ± 0.14 for control birds and 37.94 ± 0.11 for treatment birds (°C; means ± SE). Under wing temperatures were not influenced by treatment (mite infestation; F = 1.59; df = 1, 13; P = 0.23) or haplotype (F = 1.82; df = 1, 13; P = 0.20). However the treatment*temp interaction was significant (F = 35.50; df = 8, 13; P < 0.0001). The source of the interaction was one measurement of low and one high temperature in trial 1. The interaction was not stable across a wider temperature range and did not occur in experiments 2 or 3. In trial 2, average under wing temperatures were

within 0.5 ^{o}C but still were significantly higher (F = 9.23; df = 1, 13; P = 0.01) for control birds at 39.07 ± 0.12 versus 38.58 ± 0.08 for treatment birds (°C; means ± SE). No haplotype differences were detected (F = 2.62; df = 1, 13; P = 0.13). Trial 3 under wing temperatures were 37.25 ± 0.23 for control birds and 36.89 ± 0.21 for treatment birds (°C; means ± SE). Neither treatment or haplotype was a significant factor (F = 1.36; df = 1, 12; P = 0.27; and F = 1.71; df = 2, 12; P = 0.22 respectively).

Vent Skin Inflammation. Visually red and swollen vent skin (Fig. 1.1) is a result of NFM feeding and therefore was measured on treatment (NFM infested) birds only. Control (uninfested) hens never showed such inflammation, and presence and area of inflammation in treatment birds varied from week to week. The inflamed area typically was longer (parallel to hen body axis) than it was wide (perpendicular to hen body axis). The time of onset and duration of detectable inflammation are shown in Figure 1.6. There was a tendency for the mite-resistant haplotype hens (B21) to show signs of inflammation earlier (about 1 week) than mite-susceptible hens (B15). Using a sign test (inflammation present or absent) to compare the haplotypes for the pooled trials, the B21 haplotype showed significantly more inflammation on week 3 (shortly after mite infestation) (P < 0.05), but the two haplotypes did not differ on any other week. More instances of vent inflammation and redness were recorded from B15 birds (n = 73) compared to B21 birds (n = 62). Using a Mann-Whitney U test to examine the maximum duration of continuous weeks of inflammation (trials pooled), the two haplotypes did not differ significantly (W = 137, P = 0.47). The B21 haplotype had a median maximum duration of 3 weeks, while the B15 haplotype had a median maximum duration of 3.5 weeks.

A strong trend was found for area of inflammation versus hen haplotype (F = 4.20; df = 1, 20; P = 0.053, all trials pooled). The susceptible haplotype, B15, had a trend toward a greater area of skin inflammation than the B21 resistant haplotype (Fig. 1.7; Supplementary Table 2).

Energy metabolism. There was a significantly positive relationship (linear regression) between the log-transformed measures of mass and mRMR in control and treatment bird groups (control r^2 = 0.06; treatment r^2 = 0.069). These slopes were not significantly different from one another (T = 0.22; df = 102; P = 0.826), indicating that the relationship between mRMR and mass did not vary with mite infestation. Body mass was not affected by mite infestation (F = 0.11; df = 1, 46; P > 0.74; mean mass was 1239.17 ± 14.98 SE for control birds and 1249.08 ± 11.71 for treatment birds).

The mRMR values for each trial (pre-infestation, mid-infestation, late infestation) were analyzed for mite and haplotype effects. Accounting for the three time points no haplotype effects were found (F = 0.60; df = 1, 34; P = 0.443). Within the mixed model, mite infestation had no effect in trials 1 and 3 (trial 1 F = 0.73; df = 1, 17; P = 0.404; trial 2 F = 1.92; df = 1, 14; P = 0.188) but did in trial 2 (F =5.56; df = 1, 18; P = 0.03). Control birds had higher mRMR compared to mite infested (treatment) birds for each of the three metabolic readings in trial 2, as follows (mean ± SE): 1) pre-infestation, control 14.70 ± 0.64, infested 12.46 ± 0.78; 2) mid-infestation, control 13.79 ± 0.91, infested 13.35 ± 0.55 3) late infestation, control 15.71 ± 0.54, infested 14.27 ± 0.70. Apart from changes related to increasing host age and mass, mRMR did not change significantly over time with changing mite numbers.

For 6-h average metabolic rates, trial had a significant effect (F = 15.50, df = 2, 43; $P < 0.0001$), thus all trials were separated for analysis. In all trials, time point (pre-infestation, mid-infestation, late infestation) and haplotype did not affect average metabolic rates. In trial 1 there were no differences in average metabolic rates between control (17.48 ± 0.35) and infested (18.75 ± 0.52) birds (F = 0.05; df = 1, 14; P = 0.8215) and mass was the most important factor (F = 7.24; df = 1, 14; P = 0.0176). Differences in average metabolic rates between control and infested birds were detected in trials 2 and 3 (trial 2 F = 20.45; df = 1, 14; P = 0.0005; trial 3 F = 25.50; df = 1, 13; P = 0.0002). In trials 2 and 3 control birds had higher average metabolic rates (ml/min) than infested birds (trial 2: 17.04 ± 0.47 and 14.85 ± 047; trial 3: 20.60 ± 0.52 and 17.86 ± 0.55, respectively).

Production Factors

Egg Production. Trial was a significant factor on egg production (Supplementary Table 2), so treatment (mite infestation), week, and haplotype effects on egg weights (total egg weight per hen per week) were tested separately for each trial. In each of the three trials, week (reflecting increasing hen age and mass) had a significant and positive effect on egg weight (trial 1 F = 5.53; df = 10, 150; $P < 0.0001$; trial 2 F = 6.44; df = 10, 150; $P < 0.0001$; trial 3 F = 2.49; df = 10, 150; $P < 0.0001$).

The number of eggs laid per bird per week was analyzed in the same way as egg weights. Trials were separated for analysis and week again was the most important factor (trial 1 F = 3.92; df = 10, 150; $P < 0.0001$; trial 2 F = 2.54; df = 150; $P < 0.0001$; trial 3 F

= 3.92; df = 150; $P < 0.0001$). The weekly average number of eggs per treatment for each trial and percent production (seven eggs per week per hen is 100% production) was: trial 1 control 6.51 ± 0.08 (93.0%), treatment 6.38 ± 0.08 (91.1%); trial 2 control 5.55 ± 0.15 (79.3%), treatment 5.01 ± 0.18 (71.6%); trial 3 control 6.78 ± 0.05 (96.9%), treatment 6.57 ± 0.10 (93.9%). Consistently, treatment birds had about 2-8% lower average egg production than controls but these differences were not statistically significant. However, the numbers of eggs laid in trial 2 were significantly lower than in trials 1 and 3 (Tukey's test, $P < 0.0001$).

The average number of eggs laid per bird and the average mRMR were found to be correlated ($r^2 = 0.354$). Average mite levels did correlate with eggs laid, as both were related to hen age (see above).

Feed Conversion Efficiency. The few instances where no eggs were produced, but feed was consumed, were removed from the data set before analysis of feed conversion efficiency (6/528 data points). Average feed conversion for each trial was as follows: trial 1 control 1.85 ± 0.03, treatment 1.94 ± 0.03; trial 2 control 1.88 ± 0.05, treatment 2.20 ± 0.11; trial 3 control 1.75 ± 0.02, treatment 1.93 ± 0.14 (Supplementary Table 2). Average treatment feed conversion rates in each trial were higher than control feed conversion rates (indicating less efficient feed conversion) by 4.9% in trial 1, 17.0% in trial 2, and 10.3% in trial 3. Overall feed conversion was affected significantly by mite infestation ($F = 2.10$; df = 7, 507; $P = 0.042$). Within the mite infested hen group, however, specific mite scores did not significantly impact feed conversion ($P = 0.455$).

The level of mite infestation didn't have a detectable impact on the level of feed conversion efficiency.

DISCUSSION

Mite Population Trends

Mite populations in trials 1 and 3 grew steadily for 4-5 weeks, then began to decline. This pattern is typical for northern fowl mite populations, and is due to immune responses (especially host skin inflammation) which are under genetic control (Matthysse et al. 1974, DeVaney et al. 1982, Owen et al. 2009). Trial 2 did not follow the typical mite population growth pattern. Instead, mite levels increased steadily throughout the duration of the experiment, but mite densities never achieved levels as high as the other two trials. Trials 1 and 3 began in winter and trial 2 began in summer when Riverside, CA air temperatures may reach > 40 ^{o}C. An air conditioner in the chicken house and roof top sprinklers helped to mitigate high temperatures. However, temperatures in the house still sometimes exceeded 30 ^{o}C. The mites prefer on-host temperatures of about 28-30 ^{o}C, but 30 ^{o}C ambient air temperatures result in much higher temperatures (about 35 ^{o}C) in the vent feather zone where the mites live (Halbritter and Mullens 2011, De La Riva et al. 2015). Air temperatures above 30 °C also hinder northern fowl mite survival *in vitro* (Chen and Mullens 2008). These higher temperatures probably slowed mite population growth in trial 2.

Higher temperatures in trial 2 may have also contributed to the appearance of differences in weeks 7-11 between the MHC haplotypes, though there were no

statistically significant haplotype-based differences in mite densities in any of the trials. Unfortunately, the intensity of the study (very detailed and repeated observations on each hen) limited the number of hens that could be used. The relatively low n in each trial (4 hens per haplotype-mite combination) reduced our ability to detect differences, which was exacerbated by trial differences.

The temperatures in the test house were representative of commercial conditions in California's "open style" housing, and we wanted to do these trials under realistic conditions. Chickens still may have been somewhat stressed by variable temperatures, although temperatures of 30 ^{o}C, as in trial 2, should not be very stressful and are within the hens' thermoneutral zone (Arieli et al. 1980). Mashaly et al. (2004) showed increased heterophil : lymphocyte ratios (an indicator of hen stress), increased white blood cell counts, decreased egg production, and decreased antibody titers in birds kept at high heat and humidity (> 35 °C, 50% RH) for 4 weeks. If temperature variation sufficient to cause hen stress among trials did occur, it could be reflected in the mite-induced immune response. Resistant B21 birds produce a more robust immune response and may be able to keep mite levels in check during a period of heat stress better than B15 birds. Alternatively, the relatively lower temperatures in trials 1 and 3 may have masked haplotype differences which only appeared in trial 2 at temperatures closer to their thermoneutral limit. This illustrates the importance of including a reasonable range of experimental variation in order to assess possible parasite impacts, which might appear only under some conditions.

Physiological Responses

Bird vent temperatures were primarily affected by ambient temperatures. Treatment bird vents were also influenced by mite scores. Birds with higher mite scores exhibited lower vent temperatures (Fig. 1.5). As mite populations grow on a chicken, the skin in the vent area becomes red and inflamed, with mite feeding often leading to the formation of crusty scabs and karatoses as the vent area heals (Owen et al. 2009). The vent temperatures of birds in this healing phase reflect this dry scabby skin, which is devoid of vascular tissue. The scabs may insulate the vascularized and inflamed skin underneath, causing lower vent skin surface temperatures as measured by the infrared thermometer. The scab surfaces apparently still are warm enough for mites to negotiate their way to the skin surface for feeding, however, and it was common to find mites feeding underneath the looser scabs (Owen et al. 2009). Mites prefer on-host temperature zones of 28-30 ^{o}C, live in the feathers, and adjust their distance from the skin to thermoregulate (Halbritter and Mullens 2011, De La Riva et al. 2015). The mite-induced scabbing thus could both provide a sheltered microhabitat for feeding (e.g. perhaps higher humidity) and serve as a form of "heat shield" for mites and influence the size of the habitable feather zone. It was interesting that we did not detect localized vent skin surface temperature increases, which can accompany edema and may be evident at sites of skin wounding or infection (Fierheller et al. 2010). The visible edema in areas of mite damage, even without significant scabbing (e.g. weeks 2-4 post-infestation), did not result in localized increases in vent skin temperatures detectable with our instrument. Perhaps more detailed measurements could detect this. Likewise, it is possible that

arthropod feeding and introduced salivary components might result in systemic temperature changes, as for example occur with people bitten by large numbers of black flies ("black fly fever") (Adler and McCreadie 2009). However, under wing temperatures, in an area not fed on by the mites, showed no evidence of systemic fever from the mite infestations. Work conducted by Vezzoli et al. (2016) did not find hen comb temperature differences 5 or 7 weeks after infestation with NFM. However 75% of hens (n= 32) did exhibit red skin in the vent area 5 and 7 weeks post-infestation.

MHC haplotype effects were seen in regard to area of vent inflammation. The susceptible B15 haplotype exhibited larger areas of redness than were seen on B21 resistant type birds (Fig. 1.7). The vent areas were not necessarily more swollen or thickened; our visually assessments were for 'redness' as an indicator of irritation and inflammation (Fig. 1.1). Owen et al. (2009) found increased skin thickness as a result of inflammation in resistant B21 birds, but the area of inflammation was similar between the B21 and B15 haplotypes. Thus, the skin thickness data were more useful in interpreting effects of inflammation on mite fitness. We relied on visual examinations of the vent in order to non-destructively sample the birds over the duration of each trial.

Mite infestations did not affect mRMR over all trials. Differences in mRMR were attributable to variations in body mass, which is the most important factor affecting metabolic rates (Kleiber 1932, 1961, Hulbert and Else 2004). Trial 2, with its higher temperatures and delayed mite growth pattern, did show an effect on mRMR. Birds that were uninfested (controls) had higher mRMR rates than mite infested birds. However, this difference was consistent across all three readings; there was no change over time.

Because no mites were present during the first reading of trial 2, and this effect did not change as mite populations grew on the mite treatment birds, it is unlikely that the presence of mites is the factor explaining differences between the two groups of birds (treatment and control). It is possible that position effects within the hen house played a role in the mRMR differences (control birds were kept at the opposite side of the house from mite infested birds to avoid cross-contamination).

Apart from possible effects on mRMR, mites may cause disturbances to the bird's rest. Severe mite infestations can cause up to 6% blood loss per day (DeLoach and DeVaney 1981). Preening and beak condition impact parasite abundance, and birds might spend more time preening, with possible sleep interruptions, in response to ectoparasites such as lice (Clayton and Tompkins 1995, Chen et al. 2011, Vezzoli et al. 2015). Vezzoli et al. (2015) showed that while mite infested hens did not increase their total preening activity, the preening was more directed toward the vent, where the mites live. Increased preening activity, which has an energy cost, would result in increased average metabolic rate. However, we did not detect differences in average metabolic rate during the night, which again was related only to body mass. A different experimental design, including monitoring nighttime behavior directly, may be more appropriate to tease out those effects.

Production Factors

Treatment birds showed substantially increased feed conversion rates compared to control birds; they required more food per egg than control birds (were less efficient in

converting food to egg). This was not influenced by haplotype. Mite impact is consistent with previous work tracking economic damage caused by NFM (see review by Mullens et al. 2009). In the few instances where feed conversion has been quantified, it is the parameter most dependably influenced by mite infestations. Our estimate of a 4.9-17.0% reduction in feed conversion efficiency in infested hens compared to control hens is among the higher experimental losses in the literature (Mathysse et al. 1974, Arends et al. 1984). However, mite scores themselves (intensity of infestation) did not correlate well with feed conversion values. Arends et al. (1984) felt that mite numbers of < 100/hen might still cause damage, although the Mullens et al. (2009) study suggested that those lower infestations were not as important as higher infestations. In the present study, while mites overall certainly affected the amount of feed consumed and feed conversion, variable mite densities could not be used to gauge the extent of that loss.

Treatment birds produced 2-8% fewer eggs on average than control birds, though this was not statistically significant. Haplotype had no effect on egg production. Previous work (see Mullens et al. 2009) has shown decreased egg production in mite infested birds, but low numbers of hens used in our study may have prevented us from detecting significant infestation effects.

To our knowledge this is the first study tracking mite economic impact using birds with distinct MHC haplotypes. The haplotypes, which are associated with differential immune responses (e.g. more substantial skin thickening of B21 reported by Owen et al. 2009), exhibited similar feed conversion efficiency. This was surprising, since immune system responses, especially those inducing fever, may be energetically

costly to hosts (Sheldon and Verhulst 1996, Norris and Evans 2000, Sadd and Schmid-Hempel 2008). However, there may be some other haplotype effects or host tolerance effects (e.g. Råberg et al. 2009) that were beyond the scope of this study. One might expect resting metabolic rates to change due to parasite-driven energy demands, however we could not detect this effect. Parasite effects on host energy relations have been explored before, with emphasis on host fitness costs (e.g. reproductive success, body mass change, Khokhlova et al. 2002, Hawlena et al. 2006, Scantlebury et al. 2007). Experimental effects of parasitism on host metabolism are wide ranging, however, with documented cases of reduced (Hayworth and van Riper 1987), increased (Lester 1971, Meakins and Walkey 1975), or no (Munger and Karasov 1989) changes in energy metabolism, often with considerable individual variation. Booth et al. (1993) tested the effects of chewing (feather-feeding, not blood-feeding) lice on a wild population of rock doves and did find indirect effects on host metabolic rates. These lice had been presumed to be innocuous because they feed on feathers and not living host tissues. However, decreased feather mass due to louse damage caused increased heat loss and higher metabolic rate. In the present study, the mites occupy feathers and encrust them with their bodies and feces, but it is not known whether this in turn impacts insulation value of the feathers. The more likely mechanism of damage, or impact on metabolism, would be from the blood-feeding activities of the mites.

Costs of parasitism to hosts are complex, diverse, and are influenced by many factors, including species, life stage or age, and relative densities of host and parasite, environmental conditions, etc. (Sheldon and Verhulst 1996). Among domestic poultry the

effects of parasites may be more predictable because of more stable environmental conditions in captivity, or as a result of selective breeding and consequent loss of genetic diversity. Studies documenting the domestic chicken's metabolism have specifically explored breed differences (Benedict et al. 1932), seasonal changes (Dukes 1937), development effects (Beattie and Freeman 1963), temperature effects (Barott and Pringle 1946, Mission 1977), or contrasted layers versus broilers (Kuenzel and Kuenzel 1977). The effects of intestinal parasites on maximum aerobic capacity have been tested in the domestic chicken's direct ancestor, the red jungle fowl (*Gallus gallus*). Parasite-related differences were only seen in small males (Chappell et al. 1996) or chicks (Chappell et al. 1997). To our knowledge the present study is the first attempt to assess effects of ectoparasites on energy metabolism in the modern day laying hen.

Animal welfare concerns and increased consumer awareness have been influencing poultry husbandry, one major component of which is housing (Lay et al. 2011, Mench et al. 2011, Swanson et al. 2011). In traditional battery-style cages, ectoparasites like NFM are controlled using chemical pesticide sprays (Mullens et al. 2009). In furnished cages or cage-free housing, effective pesticide sprays directly to hens are difficult or impossible. Any housing changes, therefore, will influence the way pest arthropods are controlled. Selective breeding for ectoparasite resistance, such as with the B21 haplotype observed in this study, is an attractive alternative control tactic. Prior work (Owen et al. 2009) has shown that birds with the resistant B21 haplotype supported lower NFM numbers at the peak of infestation compared to B15 birds. In the present study we

saw no additional energy costs or increased feed conversion associated with B21 haplotype birds infected with NFM compared to the more susceptible B15 haplotype.

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TABLES AND FIGURES

Table 1.1. Weekly means (± SE) of bird body responses categorized by mite presence (control or infested), trial (1-3), and hen MHC haplotype (usually B2/B21 or B2/B15). Temperatures (°C) are air temperature ('Temp') and skin surface ('Vent', 'Under wing') at time of weekly hen visual examination. Area of inflammation is cm^2. 'Color' refers to % of hens showing visually inflamed (red) vents for that week.

Control

Trial 1		B2/B21 n = 4		B2/B15 n = 4	
Week	Temp	Vent	Under wing	Vent	Under wing
1	21	34.75 ± 0.13	36.18 ± 0.45	34.75 ± 0.36	36.35 ± 0.13
2	27	36.63 ± 0.46	38.55 ± 0.41	37.20 ± 0.32	38.45 ± 0.41
3	20	33.78 ± 0.97	36.98 ± 0.36	34.25 ± 0.79	37.60 ± 0.31
4	23	34.30 ± 0.41	36.65 ± 0.79	34.73 ± 0.33	37.50 ± 0.47
5	27	36.33 ± 1.53	38.15 ± 0.49	35.55 ± 0.52	38.93 ± 0.35
6	25	35.80 ± 1.14	39.45 ± 0.29	36.40 ± 0.62	39.15 ± 0.25
7	15.5	32.60 ± 0.88	37.70 ± 0.83	32.48 ± 0.16	37.90 ± 0.44
8	28	37.25 ± 0.51	39.48 ± 0.32	37.13 ± 0.40	39.48 ± 0.20
9	18	31.83 ± 3.40	37.33 ± 0.62	32.85 ± 0.22	37.15 ± 0.78
10	24	34.98 ± 1.31	38.38 ± 0.38	35.50 ± 0.51	38.30 ± 0.65
11	23	35.75 ± 1.83	39.08 ± 0.15	36.15 ± 0.30	38.58 ± 0.24

Control

Trial 2		B2/B21 n = 4		B2/B15 n = 4	
Week	Temp	Vent	Under wing	Vent	Under wing
1	31	38.08 ± 0.39	39.98 ± 0.20	37.98 ± 0.09	40.20 ± 0.59
2	30	38.08 ± 0.17	39.28 ± 0.23	37.85 ± 0.22	39.10 ± 0.21
3	30	38.20 ± 0.60	39.48 ± 0.33	38.48 ± 0.35	39.65 ± 0.62
4	30	38.45 ± 0.33	39.58 ± 0.27	37.40 ± 0.09	39.50 ± 1.05
5	29	37.55 ± 0.43	39.40 ± 0.47	38.13 ± 0.16	38.98 ± 0.20
6	29	38.15 ± 0.22	39.53 ± 0.47	37.88 ± 0.09	39.23 ± 0.57
7	29	38.75 ± 0.35	38.85 ± 0.61	36.70 ± 0.95	38.50 ± 0.32
8	30	39.03 ± 0.65	39.60 ± 0.45	38.13 ± 0.40	39.03 ± 0.41
9	26	36.43 ± 0.61	38.63 ± 0.14	37.08 ± 0.27	38.63 ± 0.50
10	28	37.43 ± 0.51	38.63 ± 0.23	36.93 ± 0.44	38.50 ± 0.72
11	25	36.53 ± 0.25	38.13 ± 0.64	36.10 ± 0.61	38.60 ± 0.56

Control

Trial 3		B2/B21 n = 4		B2/B15 n = 2	
Week	Temp	Vent	Under wing	Vent	Under wing
1	16	31.95 ± 1.35	36.40 ± 1.90	34.70 ± 0.59	35.70 ± 1.16
2	25	37.70 ± 0.10	38.40 ± 1.40	37.75 ± 0.38	38.58 ± 0.48
3	20	32.70 ± 0.30	36.80 ± 0.00	34.45 ± 1.07	36.53 ± 0.58
4	26	38.75 ± 0.35	40.15 ± 0.25	38.00 ± 0.55	39.38 ± 0.42
5	15	29.55 ± 4.85	35.30 ± 1.50	32.03 ± 0.91	35.33 ± 0.82
6	24.5	36.70 ± 0.30	40.10 ± 0.20	36.25 ± 0.48	39.23 ± 0.54
7	24	35.60 ± 0.30	36.55 ± 0.75	35.95 ± 0.85	39.03 ± 0.66
8	26	37.40 ± 0.60	39.00 ± 0.20	37.63 ± 1.01	38.90 ± 0.66
9	18	32.05 ± 1.75	32.75 ± 0.85	32.93 ± 1.63	33.05 ± 1.43
10	25	37.50 ± 1.20	38.15 ± 0.45	37.13 ± 0.48	38.45 ± 0.33
11	18	33.10 ± 2.00	35.30 ± 0.50	33.85 ± 0.26	35.88 ± 0.47

		B2/B19 n = 2	
Week	Temp	Vent	Under wing
1	16	33.95 ± 0.05	36.40 ± 0.50
2	25	37.10 ± 0.70	38.90 ± 0.50
3	20	34.90 ± 1.70	35.10 ± 1.90
4	26	37.50 ± 0.40	38.35 ± 0.15
5	15	31.80 ± 0.30	35.55 ± 0.15
6	24.5	36.85 ± 1.25	39.40 ± 0.50
7	24	35.15 ± 1.05	38.40 ± 0.05
8	26	37.65 ± 0.25	39.00 ± 0.60
9	18	31.85 ± 0.05	34.10 ± 0.70
10	25	37.30 ± 0.20	38.70 ± 0.30
11	18	33.75 ± 0.85	36.00 ± 0.10

Northern Fowl Mite Infested

Trial 1			B2/B21 n = 4			
Week	Temp	NFM score	Vent	Under wing	Area Inflammation	Color
1	21	0.0 ± 0.0	34.90 ± 0.24	37.23 ± 0.24	--	--
2	27	3.0 ± 0.7	37.10 ± 0.17	38.65 ± 0.48	--	--
3	20	5.8 ± 0.3	33.43 ± 1.06	37.43 ± 0.19	--	75%
4	23	6.0 ± 0.0	35.98 ± 0.74	37.65 ± 0.34	--	0%
5	27	6.3 ± 0.3	33.23 ± 0.51	37.80 ± 0.61	--	100%
6	25	4.8 ± 0.5	33.50 ± 0.86	37.08 ± 1.00	1663.26 ± 535.51	50%
7	15.5	5.5 ± 0.3	31.75 ± 0.76	36.85 ± 0.57	1211.36 ± 315.63	75%
8	28	5.0 ± 0.4	37.78 ± 0.37	39.13 ± 0.39	1363.65 ± 291.02	50%
9	18	4.5 ± 0.5	34.18 ± 1.52	38.20 ± 0.39	1482.69 ± 285.33	50%

10	24	3.3 ± 0.5	36.20 ± 1.07	38.55 ± 0.29	1494.69 ± 204.92	25%
11	23	3.0 ± 0.4	36.03 ± 0.32	38.00 ± 0.35	855.44 ± 304.65	50%

B2/B15 n = 4

Week	Temp	NFM score	Vent	Under wing	Area Inflammation	Color
1	21	0.0 ± 0.0	35.15 ± 0.5	37.48 ±0.40	--	--
2	27	2.8 ± 0.5	37.93 ± 0.53	38.20 ± 0.54	--	--
3	20	5.8 ± 0.3	32.85 ± 0.40	37.23 ± 0.35	--	--
4	23	6.3 ± 0.3	36.13 ± 0.31	38.03 ± 0.34	--	--
5	27	6.5 ± 0.3	33.13 ± 0.76	38.40 ± 0.40	--	50%
6	25	5.5 ± 0.5	33.98 ± 0.69	37.13 ± 0.33	1959.66 ± 308.10	25%
7	15.5	5.3 ± 0.8	32.48 ± 1.05	37.38 ± 0.72	1624.66 ± 395.23	25%
8	28	5.0 ± 0.7	37.23 ± 0.31	39.03 ± 0.28	1909.63 ± 203.83	100%
9	18	5.3 ± 0.8	33.95 ± 0.82	37.68 ± 0.63	2249.38 ± 274.34	75%
10	24	4.3 ± 0.9	36.48 ± 0.55	39.10 ± 0.26	2406.94 ± 361.01	50%
11	23	2.5 ± 0.5	36.68 ± 0.73	38.43 ± 0.39	2242.06 ± 252.67	25%

Northern Fowl Mite Infested

Trial 2

B2/B21 n = 4

Week	Temp	NFM score	Vent	Under wing	Area Inflammation	Color
1	31	0.0 ± 0.0	38.53 ± 0.30	39.80 ± 0.51	--	--
2	30	2.3 ± 0.5	36.98 ± 0.56	38.55 ± 0.25	--	--
3	30	3.0 ± 0.8	37.70 ± 0.17	39.20 ± 0.49	--	25%
4	30	3.8 ± 0.9	37.23 ± 0.55	38.83 ± 0.48	--	25%
5	29	2.3 ± 0.6	36.85 ± 0.33	38.40 ± 0.19	--	25%
6	29	3.0 ± 0.7	37.68 ± 0.65	38.55 ± 0.21	--	25%
7	29	2.8 ± 0.6	37.33 ± 0.55	38.88 ± 0.29	one infl	50%
8	30	3.3 ± 0.8	37.85 ± 0.13	38.70 ± 0.21	852.51 ± 261.32	75%
9	26	3.3 ± 0.5	36.70 ± 0.23	38.60 ± 0.12	876.75 ± 244.99	75%
10	28	3.8 ± 0.6	37.18 ± 0.22	38.03 ± 0.65	948.33 ± 87.48	75%
11	25	4.0 ± 0.0	35.83 ± 0.82	38.45 ± 0.10	872.00 ± 236.37	75%

B2/B15 n = 4

Week	Temp	NFM score	Vent	Under wing	Area Inflammation	Color
1	31	0.0 ± 0.0	37.38 ± 0.39	39.10 ± 0.43	--	--
2	30	2.0 ± 0.0	37.23 ± 0.66	38.45 ± 0.16	--	--
3	30	2.8 ± 0.5	37.90 ± 0.34	38.88 ± 0.40	--	--
4	30	3.8 ± 0.6	37.50 ± 0.16	38.98 ± 0.18	--	25%
5	29	4.0 ± 0.8	36.60 ± 0.11	38.13 ± 0.27	--	50%
6	29	3.3 ± 0.5	36.53 ± 0.38	38.78 ± 0.40	--	50%
7	29	3.8 ± 0.5	37.18 ± 0.60	37.58 ± 0.24	1486.45 ± 294.44	75%

8	30	4.3 ± 0.3	37.70 ± 0.29	38.75 ± 0.19	1294.46 ± 262.79	75%
9	26	4.5 ± 0.3	37.00 ± 0.33	38.20 ± 0.11	1063.49 ± 186.89	100%
10	28	5.0 ± 0.4	36.53 ± 0.17	38.38 ± 0.28	1280.13 ± 244.46	100%
11	25	5.3 ± 0.5	35.60 ± 0.60	37.48 ± 0.49	1364.13 ± 148.62	100%

Northern Fowl Mite Infested

Trial 3				B2/B21 n = 4		
Week	Temp	NFM score	Vent	Under wing	Area Inflammation	Color
1	16	0.0 ± 0.0	31.58 ± 0.88	35.95 ± 0.55	--	--
2	25	4.0 ± 0.0	35.58 ± 0.55	38.70 ± 0.43	--	--
3	20	6.0 ± 0.0	35.05 ± 0.43	35.90 ± 0.62	--	75%
4	26	6.0 ± 0.0	36.55 ± 0.49	38.45 ±0.41	1260.56 ± 218.44	25%
5	15	6.3 ± 0.3	30.23 ± 1.88	35.38 ± 1.21	1550.81 ± 475.63	50%
6	24.5	4.8 ± 0.5	36.60 ± 0.46	38.55 ± 0.52	1602.94 ± 323.78	0%
7	24	5.5 ± 0.3	34.90 ± 1.01	38.75 ± 0.40	2080.13 ± 187.29	50%
8	26	5.0 ± 0.6	37.08 ± 0.58	39.10 ± 0.48	1453.42 ± 263.15	50%
9	18	5.5 ± 0.5	31.33 ± 0.62	34.85 ± 0.52	2036.88 ± 60.02	100%
10	25	5.3 ± 0.5	35.95 ± 0.53	37.75 ± 0.34	2102.83 ± 306.92	50%
11	18	4.0 ± 0.8	34.60 ± 0.41	36.08 ± 0.72	2046.25 ± 368.58	50%
				B2/B15 n = 4		
Week	Temp	NFM score	Vent	Under wing	Area Inflammation	Color
1	16	1.0 ± 1.0	31.80 ± 1.66	35.00 ± 0.73	--	--
2	25	4.0 ± 0.8	35.68 ± 1.07	38.45 ± 0.41	--	25%
3	20	5.5 ± 0.5	31.68 ± 1.30	36.00 ± 0.70	--	25%
4	26	6.0 ± 0.0	34.75 ± 1.34	38.70 ± 0.24	1878.00 ± 524.77	75%
5	15	5.8 ± 0.3	31.75 ± 1.70	34.20 ± 0.96	1352.81 ± 457.49	25%
6	24.5	4.3 ± 0.3	35.68 ± 0.98	36.80 ± 0.97	1703.51 ± 448.96	25%
7	24	5.3 ± 0.5	35.50 ± 0.70	37.53 ± 0.81	1464.69 ± 290.19	75%
8	26	5.5 ± 0.5	36.40 ± 0.40	38.35 ± 0.41	1246.00 ± 265.03	25%
9	18	5.5 ± 0.5	31.35 ± 1.23	33.78 ± 0.78	1910.63 ± 483.69	100%
10	25	5.5 ± 0.3	35.79 ± 0.45	36.88 ± 0.38	1570.92 ± 166.30	50%
11	18	5.3 ± 0.5	33.75 ± 0.86	36.40 ± 0.62	1345.25 ± 314.69	75%

Table 1.2. Weekly means (± SE) of production factors categorized by trial, mite presence (control or infested), and hen MHC haplotype (usually B2/B21 or B2/B15). 'Total weight' refers to total weight of feed consumed (g). Feed Conversion is feed consumed/egg weight produced (g).

Trial 1 Control Birds

B2/B21

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	6.00 ± 0.00	354.56 ± 3.90	604.00 ± 26.72	1.94 ± 0.09
2	7.00 ± 0.00	350.08 ± 10.47	616.25 ± 31.08	1.74 ± 0.08
3	6.75 ± 0.25	366.85 ± 14.02	781.75 ± 38.81	1.94 ± 0.09
4	5.75 ± 0.25	365.73 ± 16.55	592.25 ± 31.61	1.89 ± 0.07
5	6.75 ± 0.25	355.00 ± 32.62	670.25 ± 33.71	1.84 ± 0.12
6	6.50 ± 0.50	308.58 ± 26.30	569.00 ± 56.60	1.60 ± 0.05
7	5.75 ± 0.63	368.20 ± 17.74	607.75 ± 48.55	2.05 ± 0.32
8	6.50 ± 0.29	386.23 ± 9.16	660.00 ± 21.97	1.81 ± 0.12
9	6.50 ± 0.29	371.88 ± 15.01	685.50 ± 27.73	2.12 ± 0.20
10	6.75 ± 0.25	379.48 ± 10.03	622.00 ± 28.59	1.67 ± 0.05
11	7.00 ± 0.00	305.13 ± 11.13	630.50 ± 19.47	1.66 ± 0.05

B2/B15

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	5.75 ± 0.25	359.53 ± 8.97	601.75 ± 25.29	1.92 ± 0.14
2	6.75 ± 0.25	339.95 ± 19.81	613.50 ± 34.85	1.70 ± 0.06
3	6.25 ± 0.25	405.05 ± 7.72	773.00 ± 7.85	1.98 ± 0.11
4	6.5 ± 0.29	376.95 ± 8.10	643.50 ± 20.10	1.83 ± 0.04
5	7.25 ± 0.25	377.20 ± 12.26	739.25 ± 14.41	1.96 ± 0.06
6	6.75 ± 0.25	330.20 ± 38.89	670.25 ± 28.92	1.79 ± 0.12
7	5.75 ± 0.63	414.18 ± 11.63	639.00 ± 35.68	2.00 ± 0.20
8	7.00 ± 0.00	412.65 ± 12.51	718.75 ± 20.63	1.74 ± 0.07
9	6.75 ± 0.25	389.55 ± 11.84	714.00 ± 22.50	1.90 ± 0.17
10	6.50 ± 0.50	383.43 ± 11.75	665.75 ± 23.04	1.94 ± 0.27
11	6.75 ± 0.25	371.78 ± 17.50	650.75 ± 19.84	1.70 ± 0.07

Trial 1 Treatment Birds

B2/B21

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	6.00 ± 0.00	366.78 ± 15.42	566.00 ± 45.13	1.7 ± 0.16
2	6.75 ± 0.25	333.15 ± 18.24	621.50 ± 30.86	1.69 ± 0.03
3	6.00 ± 0.41	383.48 ± 23.56	847.25 ± 15.82	2.29 ± 0.19
4	6.00 ± 0.00	362.78 ± 21.00	637.25 ± 9.86	1.88 ± 0.04

5	6.50 ± 0.50	353.83 ± 27.69	739.25 ± 5.31	2.06 ± 0.19
6	6.25 ± 0.48	339.65 ± 18.64	632.25 ± 42.79	1.8 ± 0.01
7	6.00 ± 0.41	376.08 ± 18.13	721.25 ± 28.85	2.14 ± 0.15
8	6.25 ± 0.48	376.90 ± 16.01	751.75 ± 4.89	2.01 ± 0.10
9	6.00 ± 0.41	377.58 ± 22.28	730.00 ± 12.16	2.33 ± 0.23
10	6.50 ± 0.50	346.20 ± 35.44	688.75 ± 11.66	1.85 ± 0.13
11	6.00 ± 0.58	307.43 ± 14.50	679.25 ± 16.44	1.96 ± 0.16
		B2/B15		
Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	6.00 ± 0.00	319.48 ± 24.11	582.5 ± 40.18	1.76 ± 0.12
2	6.00 ± 0.41	350.80 ± 28.19	632.75 ± 20.67	2.01 ± 0.17
3	6.50 ± 0.50	362.30 ± 23.13	830.00 ± 39.53	2.16 ± 0.24
4	5.75 ± 0.25	386.60 ± 16.78	638.50 ± 16.12	2.01 ± 0.11
5	6.75 ± 0.25	403.65 ± 6.98	715.50 ± 19.53	1.86 ± 0.09
6	7.00 ± 0.00	373.35 ± 11.47	641.25 ± 40.09	1.59 ± 0.09
7	6.75 ± 0.25	367.80 ± 23.14	682.50 ± 29.19	1.83 ± 0.07
8	6.50 ± 0.29	386.08 ± 7.78	759.50 ± 3.62	2.09 ± 0.13
9	7.00 ± 0.00	384.58 ± 18.93	786.50 ± 22.13	2.02 ± 0.05
10	6.75 ± 0.25	398.50 ± 11.44	697.00 ± 10.54	1.83 ± 0.12
11	7.00 ± 0.00	638.20 ± 16.36	689.00 ± 24.36	1.73 ± 0.07
		Trial 2 Control Birds		
		B2/B21		
Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	5.50 ± 0.65	304.45 ± 39.26	414.25 ± 40.35	1.38 ± 0.07
2	5.50 ± 0.29	306.08 ± 14.75	523.75 ± 49.07	1.70 ± 0.09
3	5.25 ± 0.75	291.28 ± 38.82	526.00 ± 59.07	1.84 ± 0.17
4	4.00 ± 0.41	232.18 ± 38.21	546.50 ± 65.90	2.44 ± 0.25
5	5.50 ± 0.50	315.48 ± 36.29	576.25 ± 47.65	1.90 ± 0.24
6	6.00 ± 0.71	342.25 ± 29.71	516.25 ± 21.68	1.56 ± 0.19
7	5.25 ± 0.48	303.90 ± 31.40	591.50 ± 40.40	2.01 ± 0.24
8	7.00 ± 0.58	391.25 ± 24.83	631.75 ± 29.85	1.63 ± 0.10
9	5.50 ± 0.50	313.55 ± 33.81	641.25 ± 18.69	2.09 ± 0.15
10	6.50 ± 0.29	389.05 ± 27.87	629.00 ± 27.18	1.63 ± 0.09
11	5.75 ± 0.25	328.63 ± 17.48	563.75 ± 24.13	1.72 ± 0.08
		B2/B15		
Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	4.50 ± 1.5	244.13 ± 82.17	399.75 ± 39.75	1.35 ± 0.08
2	4.25 ± 0.63	240.03 ± 35.33	569.00 ± 28.58	2.50 ± 0.33

3	6.25 ± 0.25	348.18 ± 9.05	603.00 ± 14.99	1.74 ± 0.06
4	5.50 ± 0.50	313.93 ± 23.17	682.00 ± 38.61	2.20 ± 0.19
5	3.75 ± 1.32	305.65 ± 25.45	669.00 ± 51.02	2.22 ± 0.23
6	7.00 ± 0.00	407.55 ± 9.18	534.75 ± 21.29	1.31 ± 0.04
7	5.00 ± 0.71	296.95 ± 43.63	626.00 ± 64.73	2.36 ± 0.63
8	6.25 ± 0.25	373.85 ± 20.27	695.50 ± 37.44	1.86 ± 0.05
9	6.00 ± 0.00	356.80 ± 7.83	699.25 ± 34.44	1.96 ± 0.09
10	6.00 ± 0.00	362.35 ± 6.87	690.50 ± 32.59	1.90 ± 0.08
11	5.75 ± 0.48	339.70 ± 24.58	620.25 ± 15.70	1.86 ± 0.18

Trial 2 Treatment Birds

B2/B21

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	4.50 ± 1.19	273.25 ± 70.19	463.25 ± 59.53	2.48 ± 1.05
2	5.25 ± 1.11	307.15 ± 63.88	587.25 ± 48.18	2.18 ± 0.45
3	4.50 ± 0.65	264.13 ± 31.70	548.00 ± 28.63	2.12 ± 0.13
4	4.50 ± 0.50	279.60 ± 28.51	615.25 ± 45.33	2.23 ± 0.10
5	3.75 ± 0.95	232.98 ± 58.04	624.75 ± 53.24	3.47 ± 1.08
6	6.00 ± 1.36	368.43 ± 77.71	550.00 ± 63.26	1.73 ± 0.39
7	4.00 ± 0.71	245.98 ± 40.93	629.50 ± 47.76	2.74 ± 0.36
8	5.00 ± 1.69	315.15 ± 107.96	625.50 ± 86.64	1.70 ± 0.08
9	5.25 ± 1.18	324.98 ± 71.76	664.75 ± 68.91	2.35 ± 0.47
10	5.00 ± 1.35	310.03 ± 84.75	639.50 ± 58.95	3.19 ± 1.41
11	4.75 ± 1.65	286.20 ± 97.94	587.50 ± 70.85	1.74 ± 0.26

B2/B15

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	4.25 ± 0.63	257.35 ± 40.72	372.00 ± 57.96	1.45 ± 0.15
2	4.25 ± 0.75	251.08 ± 47.95	548.25 ± 38.84	2.65 ± 0.83
3	5.50 ± 0.29	329.23 ± 20.05	566.25 ± 45.13	1.72 ± 0.06
4	4.50 ± 0.29	281.18 ± 22.42	665.00 ± 25.47	2.39 ± 0.11
5	5.25 ± 0.25	334.38 ± 17.28	695.25 ± 32.79	2.10 ± 0.17
6	6.00 ± 0.00	358.75 ± 15.62	556.75 ± 10.39	1.56 ± 0.06
7	5.00 ± 0.00	312.03 ± 5.90	677.50 ± 25.01	2.17 ± 0.05
8	6.00 ± 0.41	380.68 ± 25.48	725.00 ± 16.75	1.92 ± 0.09
9	4.75 ± 0.75	298.90 ± 50.32	728.25 ± 18.08	2.66 ± 0.44
10	6.00 ± 0.00	388.48 ± 3.63	733.25 ± 10.51	1.89 ± 0.03
11	6.25 ± 0.48	396.83 ± 32.62	645.75 ± 32.88	1.64 ± 0.09

Trial 3 Control Birds

B2/B21

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	6.75 ± 0.25	383.58 ± 16.21	623.75 ± 11.48	1.63 ± 0.06
2	6.00 ± 0.41	322.15 ± 25.79	636.25 ± 40.13	2.01 ± 0.19
3	6.75 ± 0.25	385.13 ± 15.88	743.25 ± 13.60	1.94 ± 0.12
4	6.75 ± 0.25	404.83 ± 14.68	743.25 ± 14.13	1.84 ± 0.09
5	7.00 ± 0.00	420.05 ± 7.72	722.00 ± 11.05	1.72 ± 0.06
6	6.75 ± 0.25	393.15 ± 16.36	613.75 ± 21.57	1.56 ± 0.03
7	7.00 ± 0.00	396.35 ± 3.48	705.50 ± 27.11	1.78 ± 0.08
8	6.50 ± 0.29	382.00 ± 21.04	721.75 ± 22.06	1.91 ± 0.19
9	7.00 ± 0.00	425.25 ± 6.70	717.00 ± 9.70	1.69 ± 0.04
10	7.00 ± 0.00	423.15 ± 8.01	684.50 ± 7.77	1.62 ± 0.04
11	7.00 ± 0.00	415.20 ± 7.81	619.00 ± 8.76	1.49 ± 0.04

B2/B15

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	6.50 ± 0.50	354.90 ± 32.30	487.00 ± 115.00	1.35 ± 0.20
2	6.50 ± 0.50	373.65 ± 21.55	673.50 ± 30.50	1.81 ± 0.18
3	7.00 ± 0.00	413.95 ± 1.25	734.50 ± 32.50	1.77 ± 0.08
4	7.00 ± 0.00	427.40 ± 1.20	756.00 ± 7.00	1.77 ± 0.02
5	7.00 ± 0.00	424.10 ± 9.80	759.50 ± 2.50	1.79 ± 0.05
6	7.00 ± 0.00	415.20 ± 0.20	656.50 ± 1.50	1.58 ± 0.0
7	7.00 ± 0.00	409.95 ± 6.15	747.50 ± 20.50	1.82 ± 0.08
8	6.50 ± 0.50	402.80 ± 27.50	768.00 ± 8.00	1.92 ± 0.15
9	7.00 ± 0.00	435.00 ± 8.00	762.50 ± 16.50	1.75 ± 0.01
10	7.00 ± 0.00	432.95 ± 5.35	731.50 ± 20.50	1.69 ± 0.03
11	6.50 ± 0.50	384.65 ± 28.05	643.50 ± 18.50	1.68 ± 0.08

B2/B19

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	5.5 ± 0.50	311.35 ± 28.45	614.00 ± 29.00	2.0 ± 0.28
2	7.00 ± 0.00	392.95 ± 8.45	627.50 ± 135.50	1.59 ± 0.31
3	6.50 ± 0.50	376.05 ± 41.55	717.00 ± 50.00	1.92 ± 0.08
4	7.00 ± 0.00	407.15 ± 11.75	757.00 ± 19.00	1.86 ± 0.01
5	7.00 ± 0.00	424.75 ± 0.65	747.00 ± 32.00	1.76 ± 0.07
6	7.00 ± 0.00	419.70 ± 4.40	702.50 ± 17.50	1.67 ± 0.02
7	7.00 ± 0.00	404.45 ± 3.55	680.50 ± 92.50	1.68 ± 0.24
8	6.50 ± 0.50	373.20 ± 23.30	733.00 ± 45.00	1.96 ± 0.0
9	7.00 ± 0.00	409.10 ± 17.80	741.50 ± 30.50	1.82 ± 0.16

10	7.00 ± 0.00	421.65 ± 4.75	728.00 ± 38.00	1.73 ± 0.11
11	7.00 ± 0.00	420.75 ± 8.35	685.00 ± 5.00	1.63 ± 0.02

Trial 3 Treatment Birds

B2/B21

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	6.50 ± 0.29	357.33 ± 12.93	556.75 ± 29.60	1.56 ± 0.10
2	6.00 ± 0.41	337.50 ± 21.76	656.50 ± 30.96	1.97 ± 0.18
3	6.75 ± 0.25	381.05 ± 10.17	681.00 ± 8.66	1.79 ± 0.07
4	7.00 ± 0.41	528.53 ± 126.80	682.25 ± 27.04	1.47 ± 0.25
5	6.75 ± 0.25	384.63 ± 14.95	689.25 ± 26.84	1.80 ± 0.06
6	6.00 ± 0.00	343.05 ± 5.26	613.50 ± 23.99	1.79 ± 0.07
7	5.00 ± 1.35	284.13 ± 76.90	679.50 ± 26.96	4.48 ± 2.59
8	6.25 ± 0.25	353.03 ± 17.63	713.50 ± 17.56	2.04 ± 0.13
9	7.00 ± 0.00	398.95 ± 2.85	708.00 ± 21.25	1.77 ± 0.05
10	7.25 ± 0.25	412.60 ± 14.88	712.75 ± 18.51	1.74 ± 0.10
11	6.75 ± 0.25	385.80 ± 15.57	664.75 ± 8.54	1.73 ± 0.07

B2/B15

Week	# Eggs/week	Total egg weight	Total weight	Feed Conversion
1	6.50 ± 0.29	372.05 ± 24.36	598.00 ± 7.91	1.63 ± 0.12
2	6.50 ± 0.65	378.95 ± 38.79	624.00 ± 71.75	1.64 ± 0.11
3	6.50 ± 0.50	363.90 ± 46.08	642.00 ± 38.18	1.84 ± 0.20
4	5.75 ± 0.95	342.38 ± 59.63	622.25 ± 96.41	1.85 ± 0.06
5	5.25 ± 1.75	313.70 ± 104.79	638.00 ± 69.76	1.68 ± 0.03
6	5.00 ± 1.68	289.15 ± 98.24	565.75 ± 71.55	1.64 ± 0.06
7	4.75 ± 1.60	284.23 ± 95.64	662.50 ± 51.21	1.88 ± 0.03
8	5.25 ± 1.11	315.73 ± 72.17	716.00 ± 31.71	2.95 ± 1.01
9	7.00 ± 0.00	417.48 ± 11.85	686.75 ± 57.73	1.64 ± 0.13
10	6.00 ± 1.00	362.75 ± 62.74	614.00 ± 118.67	1.66 ± 0.07
11	4.75 ± 1.60	287.05 ± 96.85	578.75 ± 68.94	1.7 ± 0.11



Figure 1.1. Calipers were used to measure red, visually inflamed areas within the vent regions of treatment (NFM infested) birds.

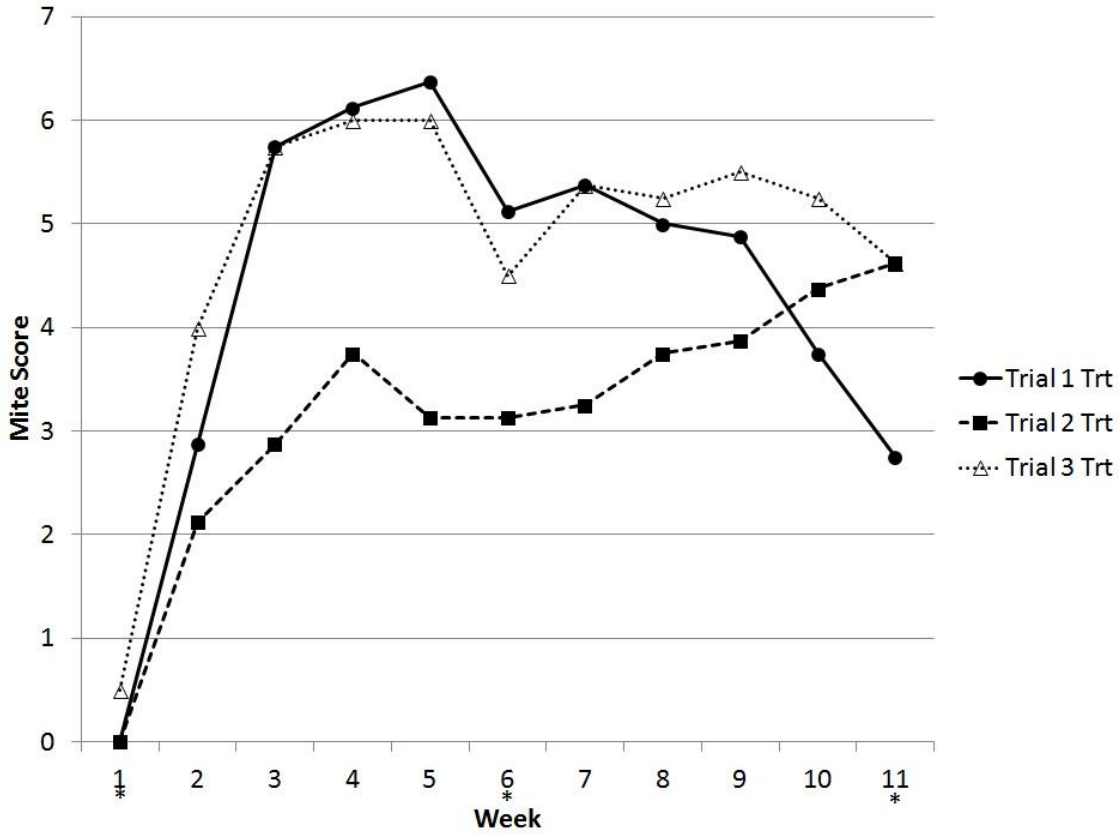


Figure 1.2. Mite population (mean) trends for each of the three trials. Mites were introduced to birds at week 2 and again at week 3. Asterisks show when metabolic was recorded. Trials 1 and 3 were conducted during winter (Feb – May and Jan – March, respectively) and exhibited typical mite population growth patterns. Trial 2 was conducted during hot summer months (Aug – Oct) and mite populations grew slowly but steadily.

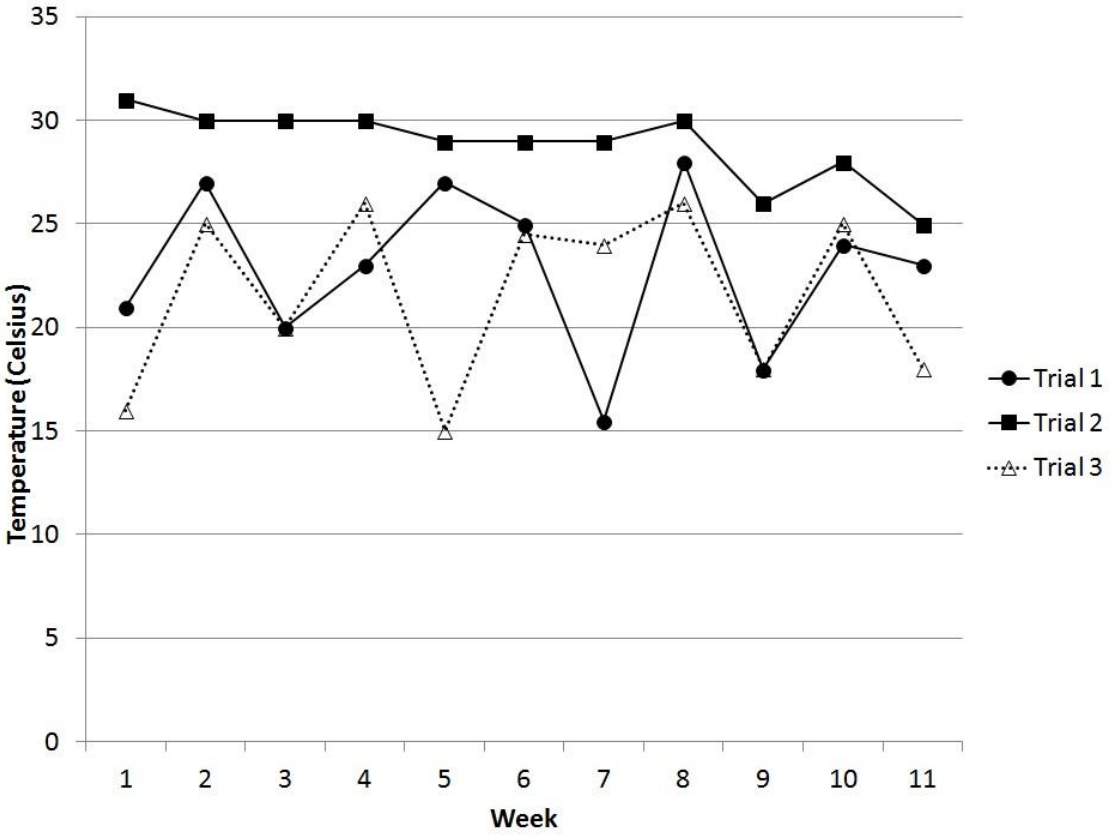


Figure 1.3. Air temperatures on the day and time of mite scoring. Throughout the duration of the study, trial 2 daily air temperatures were consistently higher than trials 1 and 3.

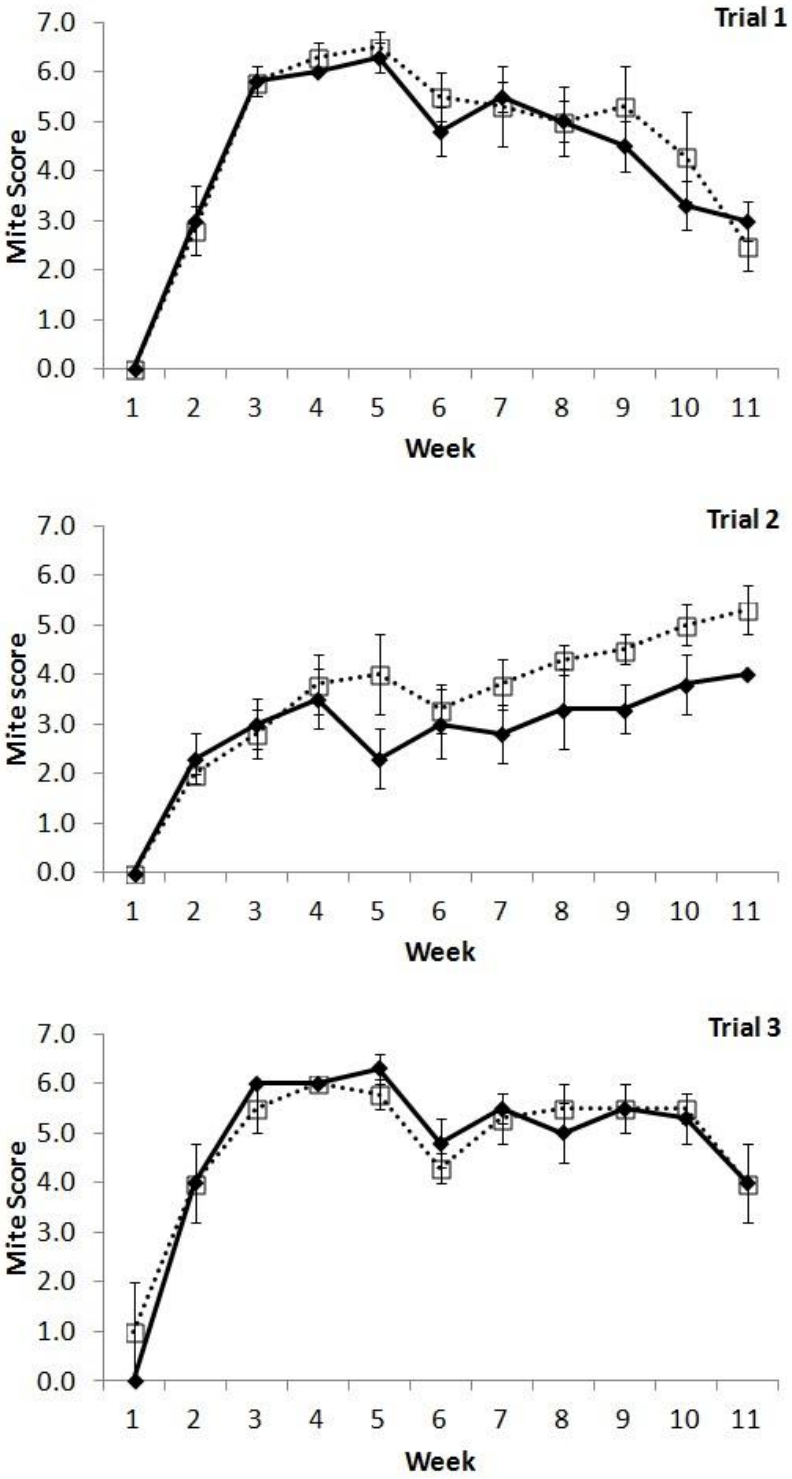


Figure 1.4. Mite populations (mean ± SE) levels for treatment birds separated by haplotype (solid = B21, dashed = B15). There were no significant differences between resistant (B21) and susceptible (B15) birds in trials 1, 2, or 3.

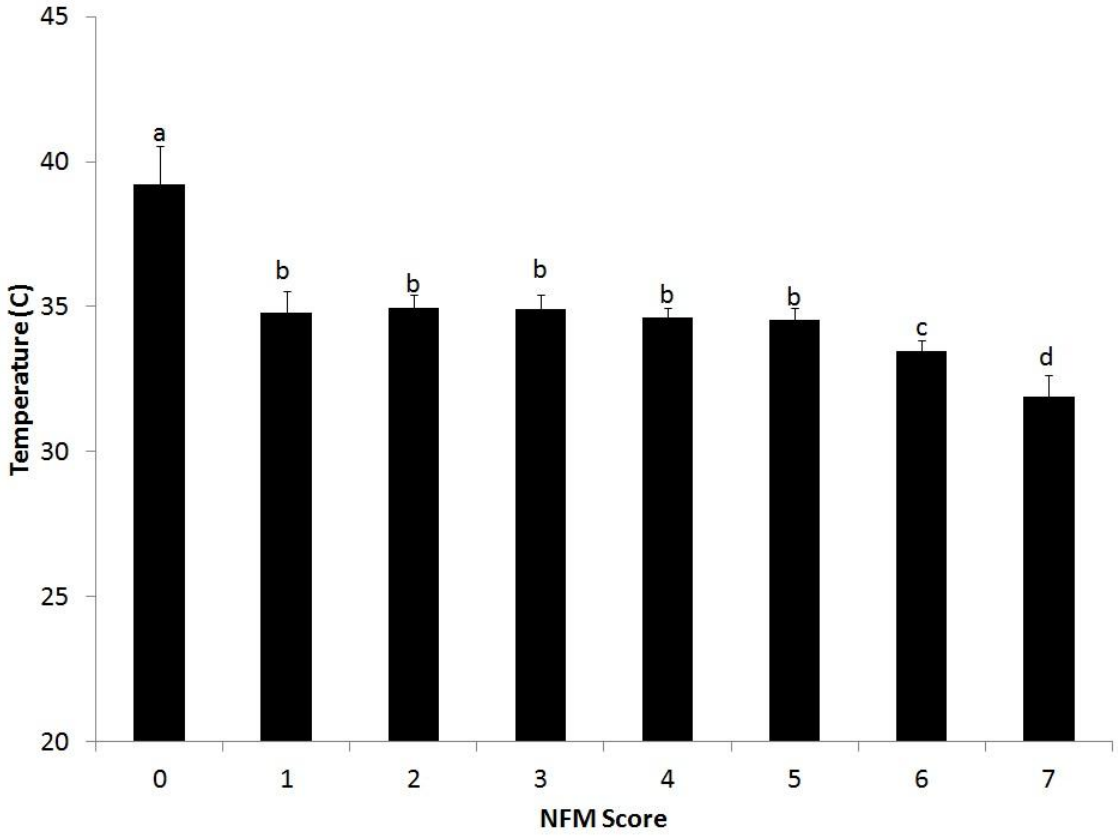


Figure 1.5. Vent skin temperature estimates were segregated by mite score with Tukey's test. Bars with different letters are significantly different ($P < 0.05$). Lower vent skin temperatures were recorded on birds with higher mite scores.

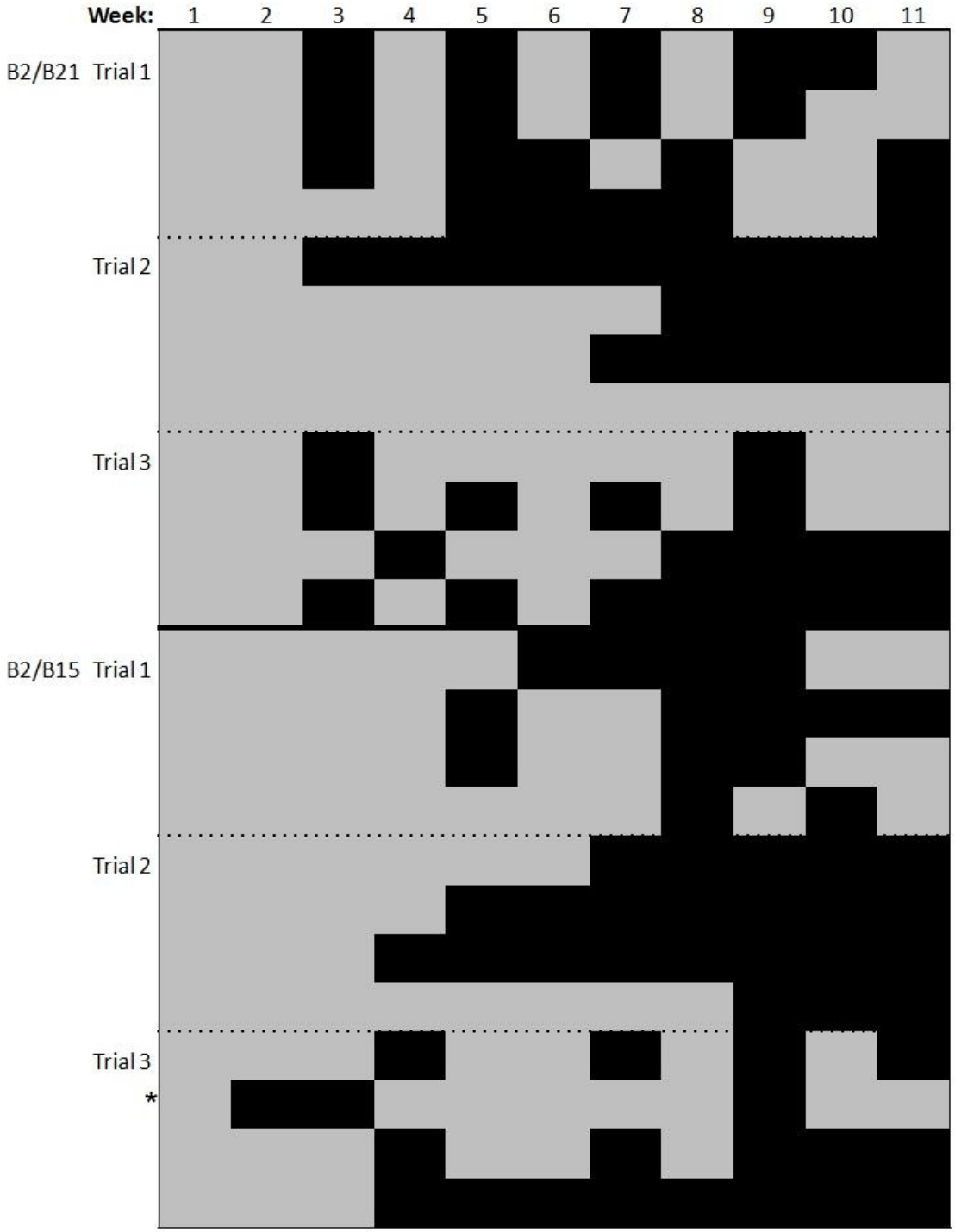


Figure 1.6. Heat map showing skin inflammation scores for either the resistant (B21) or susceptible (B15) birds for each trial. Gray indicates a bird vent that was not scored as inflamed whereas black indicates inflammation. The row marked with a star (*) indicates a bird with mites detected at week 1.

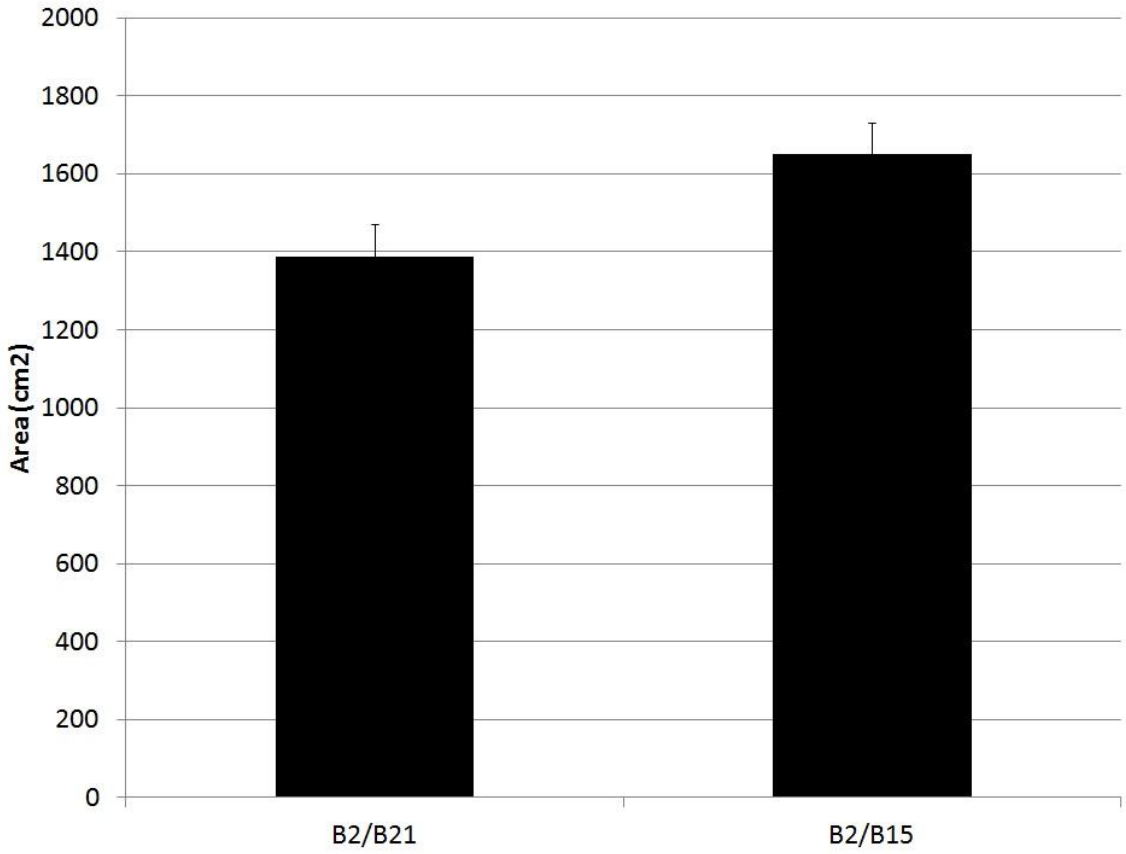


Figure 1.7. Total average skin inflammation (cm^2) in the vent region of birds (mean ± SE). Resistant (B21) birds had a trend toward less vent inflammation than susceptible birds (B15) ($P = 0.053$). All trials were pooled.

CHAPTER 2

Timing Diatomaceous Earth-filled Dustbox Use for Management of Northern Fowl Mites (Acari: Macronyssidae) in Cage-Free Poultry Systems

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ABSTRACT

Northern fowl mite (NFM) management on conventionally caged birds relies on synthetic pesticide sprays to wet the vent region. Cage-free chickens cannot be effectively treated this way, and pesticide use is restricted in organic production. Dustbathing behavior is encouraged in newer production systems for increased hen welfare. Diatomaceous earth (DE) is an approved organic insecticide that can be mixed with sand in dustboxes, suppressing mites but not excluding them, and potentially allowing development of mite immunity. We tested two hypotheses: 1) that DE-filled dustboxes placed before NFM introduction (prophylactic use) prevents mite populations from reaching economically damaging thresholds, and 2) that bird exposure to low mite numbers allows for protective hen immunity to develop and suppress mites after dustboxes are removed. Average mite densities in flocks remained below damaging levels while dustboxes were available. Average mite populations rebounded after dustbox removal (even though DE persisted in the environment) regardless of the timing of removal. There was not a powerful or immediate mite immune response in these ISA brown hens, but other hen strains might do better. Mite densities on birds where a traditional hot blade beak trimming technique was used (trial 1) were high. Mite densities in trial 2, where a newer precision infra-red trimming was used, were lower. The newer infra-red trimming method resulted in nearly intact beaks, which were better for mite control by bird grooming behaviors. The combination of early dustbox use and infra-red beak trimming should allow producers to avoid most mite damage on cage-free flocks.

INTRODUCTION

Northern fowl mites (NFM) are the most common ectoparasite of poultry in the US and cause significant economic losses in egg-laying hens (Axtell and Arends 1990, Mullens et al. 2009). Typical outbreaks occur in flocks in the first egg production cycle, and mites are introduced by infested pullets, contaminated personnel or equipment, wild birds, or carry-over mites from a previous flock (Kells and Surgeoner 1997, Axtell 1999, Chen and Mullens 2008). Once mites are established on a property they can be very difficult to eliminate and may become a predictable problem in every subsequent new flock.

The economic impact of mites can be serious for producers. Most of this damage, such as reduced feed conversion efficiency, is likely linked to physiological costs related to host immune responses. Numerous studies have demonstrated hen-immune reactions in the form of mite-specific antibodies (DeVaney and Ziprin 1980, Burg et al. 1988, Minnifield et al. 1993) and vent skin inflammation (Owen et al. 2009). Mite-infested white leghorn hens experience large immunity-related reductions in mite numbers after being infested for periods of 4-10 weeks (Owen et al. 2008, Mullens et al. 2009). However, data on economic impact in commercial egg layer flocks (Mullens et al. 2009) suggest that mite densities below a visual score of 3 (ca. 100 mites/bird) do not cause measurable economic damage. Keeping mite densities below this threshold should mitigate or eliminate economic damage caused by NFM on egg-layers, while still allowing enough low-level mite exposure for birds to develop an immune response.

Many birds including chickens perform dustbathing behavior, which has value in maintaining feather integrity by removing excess lipids (Olsson and Keeling 2005). Birds prefer to dustbathe in finer materials such as sand, as opposed to coarser substrates such as wood chips or astroturf (Olsson and Keeling 2005, Scholtz et al. 2010, Vezzoli et al. 2015). Martin and Mullens (2012) showed that birds dustbathing in a sand-diatomaceous earth (DE) mixture reduced mite numbers from an average score of 6 (ca. 5,000 mites) to an average score of 3 (ca. 100 mites) within one week. Dustboxes with substrate and DE have the potential to be used to prevent mite buildups early in the life of a flock and keep mites below economically damaging levels while still allowing naïve hens the opportunity to acquire mite immunity. Dutboxes are advantageous because they can be readily adopted in cage-free housing systems, they can use inexpensive materials, and beyond the initial set up they are not labor-intensive to maintain. In addition, DE is listed for unrestricted use by the Organic Materials Research Institute (OMRI) so this control method could be implemented in conventional or organic cage-free production.

In this study, we hypothesized 1) that DE-filled dustboxes placed before NFM introduction (prophylactic use) would prevent mite populations from reaching economically damaging thresholds, and 2) that exposure to low mite numbers would allow protective hen immunity to develop and suppress mites after dustboxes were removed. We allowed birds access to diatomaceous earth mixed with sand in dustboxes prior to experimental mite exposure to test if mite population growth would be disrupted. We then removed dustboxes at 8 and 12 weeks to determine if mites would rebound, or if sufficient immunity had developed.

MATERIALS AND METHODS

Chickens. ISA brown female chickens (18-19 weeks old) were housed in cage-free poultry houses at the University of California Riverside Agricultural Operations under UC Riverside Institutional Animal Care and Use Protocol A-20150009. Two structures (3.8 m by 5.8 m) each were divided into two separate sections, each equipped with water dispensers, feed troughs, and nest boxes. These met or exceeded USA standards for cage-free production (United Egg Producers 2010). Each of the four sections, hereafter called a house, held 18 birds (a flock) within an area 1.5 m x 3.1 m. Straw bedding ca. 5-10 cm deep was added to each house at the beginning of the study and was not removed until the study concluded. Additional straw was added halfway through the study. Lights were kept on a 16L: 8D cycle. Hardware cloth screens (6 mm openings) allowed air flow into the houses while excluding wild birds and rodents. Roof sprinklers moderated high temperatures. Each hen was uniquely marked with colored leg bands for individual identification. Control birds were kept individually in battery cages measuring 31 cm x 31 cm in a separate house on the same property. Those hens had no dustbathing opportunities and were used to check for viability of the mites used for infestation.

Mites. Northern fowl mites were aspirated using pipettes from source hens maintained at Agricultural Operations and were used to inoculate experimental birds (Martin and Mullens 2012). Once a week for the duration of the study vent feathers of all treatment and control hens were visually scored (by ACM) for level of mite infestation.

The scoring system used was as follows: 1 = 1–10, 2 = 11–50, 3 = 51–100, 4 = 101–500, 5 = 501–1000, 6 = 1001–10,000 and 7 = >10,000 (Arthur and Axtell 1983).

Dustboxes. Black PVC plastic cement-mixing tubs (Plasgad Plastic Products ACS Ltd Kibbutz, Gadot, Upper Galilee, IL) 60 cm x 90 cm x 9 cm deep were used for dustboxes (Fig. 2.1). Food grade diatomaceous earth (DE; Perma-Guard Inc., Bountiful, UT, USA) was mixed with washed play sand (Premium Play Sand®; Quikrete® Companies, Inc., Atlanta, GA, USA). A 9:1 ratio by weight of sand (16200 g) to dust (1800 g) was mixed together, which yielded a depth of ca. 5 cm of material in each box. Dustboxes were recharged with ca. 900 g DE on a weekly basis and sand was added as needed to keep the materials at a depth of ca. 5 cm.

Dustbox use was estimated for each bird weekly. A scale from 0-3 (adapted from Martin and Mullens 2012) was used to determine bird 'dustiness.' Scores were: 0 = no visible dust; 1 = slight dust on exterior feathers; 2 = dust distinctly apparent on parted/disturbed feathers in vent area; 3 = airborne dust plume obvious when feathers disturbed.

Dustbox Study. At week 1 dustboxes were placed prophylactically into each of the four houses. One week later (week 2) all birds were inoculated with 20-30 adult mites each (Martin and Mullens 2012). Five control birds, held in cages, were inoculated in the same manner. All birds (dustbox treatment and control) were reinfested individually at week 3 with 20-30 additional adult mites each to mimic continued exposure. An additional five control birds were inoculated at this time with 20-30 adult mites. Mite

numbers and dustbathing intensity were monitored weekly for individual birds. Control birds were also scored weekly to monitor mite population growth.

At week 8, after visually scoring each bird, dustboxes were removed from two of the four houses. At this time, five additional control birds (previously uninfested but from the same hen cohort) were inoculated with 20-30 adult mites and placed in cages as described for earlier controls. At week 12 dustboxes were removed from the two remaining houses (after visually scoring birds) and five more control birds were mite-infested. Birds were visually examined weekly for NFM numbers for four additional weeks, for a total length of 16 weeks per trial. Two trials (Jan-Apr 2014 and Dec-Mar 2015) were conducted.

Beak Trimming and Bit Placement. Commercial birds are routinely beak-trimmed as very young chicks to reduce feather pecking, cannibalism, and feed waste (Hester and Moore 2003, Mertens et al. 2009). Birds used in trials 1 and 2 were sourced from the same local producer. However, in the second year they had converted from using a traditional electric hot blade trimmer to using a newer infra-red (IR) trimming technology (Dennis and Cheng 2010). The IR method resulted in substantial beak regrowth (Fig. 2.2) by the time the hens reached adulthood. Many beaks in trial 2 were almost fully intact (able to close cleanly with mandible overlap at the tips) compared to the permanently and uniformly blunt tips resulting from the hot blade beak trimmer in trial 1. This difference in beak trim technique was suspected to be the cause of overall lower mite levels in trial 2.

After trial 2 was complete, 24 mite-infested birds were randomly selected and placed individually into battery cages with no opportunity to dustbathe. After one week, all birds were scored for mite infestation. To implicate the more intact beak condition (or not) in the reduced trial 2 mite numbers, birds were paired by similar mite levels and then were randomly assigned to either a control (no beak bit) or treatment (beak bit) group. Treatment birds were fitted with adult sized "ChickNBits" (Decker Mfg. Co., Keokuk, IA, USA) beak bits. The bits are used commercially to decrease feather pecking by preventing the bird's beak from closing completely (Fig. 2.3). This additionally interferes with ectoparasite grooming by birds (e.g. Clayton and Tompkins 1995). Mite populations were scored on control and beak-bitted birds for four additional weeks.

Statistical Analyses. Statistical analyses were performed using SAS® software (SAS Institute Inc., Cary, NC, 2010, v. 9.3). Proc means were used to generate averages and standard errors of mite scores.

Regression slopes (mite abundance versus time) were used to examine mite population trends (increasing, stable, or decreasing) while the dustbox was present or absent. For trial 1, houses 1 and 3 slopes were created for weeks 1-8 (dustbox present) and for weeks 9-16 (dustbox absent). Trial 1 houses 2 and 4 "present" slopes were analyzed for weeks 1-12 and "absent" slopes for weeks 13-16. In trial 2, houses 1 and 3 were combined for "present" and "absent" regression analyses because of overall low mite levels. Trial 2 houses 2 and 4 were not included in this analysis because of very low mite levels.

Mixed model repeated-measures analyses were used to examine general trends of the main effect of dustbathing on mite levels while dustboxes were present. Bird 'dustiness' scores of 0 or 1 indicated birds that did not use the dustbox (non-bathers), while scores of 2 or 3 indicated birds that were bathers for a given week. Bird was the fixed effect and week was the repeated measure. A comprehensive model was used initially, then analyses were separated by trial and house. Houses 1 and 3 were evaluated during weeks 1-8 only, while houses 2 and 4 were evaluated during weeks 1 – 12 only. Two sample t-tests were then used to examine mean mite scores in trial 1 between groups (non-bather versus bathers) for each week while the dustbox was present. Trial 2 was not included in this analysis because mite levels while dustboxes were present were too low to compare bathers and non-bathers.

RESULTS

In trial 1 one bird in each of houses 1, 3, and 4 and in trial 2 one bird in house 1 was removed (injury or death) during the course of the study and was not replaced.

Trial 1. Average mite populations varied by house (Fig. 2.4). Birds in houses 1 (n=2/18) and 2 (n=18/18) were accidentally exposed to mites sometime shortly prior to intentional experimental infestation at week 2, but mean mite densities were still low and below a score of 3. Regression slopes for houses 3 and 4 showed slow but positive mite growth (slopes 0.16, $P < 0.01$ and 0.15, $P < 0.001$ respectively) while dustboxes were present. Slopes were steeper and distinctly positive after dustboxes were removed in those houses (slopes 0.24, $P < 0.05$ and 0.75, $P < 0.05$ respectively). Mite numbers

dropped in house 2 (early mite exposure; slope -0.13, $P < 0.01$) while the dustbox was present compared to steep mite increases when it was removed (slope 1.26, $P < 0.0001$). House 1 had an increased rate of mite growth when the dustbox was available (slope 0.40, $P < 0.0001$), but mite numbers remained very low overall (scores below 2). Mites increased further when the box was removed (slope 0.18, $P < 0.05$) and eventually reached average scores of about 4 in house 1.

In all four trial 1 houses, mite levels were relatively stable when the dustbox was available, but mites increased distinctly with the removal of the dustboxes, even after infestation periods of 9-12 weeks (Fig. 2.4).

While dustboxes were available to birds in houses 1, 2, and 3, hens that were scored as non-bathers averaged significantly higher mite scores than bathers (H1: bathers = 0.75 ± 0.16, non-bathers = 2.38 ± 0.22; $F = 39.45$; df = 1, 6; $P = 0.0008$; H2: bathers = 1.31 ± 0.16, non-bathers = 2.86 ± 0.23; $F = 5.71$; df = 1, 16; $P = 0.030$; H3: bathers = 0.18 ± 0.06, non-bathers = 0.67 ± 0.18; $F = 32.62$; df = 1, 13 $P < 0.0001$). Overall house 4 average mite scores were low, and there was no significant difference between bathers and non-bathers while dustboxes were present (bathers = 0.33 ± 0.08, non-bathers = 1.73 ± 0.21, $F = 0.82$; df = 1, 16; $P = 0.38$).

Average mite levels between these two groups in houses 1 and 3 (Fig. 2.5) and houses 2 and 4 (Fig. 2.6) were compared by week using t-tests. In general, bathers had lower weekly mite levels than non-bathers. The number of bathers varied from week to week, however, but only one individual hen in each trial (n = 1/72) never showed evidence of dustbathing while dustboxes were present.

Trial 2. Average mite populations in trial 2 were overall much lower than in trial 1 ($F = 39.93$; df = 1, 142; $P < 0.0001$; Fig. 4). There was a slight positive relationship between mite growth and time for houses 1 and 3 (combined) while dustboxes were present (slope 0.03; $P < 0.05$), but mite scores were very low and averaged less than 1 (fewer than 10 mites/hen). As indicated from the slopes, mite increases were far more distinct when the dustboxes were removed (slope 0.55; $P < 0.0001$). In houses 1 and 3, mite populations increased distinctly after dustboxes were removed and eventually reached scores of 3 to 4. In house 4 mites increased but only to scores of about 1; in house 2 mites did not establish (Fig. 4).

Beak Trimming. Once the second dustbox trial was complete, mite populations on birds equipped with beak bits were compared to mites on unaltered birds (Fig. 2.7). Mite densities between the control and treatment groups were comparable before beak bits were added (week 1; $T = 0.86$; df = 22; $P = 0.40$). Mite populations on beak-bitted birds grew quickly, and after one week there was a significant difference in overall mite levels between the two groups of birds (Fig. 2.7). Beak-bitted hens harbored mite numbers approximately twice as high relative to hens that did not receive bits.

DISCUSSION

In general, mite populations grew very slowly while dustboxes were present in both trials compared to when dustboxes were absent. In trial 1 two houses (1 and 2) were accidently exposed to low mite numbers before birds were experimentally infested. In house 2 mite populations declined over time when dustboxes were present, likely because

mite levels were a little higher before the study began. In this house the dustbox addition was no longer prophylactic, but still early enough to keep mite numbers low. Trial 2 mite levels were very low in houses 1, 3, 4 and completely absent in house 2 while dustboxes were present. Once boxes were removed, mite populations increased in all of the chicken flocks, with the exception of one house in trial 2 (house 2), which never had detectable mite levels. In that case the dustbox appears to have prevented infestations from establishing even with repeated intentional mite introduction.

Despite early accidental mite exposure in trial 1, average mite scores in all houses for both trials for both trials were held at or below the target economic threshold of a visual score of 3, or ca. 100 mites per bird, while dustboxes were present. Previous work on the economic impact of northern fowl mites in commercial egg layer flocks (Mullens et al. 2009) suggests that mite densities below a visual score of 3 are probably not causing as much stress to the animals; significant economic effects (e.g. decreased feed conversion efficiency) were not consistent or obvious. Reduced mite numbers also cause fewer worker irritation concerns with the mites and fewer safety concerns associated with the application of traditional pesticides. When dustboxes were deployed before mites were experimentally introduced mites were maintained well below this threshold for damage. This was true even with variable dustbox use by individual birds. Non-dustbathing individuals generally harbored higher mite numbers in a given week, and probably served as sources of mite inoculum for the other birds in the group. Still, because most individual birds showed evidence of dustbox use at some point during the

trial period, the overall number of mites available in that house to infest new birds was suppressed.

On rare occasions even dustbathing hens could have high mite numbers. In these cases the mites occupied the tail feathers behind the vent (instead of the usual occupation zone anterior to the vent). In that case dustbathing might be less effective at exposing mites to diatomaceous earth.

It would be interesting to investigate whether dustbathing may also trigger mite dispersal from treated hens which were rendered a suboptimal host by the dust treatment. There is some chance that the dustbox could serve as a common source of contact (spread), although we saw no evidence of this. In general a mite off-host is probably less likely to survive, although in normal hen house environments mites might persist for days to weeks off-host (Chen and Mullens 2008). Northern fowl mite dispersal is not well studied and requires future work.

Despite prolonged low-level bird exposure to mites while dustboxes were available, we observed no evidence of adaptive immunity development over periods of 8 or 12 weeks. In white leghorn hens this is sufficient time to see clear suppression of mites by immune responses, with reductions of over 100x in mite densities relative to peak numbers 3-6 weeks after exposure (Mullens et al. 2009, Owen et al. 2009). As mites blood-feed they cause skin inflammation and thickening of the epidermis, primarily in the vent region. This inflammation physically blocks mites from successfully blood feeding and is linked to the major histocompatibility complex genes of white leghorn layers (Owen et al. 2008, 2009). ISA brown hens, used in the current study, are a hybrid cross

between a white leghorn and Rhode Island red, and immune responses to ectoparasite loads have not yet been examined in this strain. ISA brown hens may not mount as strong an immune response to NFM as white leghorns, or it may not occur over the same time frame. It is also possible that low mite numbers, as observed when dustboxes were present, were not sufficient to trigger a strong immune response. Martin and Mullens (2012) noted that NFM densities on ISA Brown or Hyline Brown hens would drop over time, but could remain high for 2-3 months after initial NFM exposure on some birds. Burg et al. (1988) noted that Fayoumi hens injected earlier with crude mite extracts developed lower mite numbers after live NFM exposure, while that trend did not appear with White Rock strain hens, again suggesting strain differences in immune response to NFM. Further work is needed to assess the effects of ectoparasites such as NFM on host immune responses and economic factors on breeds used in cage-free systems, such as ISA brown.

The DE in dustboxes was recharged weekly to ensure fresh material for birds to use. Sand was replaced as needed, which varied by house. In many cases, birds would try to dustbathe as soon as fresh DE was provided. Dustbathing is an innate, complex behavior that has benefits both for bird feather condition and well being (Appleby et al. 1993, Olsson and Keeling 2005). Dustbathing is also likely a socially facilitated behavior (Lundberg and Keeling 2003), and variation observed in overall dustbathing could be dependent on which individuals make up a flock. Social hierarchy may also play a role in resource use (dustbox in this case; Shimmura et al. 2008). Dominant individuals may

spend more time dustbathing or try to defend the dustbox resource from less dominant individuals.

Dustbathing is a complex behavior. It could be adaptive, in that dustbathing in certain natural substrates such as clay has ectoparasite-suppression benefits (Martin and Mullens 2012), but there is no evidence to date that dustbathing is actually driven by parasite loads. Vezzoli et al. (2015) examined the number and duration of dustbathing bouts of NFM infested birds held in furnished cages, and relative intensity of mite populations did not influence dustbathing behavior of birds provided with astroturf or sand. More work in this area is desirable to determine if frequency or duration of dustbathing in finer substrates is influenced by type or intensity of ectoparasite load (mites or other ectoparasites such as lice). In addition, an increased drive to dustbathe could reflect social hierarchy position if this is a highly desirable resource. At any rate, understanding the basic factors that influence dustbathing is important in order to best exploit this behavior for ectoparasite suppression at the flock level.

Diatomaceous earth was kicked out of the dustboxes while the birds dustbathed, and dust was observed in the bedding of each house outside the boxes. After the dustboxes were removed, DE still persisted and was visible in the environment. Birds were observed dustbathing in the straw bedding when dustboxes were not available. However, this level of residual DE was not enough to keep mite levels low after the boxes were removed. The same result was seen earlier in hens dustbathing in fine litter particles plus residual DE or kaolin clay (Martin and Mullens 2012). When birds perform dustbathing behavior they move the substrate up and into their feathers to remove excess

lipids (Olsson and Keeling 2005). From a pest control standpoint, diatomaceous earth acts as a desiccant against arthropods including mites (Quarles 1992, Cook et al. 2008, Kilpinen and Steenberg 2009). It is probable that the fine substrate (sand here) acts as a carrier to facilitate the movement of DE into the feathers where it then acts on the mites by abrasion and adsorption of surface wax, killing mites via desiccation (Ebeling 1971, Quarles 1992). The availability of DE with the fine sand substrate is important to achieving mite suppression. However, incidental exposure of birds and their mites to DE in the environment is not enough; substantial, direct exposure to DE is required for mite suppression.

One of the more interesting aspects of this study is the differences in mite populations between the two trials. Trial 2 birds maintained very low mite numbers while dustboxes were available. Yet mites were able to rebound once dustboxes were removed and reached average mites scores greater than 3 (100 mites/bird) in just six weeks. Trial 2 birds were beak-trimmed using infra-red (IR) beak trimming technology. IR-trimmed birds fitted later with beak bits showed rapid and significant mite population growth compared to unaltered controls. This helps implicate effective hen grooming behavior (with a mostly intact beak) as an important factor in suppressing mite populations. The ability of bit-free IR-beak trimmed birds to groom helped control mite populations in trial 2, relative to birds with blunter beaks in trial 1. It is interesting, however, that mite levels did not stay adequately depressed with the removal of DE dustboxes, regardless of beak condition. As producers move toward IR-beak trimming or eliminate beak trimming altogether (with docile breeds) we can expect to see a better level of ectoparasite control

through host-grooming. Our results indicate improved grooming by IR-trimmed hens may not be enough by itself to keep mites dependably below damaging levels. The combination of a cultural control tactic (beak trimming technique) and a mechanical control tactic (DE dustboxes) worked best to keep mite levels below economically damaging levels. This is beneficial to bird welfare as well.

In the current study we were able to mimic production densities but were unable to reproduce commercial flock sizes. Commercial cage-free flocks can have many thousands of birds. More on-farm work is required to elucidate actual placement, size, and number of dustboxes per flock to achieve effective ectoparasite control. Producers should keep in mind that DE particles can pose a serious inhalation risk, especially in confined spaces (Checkoway et al. 1993, Fields 1998). Using diatomaceous earth in dustboxes 1) allows birds to exhibit natural dustbathing behaviors, thus increasing animal welfare, 2) decreases the use of labor-intensive pesticide applications that can be harmful to human, animal, and environmental health, and 3) addresses ectoparasite issues proactively, before they become economically damaging.

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FIGURES



Figure 2.1. Black plastic bins contained 5-10 cm of diatomaceous earth and sand for hens to dustbathe.



Figure 2.2. Trial 1 birds (left) were beak trimmed as young chicks by a commercial breeder using a hot-blade trimmer. Note blunt beak and minimal regrowth in adult. Trial 2 birds (right) were beak trimmed as chicks using an infra-red (IR) trimmer. Note substantial beak regrowth to a sharp tip and some upper and lower mandible overlap in adult. A more intact beak allows birds to groom more effectively, thus negatively affecting ectoparasite populations.



Figure 2.3. Bird fitted experimentally with beak bit to impair ability to groom at the end of trial 2.

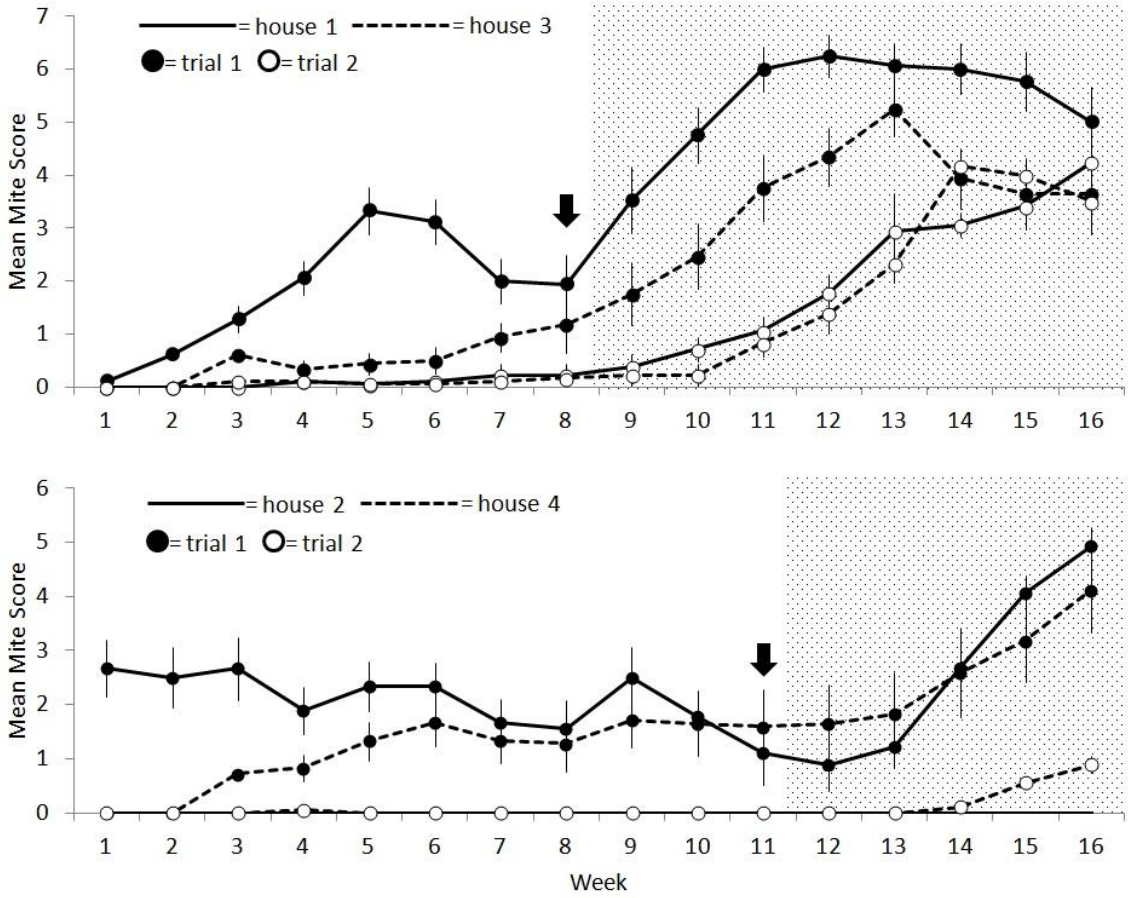


Figure 2.4. Mite population trends (means ± SE) in houses where dustboxes were removed at week 8 (top, houses 1 and 3) or week 12 (bottom, houses 2 and 4) as indicated by arrow. Shaded areas indicate mite counts in weeks when dustboxes were absent. Trial 1 birds (filled circle) generally had higher mite scores than trial 2 birds (open circle).

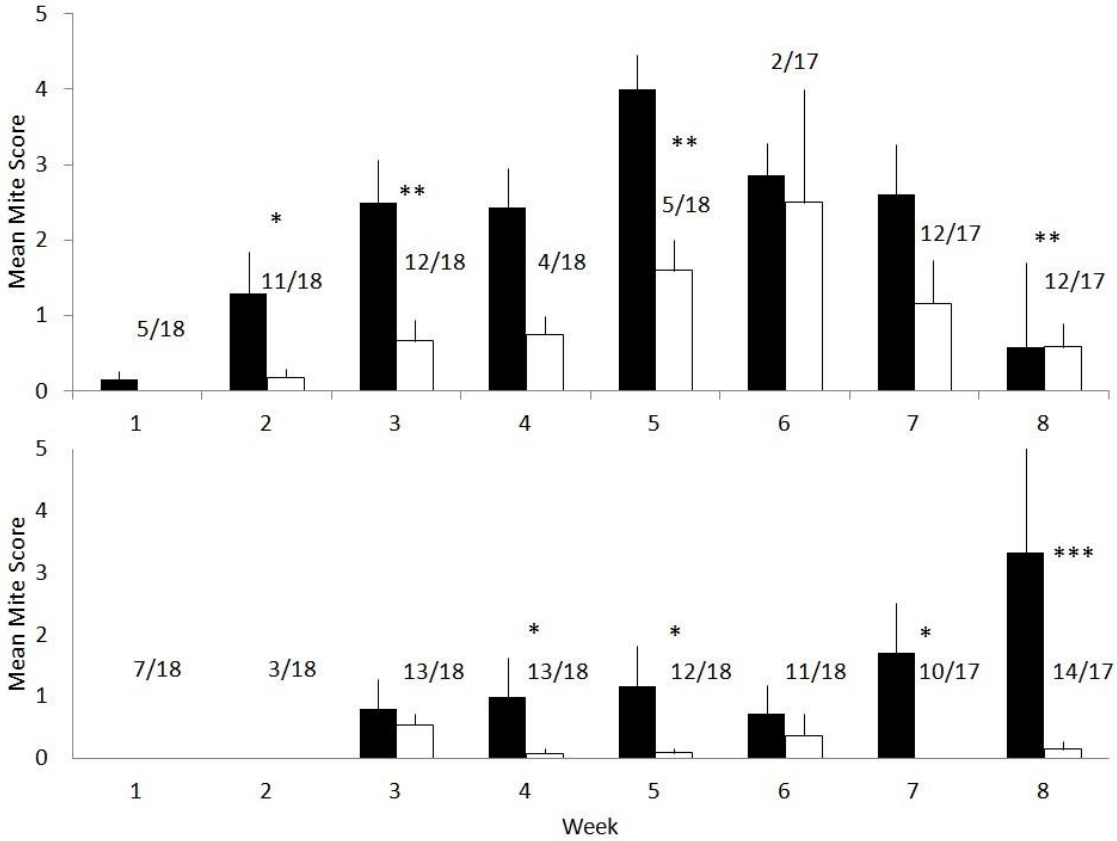


Figure 2.5. Mean (± SE) mite scores of birds scored as non-dustbathers (black) and dustbathers (white) in houses 1 and 3 (top and bottom, respectively). The numbers of hens dustbathing within each week are shown out of the total number of birds. Within a week, significant mite differences between groups are indicated with stars: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

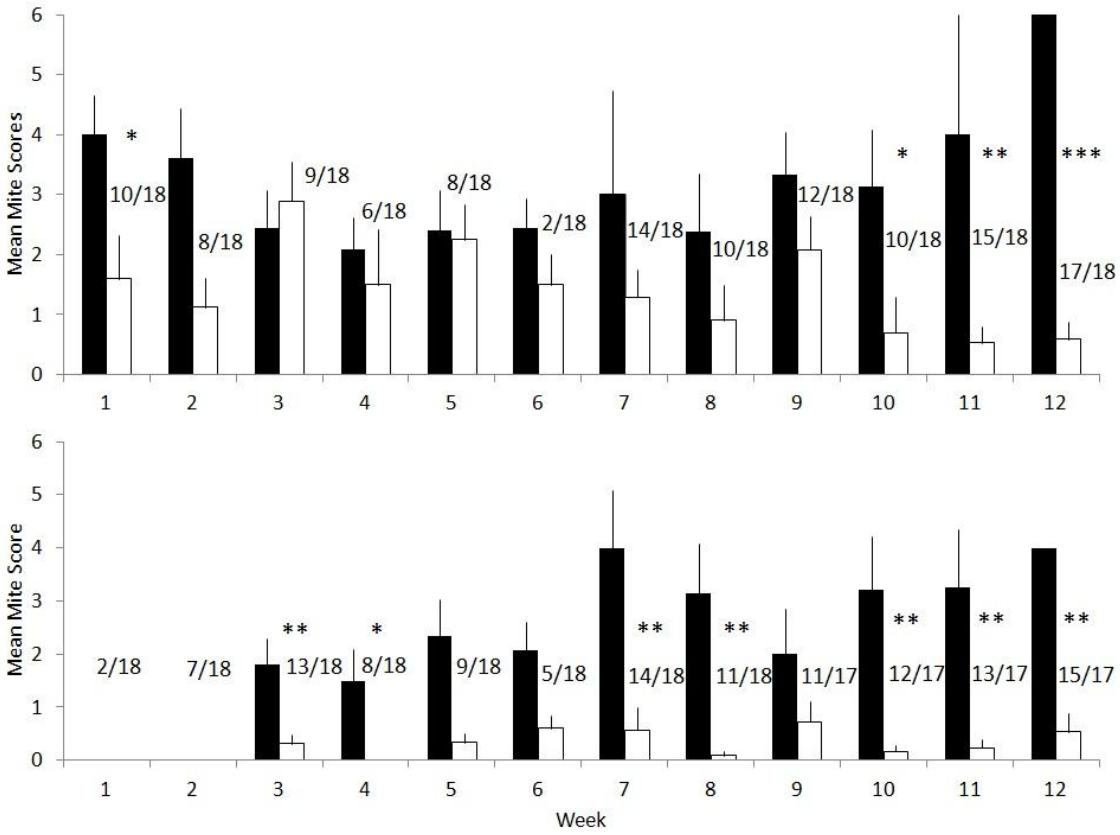


Figure 2.6. Trial 1 mean (± SE) mite scores of non-bathers (black) and bathers (white) in houses 2 and 4 (top and bottom, respectively). The numbers of hens dustbathing within each week are shown out of the total number of birds. Within a week, significant mite means between groups are indicated with stars: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

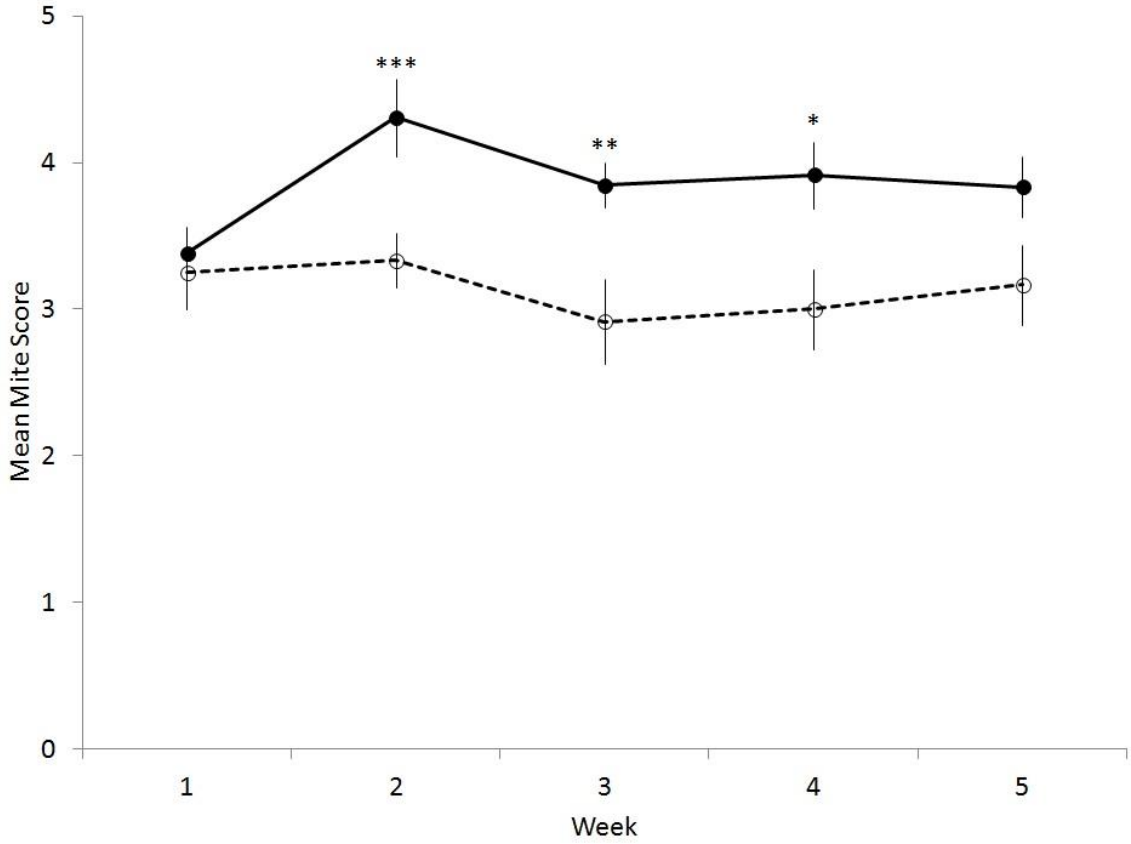


Figure 2.7. Mean (± SE) mite scores of birds with beak bits (solid line) compared to unaltered controls. Beak bits were added to birds after week 1 mite scores. Stars indicate: *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

CHAPTER 3

Sulfur Dust Bag: A Novel Technique for Ectoparasite Control in Poultry Systems

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ABSTRACT

Animal welfare-driven legislation and consumer demand are changing how laying chickens are housed, thus creating challenges for ectoparasite control. Hens housed in suspended wire cages (battery cages) are usually treated with high-pressure pesticides. This application type is difficult in enriched-cage or cage-free production. Alternatives to pesticide sprays are needed in enriched-cage or cage-free systems. In this study we tested the efficacy of sulfur dust deployed in "dust bags" for control against the northern fowl mite (*Ornithonyssus sylviarum*), which causes host stress, decreased egg production, and reduced feed conversion efficiency. Dust bags were hung from the tops of cages or were clipped to the inside front of cages. We also tested permethrin-impregnated plastic strips, marketed for ectoparasite control in caged or cage-free commercial and backyard flocks. Previous work has shown sulfur to be very active against poultry ectoparasites, however we found that the placement of bags was important for mite control. Sulfur in hanging bags reduced mites on treatment birds by 95 or 97% (depending on trial) within one week of being deployed, and mite counts on these birds were zero after 2 weeks. Clipped sulfur bags acted more slowly and did not significantly reduce mites in one trial, but reduced mite counts to zero after 4 weeks in trial 2. Permethrin strips had no effect on mite populations. This may have been due to mite resistance, even though this mite population had not been exposed to pyrethroids for several years. Sulfur bags should be effective in caged or cage-free systems.

INTRODUCTION

Animal welfare concerns are driving poultry production away from traditional battery cages to larger enriched-cage or cage-free production systems. Enriched cages allow for more space per bird and include upgrades such as perches, nest boxes, and scratch pads. "Cage-free" birds may include several types of production systems (e.g., aviary, free-range, pasture), but all provide birds with some type of nest boxes and perches. Ectoparasite control in wire battery cages is achieved by high-pressure insecticidal sprays (Mullens et al. 2009). These products must penetrate the feather layer from under the birds to treat mites, which are found primarily in the vent region of the bird. It is difficult or impossible to spray birds in this way in enriched-cage or cage-free systems because of the complex environments or solid floors. In addition, pyrethroid and organophosphate resistance is a growing concern (Mullens et al. 2004). As poultry production moves away from battery cages due to legislation (e.g. California's Proposition 2 "Standards for Confining Farm Animals;" Sumner et al. 2008) or increased consumer demand, new pesticide delivery systems for use in non-battery cage production systems will need to be developed.

The most damaging ectoparasite of US laying hens is the northern fowl mite, *Ornithonyssus sylviarum* (Canestrini & Fanzango) (Axtell and Arends 1990). These blood-feeding mites induce skin inflammation, immune system activation, irritation, anemia, and even rarely death as a result of exsanguination (DeVaney and Ziprin 1980, Burg et al. 1988, Minnifield et al. 1993, Mullens et al. 2009, Owen et al. 2009). This mite is a permanent ectoparasite (all life stages live on-host) that is found on chickens

regardless of housing type (Lay et al. 2011). Mites can be introduced to flocks by wild birds or rodents, or contaminated equipment or people, and are very difficult to eradicate once introduced to a poultry facility (Kells and Surgeoner 1997, Axtell 1999).

One option for enriched-cage or cage-free production is self-treatment of animals. Self-application of insecticidal dust by rubbing or bumping against large dust bags has been used for the control of pests on cattle for several decades (Turner 1965). For poultry, sulfur has proven to be a highly effective acaricide at low doses in dustboxes in cage-free settings, eliminating ectoparasites even on birds that did not dustbathe in the material (Martin and Mullens 2012). Sulfur is also effective for mites in a water slurry sprayed onto a bird's body (Mullens et al. 2012). Self-treatment reduces labor costs and treatment-associated stress to the birds (Jones and Kissam 1983). Previous work suggests that mite populations below a score of 3 (ca. 100 mites per bird) are not as economically damaging to producers as higher mite numbers (Mullens et al. 2009).

In this study we tested sulfur dust bags for mite control in caged birds. Dust bags were either clipped onto the front of the cage or hung from the top of the cage. We also tested permethrin-impregnated plastic strips, which are available for commercial and home use on northern fowl mites (Hall et al. 1983).

MATERIALS AND METHODS

Birds. Ectoparasite-free white leghorn (Highline strain CV20) chickens (17-19 weeks old) were acquired from local commercial producers. All birds were beak trimmed before they arrived, which is routine for commercial chickens to reduce feather pecking,

cannibalism, and feed waste (Mertens et al. 2009). Birds were housed individually at the University of California Riverside Agricultural Operations (AgOps) in plain wire cages measuring 31 cm x 31 cm with a 16:8 light:dark cycle. Animal care was approved under University of California Riverside Institutional Animal Care and Use Protocol A-20150009.

Mites. Northern fowl mites were maintained on white leghorn chickens at AgOps. For inoculation, mites were aspirated with pipettes from those source birds. Mites then were applied to the vent region of each experimental bird (Martin and Mullens 2012). Once a week for the duration of the study all treatment and control birds were visually scored (by ACM) for level of mite infestation. The scoring system used is as follows: 1 = 1–10, 2 = 11–50, 3 = 51–100, 4 = 101–500, 5 = 501–1000, 6 = 1001–10,000 and 7 = >10,000 (Arthur and Axtell 1983).

Dust bags. Cloth bags measured ca. 7.5 cm x 12.7 cm and were constructed from a single layer of 100% cotton gauze (ca. 20µm openings; Hobby Lobby, Inc, Oklahoma City, OK, USA). Dust bags were filled with 30 g (± 1) of 90% wettable sulfur (Yellow Jacket®; Georgia Gulf Sulfur Corp., Valdosta, GA, USA) then stapled shut. Bags were either hung from the top of the cage with a paper clip which was inserted straight through the gauze material ("hanging bag") or were folded over the front inside of the cage at the bottom of the cage door and affixed with a large binder clip ("clipped bag"; Fig. 1). Each clipped bag also was filled with 30 g (± 1) of 90% wettable sulfur. In trial 2 the dust bags were weighed weekly (± 0.1 g) to determine weight of sulfur lost.

Permethrin strips. Plastic permethrin-impregnated strips measured 22 cm × 1.5 cm × 1.5 mm (Die No Mite Strips™; Car Mac Products, Houston, TX, USA). Each strip weighed 6.5 g and contained 10% permethrin (active ingredient; AI) and 6% piperonyl butoxide (synergist). The labeled rate was 1 strip per 5 birds; we provided each bird with 1 strip which was hung from the inside top of the cage (Fig. 1). The strips must make contact with the birds for the active ingredient to be effective. Because of the small cage size, contact with the strip was frequent.

Dust bag studies. At week 1, twenty-four birds in each of two trials were experimentally infested with 20-30 adult mites each (described above). Mite populations were allowed to develop for four weeks, with weekly visual mite scores recorded for each animal. This was the pre-treatment period (weeks 1-5). At week 5, birds were divided into four groups of six adjacent cages. Then one of four treatments was assigned to each bird's cage: hanging bag, clipped bag, permethrin strip, or control (no treatment). Cage groupings were separated into different cage rows or by at least 3-6 empty cages to prevent cross-contamination and minimize mite movement among treatment groups. When performing daily hen care duties or scoring birds, nitrile gloves were changed between each treatment group. Mite levels were monitored weekly for four additional weeks (post-treatment) and the study was concluded at week 9. Two complete trials were conducted (Nov-Dec 2014 and Dec 2015- Feb 2016).

Statistical Analyses. Statistical analyses were performed using SAS® software (SAS Institute Inc., Cary, NC, USA, 2010, v. 9.3). Proc means were used to generate averages and standard errors of mite scores. Mixed model repeated-measures analysis

was used to examine the effect of treatments (hanging bag, clipped bag, permethrin strip, control) on mite score. Bird was the fixed effect and week was the repeated measure. Trials were combined for this part of the analysis. Analyses were separated into pre- and post-treatment time frames (weeks 1-5 and 6-9 respectively). Tukey's test was used to separate treatment means (by trial) for each week post-treatment.

RESULTS

Mean mite scores for trials 1 and 2 are shown in Figure 2. No significant differences were found between trials ($F = 0.01$; df = 1, 40; $P = 0.92$) or treatment groups ($F = 0.04$; df = 3, 40; $P = 0.99$) during the pre-treatment period (weeks 1-5). During the post-treatment period (weeks 6-9) significant differences among treatments were observed ($F = 45.96$; df = 3, 40; $P < 0.0001$; Fig. 2). Control birds in both trials had mite populations that grew steadily. At week 5 (pre-treatment) control bird mite scores were 5.33 ± 0.42 and 5.67 ± 0.33 (trials 1 and 2, respectively.) In both trials chickens treated with sulfur hanging bags had significantly lower mite populations after just 1 week of treatment. Birds with sulfur clipped bags had more variable results. Mean mite scores on the permethrin strip-treated birds never differed significantly from the control group.

In trials 1 and 2, mean mite scores on hanging bag-treated birds fell from a score of around 6.00 to 2.17 ± 0.48 (mean ± SE) and 1.67 ± 0.42, respectively, one week post treatment. There was a 95% or 97% reduction in mite scores (versus pre-treatment) just two weeks after treatment in trials 1 and 2, respectively, while control mite scores

remained high (above a score of 5). In both trials mean mite scores were 0.00 ± 0.00 by week 8, 3 weeks post-treatment.

The sulfur clipped bags had more variable results; trial 1 birds had decreased mite levels after treatment, though those did not differ significantly from control birds (5.67 ± 0.33 at week 5 [pre-treatment] compared to 5.00 ± 0.82 at week 6 [1 week post-treatment]. Average mite scores for trial 1 clipped bags were lowest at week 9 (3.67 ± 1.05). In trial 2 birds treated with sulfur clipped bags had significantly fewer mites compared to controls two weeks after treatment. Two weeks post treatment mean mite scores were 1.83 ± 0.95 for birds with clipped bags compared to 6.00 ± 0.26 at week 5 (pre-treatment). Average mite scores for trial 2 clipped bags were lowest at week 9 (0.00 ± 0.00).

The amount of sulfur dispensed per bag per week varied greatly among birds, and over weeks 5-9 release levels were 0.2 – 18.9 g for hanging bags and 0.3 – 14.9 g for clipped bags (trial 2). One week post-treatment, trial 2 hanging bags had lost on average 7.4 g of sulfur (range: 1.6-16.5 g) while clipped bags lost on average 3.4 g of sulfur (range: 0.3-8.5 g). Some sulfur remained in nearly all bags at week 9. Despite high variation in rates of sulfur release, mites were dramatically reduced in all hanging bag cages 1 week after bags were added. This included the cage with the lowest sulfur release, where the hen's mite score dropped from a 6 to a 2 after a release of only 1.6 g of sulfur.

DISCUSSION

Treatment with hanging sulfur dust bags decreased mite populations in both trials (Fig. 2). Within one week of treatment, mite scores were on average below 3 and were at 0 three weeks post-treatment. Previous work suggests that visual mites scores of ≤ 3 (≤ 100 mites/bird) are not as economically damaging to laying hens on a commercial scale as are higher mite numbers (Mullens et al. 2009). In trial 1, clipped bags did not reduce mite populations significantly from control (untreated) birds. However in trial 2 we did observe significantly reduced mite populations two weeks post-treatment using the clipped bags. The hanging sulfur bags reduced mite levels more quickly and consistently between the two trials compared to the clipped bags. The frequency of bag contact by individual hens may have contributed to this variation for clipped bags. Birds had to come into contact with the hanging sulfur bags in order to reach the feeder. When birds came in contact with these bags, much of the sulfur that came out would fall directly onto the bird. The clipped bags were in the front of the cage near the feed trough, and when sulfur released from the clipped bags much of it would fall down and through the wire floor of the cage.

We were unable to measure how much sulfur actually got onto the birds. We did measure the amount of sulfur lost in each bag (clipped and hanging) each week in trial 2. Weekly sulfur loss varied from 0.2 g – 18.9 g per bag. Loss was difficult to correlate directly with mite reduction, since bag weight measurements were taken weekly but the hanging sulfur bag worked more quickly than that. Preliminary data (unpublished) showed large reductions in mite numbers in as little as 5 days post-treatment. Daily mite

and sulfur measurements would yield a better idea of how much sulfur is required to decrease mite populations. Mullens et al. (2012) applied a sulfur and water slurry to directly to the vent region to treat northern fowl mite infested birds. Just 0.2 g of sulfur eliminated mites for 4-5 weeks, demonstrating that very small amounts of sulfur are effective against mites (Mullens et al. 2012). The mode of action of sulfur against mites is currently unknown. It probably acts as a fumigant in the feather layer of hens by reducing to hydrogen sulfide at temperatures > 21° C (Ware 1993, Mullens et al. 2012). Hen feather temperatures in the mite habitation zone are usually well above that, and temperatures at the feather base generally are above 30° C (De La Riva et al. 2015).

Mean mite scores on permethrin strip-treated birds did not differ significantly from control birds at any time post-treatment in either trial (Fig. 2). Each bird was treated with 1 strip (greater than label rate of 1 strip/5 birds) and made frequent contact with the strip because of the limited space per cage. Thus the failure of this product to control northern fowl mites is likely due to resistance. The mite population used in this study (maintained on amplifier hens at AgOps) is derived from mites collected from a commercial poultry facility over a decade ago, and has not been exposed to permethrin or other pesticides since being establish in laboratory colony. Mullens et al. (2004) found 50% of northern fowl mite field populations in southern California to be very resistant (> 1000x) to commercial formulations of permethrin. The mechanism conferring permethrin resistance in our northern fowl mite population likely has low fitness costs to maintain. Alternatively the resistance may be fixed in the population. It appears to have persisted over time even in the absence of selection pressure. Recent, independent laboratory

bioassays with technical permethrin confirmed our mite population is resistant to this chemical class, so it probably was not a failure of the permethrin strip treatment to release acaricide (Mullens, unpublished data). Currently there is very limited availability of registered products for northern fowl mite control in California, with most products containing the active ingredient permethrin. This highlights the need for alternative control options for northern fowl mite in commercial poultry.

Sulfur deployed via dust bags could be a great alternative to traditional pesticide applications. Sulfur is a natural material that is active against mites in very small doses (Mullens et al. 2012). Dust bags allow the birds to effectively self-treat, eliminating the need for large-scale deployment of dust, which can be an inhalation or combustion risk (Mullens et al. 2012). Sulfur treatments also effectively control poultry lice (Creighton et al. 1943, Martin and Mullens 2012) and possibly other poultry ectoparasites, such as *Dermanyssus gallinae* or *Echidnophaga gallinacea*, that are reemerging (Murillo and Mullens 2016). In this study each bird was held in an individual cage with a single dust bag, but on a commercial scale one bag per cage holding multiple birds would probably be sufficient. In Martin and Mullens (2012) sulfur deployed in dustboxes in cage-free houses achieved mite control even on birds that did not dustbathe, further highlighting the effectiveness of sulfur.

Overall, hanging sulfur dust bags worked very well to control northern fowl mites below economically damaging levels in just one week. This technique could be successful in either cages or conventional cage-free facilities. The sulfur used in this study is currently labeled for use in poultry at a rate of 200-250 lbs per 20,000 ft^2 applied

as a dust or 25-50 lbs per 100 gallons of water applied as a spray. Sulfur only carries the signal word "caution," though there may be an ignition risk when it is suspended densely in air. Applying sulfur via dust bags is a more targeted approached and eliminates the need to apply to large areas, which could be hazardous to workers and may miss birds. In caged or cage-free poultry, dust bags could be hung near shared resources such as in nest boxes, near feeders, or in doorways to coops to achieve ectoparasite control. Future work on commercial properties would be useful in refining how best to use sulfur dust bags, including how frequently they should be deployed, replaced, or recharged. Using dust bags at the beginning of a flock cycle, when mite naïve birds are most susceptible to economic damage by mites (Mullens et al. 2009), would likely be the most economical and efficient method of mite control. It may even be possible to eliminate mite populations on a poultry operation in this manner, which is difficult to achieve with most insecticidal sprays. The dust bag material should be investigated further to optimize durability and release of sulfur dust over time. The cotton gauze material worked well enough in this study, but would not likely hold up in a commercial flock.

Sulfur dust is one of the oldest pesticides and has been used for northern fowl mite control since the mite was first described on domestic poultry (Cleveland 1923). Bishop and Wood (1931) recommended "flowers of sulphur" in either a liquid dip form or as a dust and claimed it would eradicate "feather mites" (northern fowl mites), which then were rather new North American poultry pests. However the use of a slow release self treatment dust bag for deployment in poultry systems is, to our knowledge, a novel technique for controlling ectoparasites on poultry. Sulfur dust bags 1) increase worker

safety by eliminating broadcast sprays, 2) decrease the environmental impact of pesticides by using small amounts of targeted sulfur, and 3) have the potential to be used in caged and cage-free poultry production.

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FIGURES



Figure 3.1. Cotton gauze bags filled with sulfur were deployed by either clipping bags to the bottom of the inside of the cage door (top left) or by hanging from the top of the cage (top right). Permethrin-impregnated strips were hung with metal rings from the top of the cage (bottom).

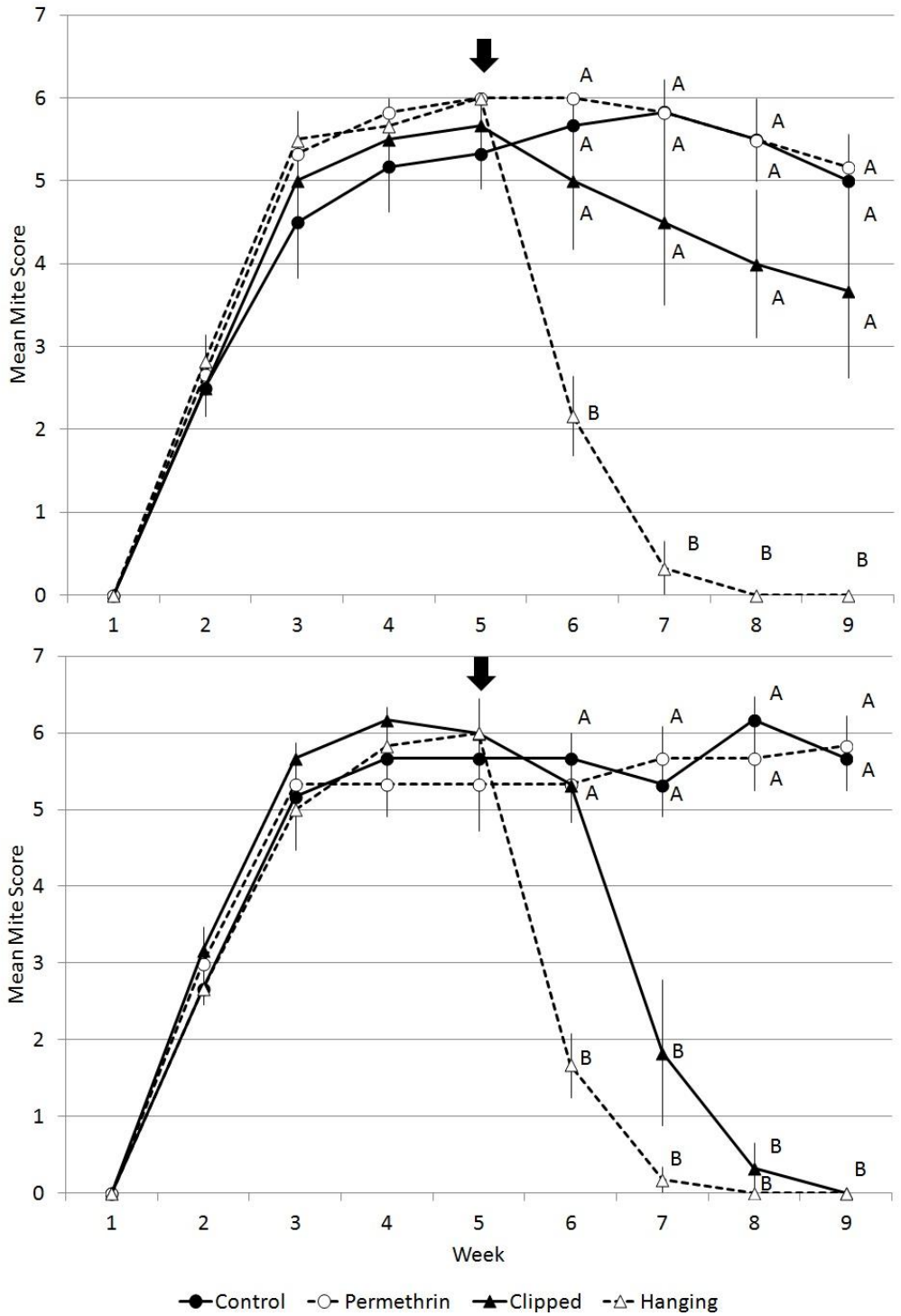


Figure 3.2. Weekly mean (± SE) mite scores for trial 1 (top) and 2: control, permethrin strip, clipped bag, hanging bag. Treatments were deployed after mite counts at week 5 (arrow). Weekly means after treatment date with the same letter are not significantly different ($P = 0.05$).

CHAPTER 4

Diversity and Prevalence of Ectoparasites on Backyard Chicken Flocks in California

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ABSTRACT

Peridomestic ("backyard") chicken flocks are gaining popularity in the developed world (e.g. North America or Europe), yet little is known regarding prevalence or severity of their ectoparasites. Therefore, five birds on each of 20 properties throughout southern California were surveyed in summer for on-host (permanent) and off-host dwelling (temporary) ectoparasites. Only four premises (20%) were entirely free of ectoparasites. In declining order of prevalence (% of premises), permanent ectoparasites included six chicken louse species: *Menacanthus stramineus* (Nitzsch) (50%), *Goniocotes gallinae* (De Geer) (35%), *Lipeurus caponis* (L.) (20%), *Menopon gallinae* (L.) (15%), *Menacanthus cornutus* (Schömmer) (5%), and *Cuclotogaster heterographus* (Nitzsch) (5%). Only one flea species, *Echidnophaga gallinacea* (Westwood) (20%), was found. Three parasitic mite species were observed: *Ornithonyssus sylviarum* (Canestrini & Fanzago) (15%), *Knemidocoptes mutans* (Robin & Lanquetin) (10%), and *Dermanyssus gallinae* (De Geer) (5%). Many infestations consisted of a few to a dozen individuals per bird, but *M. stramineus*, *G. gallinae*, *M. cornutus,* and *E. gallinacea* were abundant (dozens to hundreds of individuals) on some birds, and damage by *K. mutans* was severe on two premises. Off-host dwelling ectoparasites were rare (*D. gallinae*) or absent (*Cimex lectularius, Argasidae*). Parasite diversity in peridomestic flocks greatly exceeds that is routinely observed on commercial chicken flocks and highlights a need for increased biosecurity and development of ectoparasite control options for homeowners.

INTRODUCTION

Peridomestic, or "backyard" poultry flocks, have been steadily increasing in popularity in the United States (Elkhorabi et al. 2014). Some arthropod parasites, such as the northern fowl mite (*Ornithonyssus sylviarum* [Canestrini & Fanzago]), chicken body louse (*Menacanthus stramineus* [Nitzsch]), and chicken red mite (*Dermanyssus gallinae* [De Geer]) are listed as common pests in commercial chicken flocks (Axtell and Arends 1990). However, one would expect a different ectoparasite fauna among backyard flocks due to many factors including 1) biosecurity and housing differences, 2) bird age and strain mixing, 3) decreased prevalence of pesticide use, 4) ready exchange of birds among owners or feed stores selling or showing birds, and 5) lack of experience with poultry parasites by homeowners. Free-ranging birds often have a greater chance of acquiring a more diverse parasite population (Lay et al. 2011). The aim of this study was to gather information regarding parasite diversity and relative abundance on backyard chicken flocks in southern California.

MATERIALS AND METHODS

A survey was advertised at backyard flock workshops in San Bernardino and Pomona, California, as well as at local feed stores (Riverside and San Jacinto, California). Survey participants had to have 5-50 birds for at least 6 months. The survey was not random, and such a survey probably could not have been conducted. The occurrence of backyard flocks is not well known or monitored, and some owners might consider such an activity a governmental intrusion. This survey instead relied on

interested and willing homeowners. Only adult female birds at or near egg-laying age (≥ ca. 20 weeks) were included in the study.

Five birds on each of 20 different properties were surveyed in summer (June-September 2015) throughout southern California (Fig. 4.1). We never visited multiple flocks per day, and strict sanitation/biosecurity protocols were followed. Five birds from each flock were arbitrarily selected for ectoparasite collections and birds were handled and parasites collected in accordance with UCR animal use protocol A-20150009. First the animal was examined visually by looking closely at the skin and feathers. When appropriate, individual parasites were collected by forceps and placed into 70% EtOH. After notation of general parasite numbers and likely species observed, ectoparasites were removed using one of two methods, according to the owner's preference (Fig. 4.2): Method #1 included spraying the chicken's feathers in 8-10 body locations with a total of ca. 10 ml of liquid pyrethrin solution (0.18% pyrethrins, Bayer, Whippany, NJ, USA). Body locations included anterior, lateral and posterior sides of the abdomen, under each wing, base and anterior back of neck, and central-rear dorsum. The solution was gently distributed into the surrounding few centimeters of skin and feathers using a gloved hand. This method killed or stunned the parasites, which could then be dislodged by gently ruffling the feathers over a dry, clean, plastic dishpan. This debris was searched visually, and all suspect arthropods were collected using a small paintbrush or forceps and placed in a vial of 70% EtOH labeled by chicken number and premise. Method #1 was most often used. Method #2 included gently bathing the chicken (except for the head), disturbing the feathers and skin for ca. 2 minutes in a dishpan with ca. 1.5-2 L of water

containing 1 ml of dishwashing soap (Dawn, Proctor & Gamble, Cincinnati, OH, USA). The hens were then rinsed in fresh water, and parasites in the pan were separated with a fine #100 mesh sieve and then backwashed into a clean pan for collection as above. All parasites collected were placed into 70% EtOH.

In addition to on-host collections, chicken nestboxes and coops were examined. Cracks and crevices were especially searched for evidence of temporary, blood-feeding parasites. At least four environmental samples of approximately 1 liter each were collected near likely parasite harborage (nestboxes, food and water, perches) into plastic bags, which were sealed and transported to the laboratory in an ice chest. Samples often included straw or other bedding material (e.g. from the edges of nestboxes), litter, or moist soil under feed or water containers. Berlese funnels (25 W incandescent light) were used for 24 h to separate live arthropods from collected debris into 70% EtOH.

Species identification was confirmed using a dissection microscope and when necessary specimens were slide-mounted, cleared in Hoyer's medium, and identified using phase-contrast microscopy and the keys and illustrations in Emerson (1956), Furman and Catts (1982), and Price and Graham (1997). Voucher specimens were deposited in the UC Riverside Entomology Museum. Birds ranged from quite large (e.g. >2-3 kg meat hens) to smaller bantam breeds (< 0.5 kg). Therefore parasite loads for each individual bird were not strictly quantitative or comparable; for example a higher proportion of parasites were likely removed from smaller hosts. Field notes were made to include parasite abundance on individual birds.

RESULTS AND DISCUSSION

Overall the diversity and prevalence of observed ectoparasites is presented in Table 4.1. Surveys of four sites revealed no parasites on or around the birds. Parasites collected on the remaining premises (80%) included lice (Phthiraptera: Ischnocera and Amblycera), fleas (Siphonaptera), and mites (Acari: Astigmata and Mesostigmata). Lice were the most prevalent and abundant of all ectoparasite groups (Fig. 4.). The chicken body louse, *M. stramineus*, was collected on 50% of premises and 36% of birds. It was the most abundant species recovered and sometimes was quite dense on individual birds, with dozens to hundreds of specimens seen. This species was followed in abundance by the fluff louse, *Goniocotes gallinae* (De Geer), collected on 35% of premises and 20% of all birds. It was found in numbers up to hundreds per hen and was most often observed in the fluffy vent feathers. The wing louse (*Lipeurus caponis* [L.]) was most often collected in the primary wing feathers, when backlit with natural light. Rarely were more than a dozen or so collected from any one bird. The shaft louse (*Menopon gallinae* [L.]) was collected on multiple properties. It may have been common (dozens per hen), but superficially resembled *M. stramineus*, making it difficult to differentiate from the body louse. The head louse (*Cuclotogaster heterographus* [Nitzsch]) was collected on bird head feathers (dozens per bird) in one flock. Some *M. cornutus* (Schömmer) were observed in only one flock, with dozens to hundreds per bird.

Sticktight fleas (*Echidnophaga gallinacea* [Westwood]) were found on 20% of properties and this was the only flea species recovered. Some birds had up to hundreds of

fleas attached to areas with little to no feather coverage such as the comb, wattles, and around the eye.

The northern fowl mite (*O. sylviarum*) was the most common parasitic mite encountered (15% of premises and 7% of birds). It was usually present in the vent region and at densities of less than 20 mites on a bird, but this was summer and the mite is more successful in cooler weather (Hall 1979). The scaly leg mite (*Knemidocoptes mutans* [Robin & Lanquetin]) was identified on two properties with the assistance of a veterinarian. Signs of scaly leg mite infestation are not absolutely characteristic and are complicated by factors such as secondary infections and gross tissue inflammation. Therefore positive diagnosis is invasive and difficult to confirm. We report here only the properties where the mite was confirmed and do not present data on individual birds, although several birds exhibited what appeared to be severe scaly leg infestations (swollen and deformed toes and shanks) on each of those sites. The chicken red mite (*D. gallinae*), collected on one property closer to the coast (cooler temperature) and in the nestbox area, is not reported on individual birds because it is only a temporary parasite, found off-host during the day. It was abundant at the infested site, with a hundred or more in the one nestbox.

Dermanyssus gallinae is a very common and devastating poultry parasite in European flocks (Sparagano et al. 2014) but seems uncommon in commercial caged-layer egg flocks in the United States, probably due to lack of sufficient harborages in battery-style cages. As flock producers in the US move toward furnished and cage-free housing, it may provide an opportunity for *D. gallinae* to become problematic. This is true also for

the sticktight flea, which to our knowledge is not a major pest in commercial caged flocks. This flea requires organic material (soil or litter) for immature development. This may be available in cage-free or free-range flocks, but is prevented in suspended battery cage systems.

The louse diversity observed on backyard birds was high and resembles louse diversity found in previous surveys of backyard chicken flocks in other countries (Permin et al. 2002, Shanta et al. 2006, Syrcha et al. 2008). These species are described in the earlier literature (Emerson 1956) but little or no economic data are available for most of them. Most of these louse species are probably not of serious economic importance, with the exception of those that can blood-feed (*Menacanthus* and possibly *Menopon*; Crutchfield and Hixson 1943; Price and Graham 1997).

Nearly a third (31%) of birds surveyed were infested with at least two species of ectoparasites (Table 4.2). Most co-infested chickens had only two species collected from them (65%) with a maximum diversity of four ectoparasite species on one bird. Most co-infestation was with multiple louse species and the sticktight flea, though one single bird had both northern fowl mites and lice. This is consistent with previous work that suggests mites have a difficult time surviving on chickens infested with large numbers of body lice (Chen et al. 2011).

Absent from our survey were argasid ticks or bed bugs, which can be found in cracks and crevices during the day and feeding on birds at night. The hot, dry weather may have influenced these findings. However, discussions with local poultry

veterinarians suggest that *Argas* spp. do become problems at times in inland southern California for chickens in backyard settings.

Backyard flock surveys in other countries have been conducted (Permin et al. 2002, Shanta et al. 2006, Syrcha et al. 2008), but we are not aware of published, scientific backyard ectoparasite surveys in the US. Rather, our knowledge is based on self-reporting of ectoparasite problems by owners (Garber et al. 2007, Elkhorabi et al. 2014). In this survey, experienced entomologists conducted all collections and identification.

Backyard flocks can be used as an indicator of potential species richness and diversity on chickens in an area. With the exception of *O. sylviarum* and *M. stramineus*, the species we collected in backyard flocks have been rare or absent in commercial battery cage layer flocks in southern California over the past 30 years (Mullens, personal observation). Most commercial egg-laying chickens over the past several decades have been primarily maintained in battery or conventional cages, although this is changing. Increased consumer awareness and new animal welfare legislation are driving changes in chicken housing in the United States, especially in commercial industries. For example Proposition 2 in California, requiring enhanced space per bird, was passed in 2008 and implemented in 2015. Subsequently chicken egg production is shifting to furnished cage, cage-free, free-range, or pasture operations, with the latter mimicking many backyard flock habitats. These more open habitats will likely increase the risk of ectoparasite acquisition and transmission. Ectoparasite infestation increases bird stress and perhaps economic damage such as decreased egg production and feed conversion efficiency (Mullens et al. 2009). Backyard chicken flocks are likely to continue increasing in

popularity. Therefore more information regarding the biology and ecology of these lesser-known parasites is critical to developing effective management options.

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TABLES AND FIGURES

Table 4.1. Flock and bird prevalence (%) of ectoparasites found on chickens in southern California (2015).

		Flock Prevalence (%) n = 20	Bird Prevalence (%) n = 100
Phthiraptera	*Menacanthus stramineus*	50	36
	Menacanthus cornutus	5	5
	Menopon gallinae	15	11
	Cuclotogaster heterographus	5	5
	Goniocotes gallinae	35	20
	Lipeurus caponis	20	8
Siphonaptera	*Echidnophaga gallinacea*	20	18
Acari	*Ornithonyssus sylviarum*	15	7
	Dermanyssus gallinae	5	-
	Knemidocoptes mutans	10	-

Table 4.2. Ectoparasite species richness on individual chickens which had infestations of multiple ectoparasite species. The left hand column shows the percent of birds with each parasite combination.

% n = 100	*Menacanthus stramineus*	*Menopon gallinae*	*Cuclotogaster heterographus*	*Goniocotes gallinae*	*Lipeurus caponis*	*Echidnophaga gallinacea*	*Ornithonyssus sylviarum*
5	X			X			
3	X		X				
1	X			X			
3	X					X	
4		X				X	
3				X		X	
1					X	X	
1	X			X	X		
1	X	X				X	
2	X			X		X	
1		X		X	X		
2	X	X		X	X		
3	X	X		X		X	
1	X				X	X	X



Figure 4.1. Map (via Google Earth) of southern California showing study sites (pins) relative to the University of California, Riverside (star) and Los Angeles (diamond).



Figure 4.2 Ectoparasites were collected by either spraying birds with liquid pyrethrin (left) or bathing birds in soapy water (right).

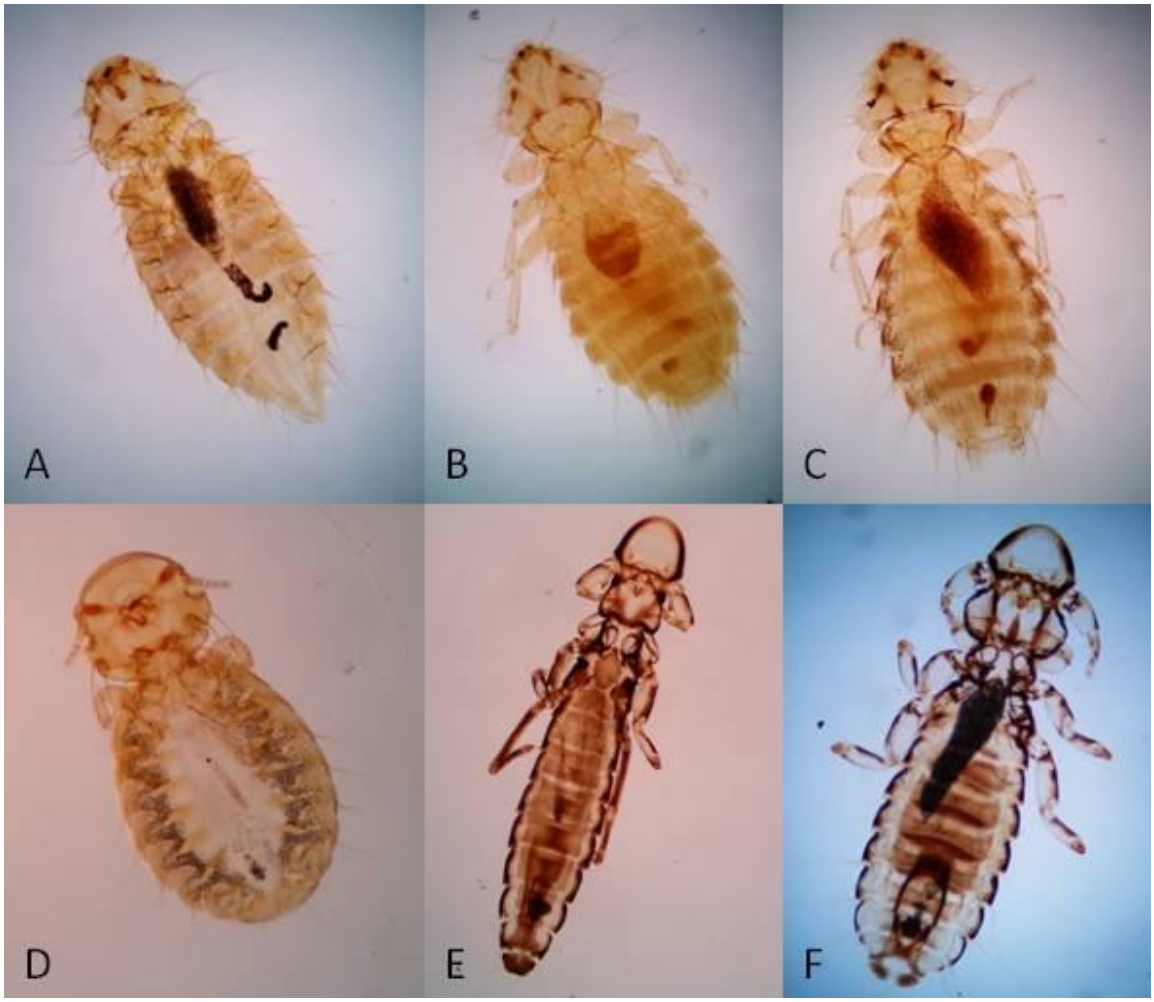


Figure 4.3. Chicken lice (not to scale) collected in survey. (A) *Menopon gallinae;* (B) *Menacanthus cornutus;* (C) *Menacanthus stramineus;* (D) *Goniocotes gallinae ;* (E) *Lipeurus caponis;* (F) *Cuclotogaster heterographus.*

CONCLUSION

The northern fowl mite, *Ornithonyssus sylviarum*, is the most damaging ectoparasite on egg-laying hens in the US and is likely to remain problematic despite changing poultry housing. However current methods for managing this ectoparasite are unlikely to work in enriched, cage-free, or organic operations.

One potential strategy for management is breeding for mite-resistance. Genes of white leghorn chickens linked to the major histocompatibility complex (MHC) were previously identified as conferring more (B21 haplotype) or less (B15 haplotype) mite-resistance. However, immune responses can be energetically costly to the host. We tested energy costs (resting metabolic rate) of mite infestations on egg-laying birds of both MHC B-haplotypes. Resting metabolic rates were documented before (pre-) mite infestation, during (mid-) infestation, and after peak (late) mite infestation. Mite scores, economic parameters (egg production, feed consumption) and physiological aspects such as skin inflammation and skin temperature were recorded weekly. Across experiments and different infestation time points, resting metabolic rates generally were not affected by mite infestation or haplotype, although there were instances of lower metabolic rates in infested versus control hens. Feed conversion efficiency was significantly worse in birds infested with mites. Overall egg production and average egg weight were not affected significantly, although there was a trend toward reduced egg production by infested hens.

No significant differences in resting energy expenditure related to mite infestation or immune responses were detected. There were no obvious negative effects of hen

haplotype/immune response on metabolic or economic factors; mite presence was the most important effect. Breeding for resistance to mite infestation or developing vaccines using these two haplotypes appears to be neutral (no detectable negative effects) in terms of impact on hen energy costs or production efficiency, and may be an attractive option for future mite control.

As we transition away from pesticide sprays for control of poultry ectoparasites, management tactics that can be implemented quickly will be needed for ectoparasite control. Dustbathing behavior is encouraged in newer production systems for increased welfare and has potential for ectoparasite control when substrates with insecticidal dusts, like diatomaceous earth, are included. Diatomaceous earth (DE) is an approved organic insecticide that can be mixed with sand in dustboxes, suppressing mites but not excluding them, potentially allowing development of mite immunity. We tested if DE-filled dustboxes placed before NFM introduction would prevent mite populations from reaching economically damaging thresholds, and to see if bird exposure to low mite numbers would allow protective hen immunity to develop and suppress mites even after dustboxes were removed.

Average mite densities in flocks remained below damaging levels while dustboxes were available. Average mite populations increased rapidly after dustbox removal (even though DE persisted in the environment) regardless of the timing of removal. There was not a powerful or immediate mite immune response in these ISA brown hens, but other hen strains might have a more robust response.

We found that mite densities on birds where a traditional hot blade beak trimming technique was used (trial 1) were high compared to trial 2 mite densities where a newer precision infra-red trimming was used. The newer infra-red trimming method resulted in nearly intact beaks, which were better for mite control by bird grooming behaviors. The integration of early dustbox use and infra-red beak trimming should allow producers to avoid most mite damage on cage-free flocks.

While dustboxes filled with desiccant dusts have proven to be a promising parasite management tactic in cage-free systems, alternative controls are still needed in furnished or enriched cage systems. We tested the efficacy of sulfur dust deployed in "dust bags" for control against the northern fowl mite. We also tested permethrin-impregnated plastic strips, marketed for use in caged or cage-free commercial and backyard flocks. Sulfur has been shown to be very active against this mite species, however we found that the placement of bags was important for mite control. Sulfur in hanging bags reduced mites on treatment birds significantly within one week of being deployed, and mite counts on these birds were zero after just 2 weeks. Sulfur bags clipped to the front of the cage near the opening to the feeder acted more slowly and reduced mites in only one of two trials. In the trial where clipped sulfur bags reduced mites, counts had dropped to zero after 4 weeks. Permethrin strips had no effect on mite populations. This is probably due to mite resistance, even though this mite population had not been exposed to pyrethroids for several years. Sulfur bags should be useable in cage or cage-free systems.

While northern fowl mites have historically been the most important ectoparasite in domestic poultry, this could change as birds move into more complex environments with access to soil, feces, and wild animals. We surveyed backyard chicken flocks in southern California as an indicator of what species cage-free birds are encountering. Biosecurity in backyard flocks is generally minimal or absent, thus we expected to find more ectoparasite species than what would perhaps be found on commercial flocks.

Of the 20 properties surveyed in summer for on- host (permanent) and off-host dwelling (temporary) ectoparasites, only 4 properties (20%) were entirely free of ectoparasites. Permanent ectoparasites found at remaining properties included six species of chicken lice, one species of flea, and three parasitic mite species. Off-host-dwelling ectoparasites were rare. Nearly one-third of birds were parasitized by multiple species.

Parasite diversity in the backyard flocks surveyed greatly exceeds what is routinely found on commercial caged-chicken flocks and highlights a need for increased biosecurity and development of ectoparasite control options for homeowners. All of the species collected in this survey are cosmopolitan in distribution and are likely potential threats for commercial poultry. Little is known about the life histories, economic damage, or potential control options of many of the parasites collected in this survey. In addition, we do not know how infestations with multiple parasite species may affect chicken health, economic impact, or interact with one another. Studies have shown that infestations with northern fowl mite decrease to zero with the introduction of the chicken body louse. Understanding how ectoparasite interactions with one another and the host can possibly lead to the development of better management tactics, including even

biological control. Future surveys of commercial cage-free, free-range, and pastured flocks will help to identify specific industry ectoparasite threats.

The US is undergoing an unprecedented shift in poultry production. Changes in how poultry are managed have historically been driven by economics and evidence-based research and technology. Now, consumer demand is the driving force telling producers how they want their egg-laying chickens raised. Retail and commercial egg buyers are trending towards cage-free egg consumption. The amount of food grown in this country is increasing while the number of farmers is decreasing, which has led to a huge disconnect from farm-to-table; there is no "right" answer when it comes to which poultry housing system is best for hen health and welfare.

Concerning animal health, ectoparasite threats are generally lessened in caged-birds. Many of the ectoparasites that were found in the backyard flock survey have not been found in commercial flocks for decades, primarily due to housing shifts. Any successful ectoparasite control will likely have to integrate multiple control tactics to develop an effective strategy.

Poultry housing systems are becoming increasingly complex and will require entomologists to work in concert with poultry scientists, veterinarians, and producers to optimize and balance bird health and welfare.