IMPACT OF THE NORTHERN FOWL MITE ON LAYING HEN PRODUCTION AND WELFARE BEGINNING AT 17 WEEKS OF AGE

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Dedicated to my friends, family, and colleagues

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ABSTRACT

The northern fowl mite (*Ornithonyssus sylviarum*) is an obligate blood-feeding ectoparasite of poultry that can cause decreased egg production, profit loss, anemia, irritation to flocks and personnel, and death to hens in extreme cases. This study aimed to investigate the effects of the northern fowl mite (NFM) on laying hen performance and welfare quality beginning at 17 weeks of age. Two flocks (Trials 1 and 2) involved 800 Tetra Brown hens (n=200 per room) housed in four cage-free rooms at the Purdue University Poultry Unit. Two rooms were infested with NFM and two rooms served as controls. In Trial 1, initial NFM infestation occurred at 24 weeks on 2% (4 hens) in each of the NFM rooms. Because NFM populations were scarce, a second infestation on 2% of hens occurred at 35 weeks. A final attempt to infest with NFM occurred at 41 weeks with all hens in NFM rooms being infested. In Trial 2, initial NFM infestation occurred at 24 weeks on 2% (4 hens) in each of the NFM rooms and a second infestation took place on 2% of hens at 30 weeks of age to boost the NFM population. Egg production and mortality were recorded daily and case weights were recorded weekly. Monthly Welfare Quality® assessments were taken, as well as monthly mite counts on all birds beginning at 28 weeks. Mite checks were conducted periodically on 25% (50 hens) in all rooms between weeks 25 and 38. Data were analyzed in SAS® using the GLM procedure and an ANCOVA and all significant statistical differences were reported at $P < 0.05$. Proportion of hens with a mite infestation was treated as the covariate. In Trial 1, regardless of treatment, mortality increased dramatically after 21 weeks, leading to a loss of 473 hens by period 7. NFM hen-day production percentage (HD) was approximately 2% lower than the Tetra management guide and control HD was 7% higher than the Tetra management guide. In Trial 1, treatment had an effect on HD, case weights, and feather damage on the belly ($P < 0.05$). Proportion of hens infested with mites had an effect on percent shell and feather damage on the belly. Cannibalism and pecking in Trial 1 led to extreme feather loss, high mortality, and negatively impacted production parameters. Feather loss and high mortality contributed to the low NFM populations. In Trial 2, percent livability remained high (approximately 97% for both groups) and HD remained slightly lower than the Tetra management guide (9% and 8% lower for control and NFM), respectively. In Trial 2, treatment had an effect on eggs per hen housed, mortality, feather damage on the head and neck, and skin lesions ($P < 0.05$). Proportion of birds infested with mites had an effect on feather damage on the back, crop, head, and neck ($P < 0.05$).

CHAPTER 1. REVIEW OF THE LITERATURE

1.1 Introduction

The northern fowl mite (*Ornithonyssus sylviarum*) (Canestrini and Fanzago) is the most common ectoparasite of poultry in the United States and causes significant economic losses through the mites' direct blood-feeding on the bird. Due to the bite a host immune response is induced, which results in reduced egg production and feed conversion efficiency (Harris et al. 2000, Murillo and Mullens 2016a, Vezzoli et al. 2016). A study conducted by Mullens et al. (2009) showed economic losses of $0.07-0.10 per hen over a 10-week period or a 2.1-4.0% decrease in hen-day production due to a northern fowl mite infestation. Severe infestations occur mostly in laying hens or breeding facilities because flocks are raised for a longer period of time, which allows mite populations to grow and flourish (Mullens et al. 2009), and mites can be introduced into a flock via infested pullets, personnel, and equipment (Axtell and Arends 1990, Kells and Surgeoner 1997, Murillo and Mullens 2016a). These mites cause irritation to flocks and personnel working in poultry houses, and the newly reintroduced trend toward non-cage housing or exposure to the outdoor environment may increase risk of parasite burden, which reduces hen welfare (Harris et al. 2000, Klipinen et al. 2005, Lay et al. 2011). The U.S. egg production industry is undergoing a shift away from conventional caged egg production towards alternative housing systems such as cage-free or enriched colony cage housing (Petrik et al. 2015, Campbell et al. 2016, Ochs et al. 2018). Consumers, retailers, and legislation in the U.S. and elsewhere (such as the European Union) have encouraged the shift as concern for animal welfare in confined production systems grows (Lay et al. 2011, Zhao et al. 2015, Ochs et al. 2018).

1.2 Northern Fowl Mite (*Ornithonyssus sylviarum*)

1.2.1 History and Biology of the Arthropod Ectoparasite (Northern Fowl Mite)

The northern fowl mite (NFM) was first discovered in 1917 in Beltsville, Maryland and then appeared in Raymond, Illinois in 1919 (DeVaney et al., 1977). This ectoparasite began to spread throughout the United States and was recognized as a potentially detrimental poultry pest by Wood in 1920 and has been flourishing for nearly a century (Murillo and Mullens 2017). NFM are hematophagous (blood-feeding) and are a permanent ectoparasite, meaning that they complete their life cycle on-host, which ranges from 5 to 12 days (Loomis et al. 1970, DeVaney 1979, Mullens et al. 2009). Mites harbor around feathers of the vent region on birds due to the lower skin temperature and higher humidity (De La Riva et al. 2015), and there they will lay eggs and shed skin and waste. The abundance of down-like feathering in the vent region creates a nest-like environment for the mites and will result in clusters of matted feathers that appear to be gray-black or "dirty". This is a result of clusters of mites, their feces, eggs, and egg cases in the vent region and is a tell-tale sign of an infestation (DeVaney and Augustine 1987, Vezzoli et al. 2016). The mites primarily reside on these vent feathers and migrate to the skin surface to complete a blood meal (Owen et al. 2009).

Because their life cycle is short, NFM can build up a large population very quickly (Axtell and Arends 1990, Harris et al. 2000, Mullens et al. 2004). NFM have an advantage in reproduction because they use oedipal mating, which means that unmated females lay unfertilized eggs that produce male offspring. They then mate with the male offspring, which produces female offspring, so essentially only a female mite is needed to continue the life cycle (McCulloch and Owen 2012). Within 48 hours of taking a blood meal, the adult female will lay an average of 2 to 3 eggs (Chen and Mullens 2008). NFM have five life stages: egg, larva, protonymph, deutonymph, and adult (Lemke and Collision 1985). Larvae are hexapod with undeveloped mouthparts and do not participate in blood-feeding; only protonymphs and adults are the blood-feeding life stages (Lemke and Collision 1985, Harris et al. 2000, Chen and Mullens 2008).

NFM can survive off-host for up to 1 to 3 weeks depending on temperature and humidity, which enhances their chance for transmission between flocks (Axtell and Arends 1990, Kells and Surgeoner 1997, Mullens et al. 2009). Chen and Mullens (2008) investigated temperature and humidity effects on off-host survival of NFM. The study found that the protonymph stage survived longer on average than the adult stage, and higher temperatures and lower humidity levels significantly reduced survival of both adult and immature stages of NFM. Therefore, populations of NFM tend to decrease in the summer months when temperature increases. They found maximum survival to be 35 days and 29 days for adults and protonymphs, respectively.

1.2.2 How the Northern Fowl Mite Affects Production Parameters

The northern fowl mite has the potential to cause significant economic loss in egg production systems through direct blood-feeding. These economic losses can equate to \$0.07-0.10 per hen, which is significant on a large production scale (Mullens et al. 2009). NFM cause a host immune response in hens once infestations increase and this leads to decreased egg production, reduced feed conversion efficiency, depressed body weight gain, and even anemia or death in severe infestations (Owen et al. 2009, Mullens et al. 2009, Loomis et al. 1970, Vezzoli et al. 2016). They cause inflammation and scabbing in the vent region on hens, which leads to bird irritability (Loomis et al. 1970). Mullens et al. (2009) found that northern fowl mites depressed individual hen weight gain in a high or moderate infestation, but heavier hens tended to harbor more mites than those that were lighter.

Vezzoli et al. (2016) found that NFM infestations can negatively impact interior egg quality as mite populations increase. Vezzoli et al. (2016) found a reduction in egg weights and egg specific gravity. DeVaney (1981) conducted a study that examined effects of NFM on egg quality in White Leghorn hens and found that eggshells of infested hens were thicker; however, eggshell thickness still fell within the normal biological range. DeVaney also found that in White Leghorn hens, a heavy infestation causes a 5-15% decrease in egg production without decreasing fertility or hatchability.

Northern fowl mites are most damaging early on in a new flock during the first egg production cycle, when hens are immunologically naïve and infestations above 25% of the flock population are considered to be economically damaging (Harris et al. 2000). DeLoach and DeVaney (1981) found that a heavy infestation can result in a daily loss of 6% of a hen's blood per day. Increasing populations of NFM in the study ingested up to 200 microliters of blood per day per 100 mites.

1.2.3 Poultry Host Immune Response to the Northern Fowl Mite

A study conducted by DeVaney and Augustine (1987) found that there was a rapid increase and then decline in northern fowl mite populations on infested hens, which suggested hens mounted an immune response to the ectoparasite. The authors noticed that mite populations declined more quickly in hens infested with larger populations of mites. When examining the sera, they found that the northern fowl mite induced an antibody response in the hens and a steady reduction in mite populations began very shortly after this antibody was detected. Minnifield et al. (1993) noticed that serum antibodies were produced in hens to combat Northern Fowl Mite proteins, which suggests a natural immunity that forms in hens as a result of the mites taking a blood meal. Because northern fowl mites feed multiple times, this could contribute to a decline in mite populations over time. While an infestation causes reduced egg production and egg weights and depressed feed conversion efficiency, it causes red skin and scabbing in the vent area of hens where mites reside (Vezzoli et al. 2016). Owen et al. (2009) investigated this skin inflammation on infested hens and how it related to the hen immune response to the northern fowl mite. The study reported that the host exhibits an inflammatory response to the mites, which increases distance to blood vessels beyond the length of the NFM mouthparts. This prevents both the protonymph and adult stages from blood-feeding, which further prevents development and reproduction. The present study found mite-specific antibodies in hens that increase with higher intensities of infestation. Northern fowl mite populations moved as host inflammation occurred and spread – mites would move away from inflamed areas but as the skin recovered, they would recolonize those areas.

1.2.4 Northern Fowl Mite Spread and Control within Poultry Systems

Northern fowl mite infestations typically occur in egg production or breeder systems, where hens live for long periods of time, giving mites an opportunity to grow and flourish (Axtell and Arends 1990). Northern fowl mites can be transmitted via personnel, wild birds and rodents, contaminated equipment, and can remain in poultry houses if the cleanout process between flocks is insufficient because mites can survive for weeks without a host (Kells and Surgeoner 1997, Mullens et al. 2001). Multiple studies have shown that northern fowl mites can disperse well to hosts across cages by walking along cage equipment; however, they generally will not spread between hens until population on an individual hen becomes moderate or above 50 mites per hen (Mullens et al. 2001, Mullens et al. 2009). They can be found on eggs and can migrate via egg belts, where they will crawl off eggs (Mullens et al. 2009).

Northern fowl mites experience their longest longevity in 75-80% humidity and at lower temperatures. Mites in all stages of development are found on the host during an infestation, and individual hen NFM populations usually peak at 3 to 6 weeks post-infestation; however, mite populations vary among birds of the same age and strain (Abasa 1969). Severe infestations can reach above 50,000 and even 100,000 mites per bird in some cases, which can cause severe hen blood loss (Matthysse et al. 1974, DeLoach and DeVaney 1981, Owen et al. 2009). When mite populations become high on an individual hen, mites will migrate to other areas of the bird (such as the neck or breast) or will fall off and persist in the environment (DeVaney et al. 1977, Lemke and Collision 1985, DeVaney and Augustine 1987, Mullens et al. 2009).

Because the entire northern fowl mite life cycle can be completed on their host in 5 to 12 days, a population can spread rapidly in confined systems, which is what the industry typically uses (Mullens et al. 2001). DeVaney (1980) found that in a conventional cage system, mites spread along the length of a poultry house within one month after one hen was identified to have a low NFM population. As the poultry industry moves away from traditional conventional cages and toward enriched-cage or cage-free settings, ectoparasite control becomes more challenging because the traditional high-pressure insecticide treatments are difficult to use in these more complex systems and because these products must reach below the feather layer to be effective (Murillo and Mullens 2016b). Ectoparasite management and control is related to the type of housing and equipment used in poultry housing, manure disposal, and environmental quality in the house (Axtell and Arends 1990), and environments where hens are exposed to litter or soil (cage-free or outdoor systems) provide a greater opportunity for disease and parasites (Lay et al. 2011). Non-cage or cage-free poultry production systems offer environments where ectoparasites are able to flourish because of the complex environments that offer daytime harborage (Lay et al. 2011) and mite population levels are influenced by factors such as hen physical condition and stocking density (Lemke and Collision 1985).

Prevention is a helpful means of controlling ectoparasite populations through disinfection of poultry houses between flocks, preventing wild bird or rodent access, and monitoring movement of equipment or personnel between houses (Mullens et al. 2004). Early treatment is more effective at controlling mite intensity (Mullens et al. 2009), and some practices such as providing dust boxes for hens or beak trimming have proven to be effective (Mullens et al. 2010, Lay et al. 2011, Martin and Mullens 2016). Dustbathing is a natural behavior in poultry that aims to maintain feather quality through removal of excess lipids, and this behavior is encouraged in newer production systems for hen welfare (Martin and Mullens 2012, Murillo and Mullens 2016a).

While early treatment is a better option in ectoparasite control, dust boxes have also shown to reduce mite populations even after an infestation has already occurred (Murillo and Mullens 2016a). Diatomaceous earth and sulfur are effective in dust boxes; however, sulfur has proven to be most effective because it causes a decline in mite populations on all hens, even those that do not use the dust box, and even after dust boxes are removed (Martin and Mullens 2016). Murillo and Mullens (2016b) conducted a study using powdered sulfur in hanging bags in a caged egg production setting. This study found that sulfur reduced mites by 95-97% within one week of being added, so sulfur is an extremely effective means of mite control. Beak trimming has a large effect on ectoparasite populations, and birds with intact beaks are able to groom themselves more efficiently, which dramatically reduces northern fowl mite populations (Mullens et al. 2010). Murillo and Mullens (2016a) found that mite density was high on birds beak trimmed using a hot blade but were lower on infrared beak trimmed birds because the beak was nearly intact with infrared trimming. Typically, beak trimmed birds harbor 3 to 10 times more mites than birds with intact beaks because feather grooming ability is impaired when beaks are not fully intact (Lay et al. 2011).

1.2.5 The Northern Fowl Mite as a Vector of Disease

Valiente Moro et al. (2007) conducted a study that examined infection of *Salmonella enteriditis* of the poultry red mite (*Dermanyssus gallinae*), both through a blood meal or cuticular contact. The study found that mites became carriers of *Salmonella* immediately after infection, and the pathogen multiplied inside previously infected mites. This brings to question whether the Northern Fowl Mite, *Ornithonyssus sylviarum*, could be a vector of *Salmonella* spp.

1.3 Cage-Free Egg Production

1.3.1 History and Industry Trend Toward Cage-Free Egg Production

In recent years, egg production systems have been subject to scrutiny as consumers have grown more concerned about where their food comes from, how it is produced, and the effects that production may have on animal welfare and environmental sustainability (Lay et al. 2011, Ochs et al. 2018). This pressure placed on the egg production industry has led to a shift from traditional conventional cages to alternative housing systems, and the pressure is due largely in part to legal changes in the U.S. and responses from retailers and restaurants (Petrik et al. 2015, Campbell et al. 2016, Ochs et al. 2018).

With the increasing concerns about hen welfare in caged systems, some U.S. states have passed legislation requiring increases in hen space allocations, or even phasing out conventional cage systems altogether (Zhao et al. 2015, Ochs et al. 2018). Some food retailers and restaurants in the

U.S. have pledged to source only cage-free eggs (Oliveira et al. 2019); McDonald's began using cage-free eggs in 2015 and by the end of April 2016, nearly all of the top U.S. grocery chains developed timelines for shifting entirely to cage-free eggs (Ochs et al. 2018).

The European Union moved to ban conventional cages in 1999, and by 2012 the ban had taken total effect. This ban led to development of alternative, non-cage housing systems such as aviaries or single-tier floor systems as a replacement for battery cages (Mertens et al. 2009, Mench et al. 2011). These alternative systems are more extensive and provide larger living spaces with perches, nest boxes, and areas for foraging and dustbathing, where hens' natural behaviors can be accommodated (Mench et al. 2011, Zhao et al. 2015). In the U.S., the majority of egg production takes place in conventional cage systems; however, production in alternative systems is growing (Mench et al. 2011, Zhao et al. 2015). Eggs are one of the primary sources of protein worldwide. As of November 2019, the U.S. national egg production flock was comprised of 340 million commercial laying hens, of which 19.6% was housed in a cage-free setting (Egg Industry Center 2019). Cage-free egg production is increasing – nearly 18% of the U.S. commercial flock (328 million hens) were housed in a cage-free setting in 2018, and as of March 2019 that number had increased to 18.4% (United Egg Producers 2019). According to United Egg Producers (2019), the USDA's Agricultural Marketing Service estimated that 71% of the U.S. commercial flock must be in cage-free production by 2026 in order to meet demand.

1.3.2 Conventional Cage Systems

The conventional cage system was developed in the 1930s, and widely adopted on a large scale in the 1950s with the intent to separate hens from feces in order to prevent transmission of disease and to increase cleanliness of eggs (Mench et al. 2011). These cages allowed for automatic feeding, watering, and egg collection, which increased efficiency, and this large-scale conventional egg production made eggs an inexpensive source of protein (Mench et al. 2011, Thompson et al. 2011). Originally, conventional cages were designed to hold only one hen per cage, but larger cages for groups of hens took their place to increase economic efficiency by increasing stocking density (Craig and Swanson 1994, Mench et al. 2011). Conventional cages typically house at least four hens per cage with a minimum of 432.3 cm^2 (67 in^2) of space per hen. Each poultry house may contain thousands of cages per house for more than 100,000 hens per building. In an alternative system, such as cage-free aviary housing, which allows hens to roam throughout different levels or sections of the building, hens have a minimum of 929.03 cm^2 (144 in^2) of individual space. A typical aviary system holds around 80,000+ hens per building due to the increased space per hen (Karcher and Mench 2018, Ochs et al. 2018, United Egg Producers Certified 2019).

1.3.3 Non-Cage Alternative Housing Systems

There are many variants of non-cage, alternative housing systems; however, indoor non-cage systems fall either into the aviary or barn category and these systems house thousands to tens of thousands of hens per building (Mench et al. 2011, Karcher and Mench 2018). Aviary systems allow for use of the height in a building and involve multiple platforms, or tiers, where birds can move throughout the system (Mench et al. 2011). In an aviary system, the tiers provide space where hens can stand and exhibit natural behaviors such as perching, and ramps are provided within the system to allow hens to move throughout each tier. The floor is covered with a litter substrate that hens can access for foraging and dustbathing. Some aviary systems have additional doors that allow hens to be confined within the aviary system (away from the littered floor area) for a period of time in order to train hens to lay eggs within the system rather than on the floor (Karcher and Mench 2018). Each tier or platform is made of a wire or slatted floor with a manure belt underneath to allow for ease of manure collection (Xin et al. 2011, Karcher and Mench 2018).

A barn system is an open, indoor system with sections of raised slatted or wire flooring. Above this flooring are feeders, waterers, and nest boxes where hens can go to lay eggs and a manure belt is positioned below for ease of collection. The rest of the floor is covered in a litter substrate, where hens can dustbathe and forage and perches are often included in the house (Karcher and Mench 2018). These non-cage systems allow hens more space and opportunities to perform natural behaviors such as running, wing flapping, flying, nesting, dustbathing, and perching (Heerkens et al. 2015).

1.3.4 Costs and Benefits of Conventional Cage and Cage-Free Systems

Cage size and high stocking density in conventional cages does not allow for full expression of natural behaviors, and hens in a small cage will likely work to increase their individual space to perform certain behaviors (Lay et al. 2011). Lay et al. (2011) wrote that hens do not fully acclimate to spatial restriction because they will revert to behaviors like stretching and wing flapping when moved to larger spaces after periods of confinement. In addition to suppression of natural behaviors, high stocking density in conventional cages can increase difficulty of access to food and water and can also increase prevalence of negative behaviors such as smothering, feather pecking, and cannibalism – some of which can be combatted with the practice of beak trimming (Lay et al. 2011). Cunningham and Mauldin (1996) wrote that crowding and high-density situations have negative effects on production and welfare regardless of housing system used.

Bone fragility is another concern with conventional cages – hen bone strength is better maintained when birds have an opportunity to live a more active lifestyle (Craig and Swanson 1994, Newman and Leeson 1998). Osteoporosis in caged layers can become a problem, especially because today's high-producing hens are under a negative calcium balance when they enter the laying phase (Regmi et al. 2016). Alternative housing systems for laying hens can provide opportunity for behaviors such as running, flying, and jumping that allow for mechanical loading on the bones, which can help reduce bone loss (Regmi et al. 2015). In the study conducted by Regmi et al. (2015), pullets housed in an aviary system were found to have greater bone width and cortical thickness when compared to pullets housed in a conventional cage system, and this study indicated that mechanical loading activities on the skeleton can improve load-bearing capability on the tibia and humerus, specifically.

Conventional cages lack foraging materials for ground pecking and dustbathing, resulting in sham dustbathing. When hens are unable to perform dustbathing, plumage is likely to be dirtier and less functional from an insulative standpoint (Lay et al. 2011). In conventional cage systems, some strains of hens may perform stereotypic behaviors, such as pacing prior to oviposition due to frustration because of the lack of access to nests or nest building materials, and the presence of stereotypic behaviors may be an indicator of reduced welfare (Craig and Swanson 1994, Lay et al. 2011). A study conducted by Mench and Blatchford (2014) involved a kinematic analysis of space use by White Leghorn hens to stand, turn around, lie down, and wing flap. The study concluded that providing different amounts of space could affect the expression of certain behaviors due to the amount of space required to perform those behaviors and due to social interactions of hens.

Lay et al. (2011) indicated that multiple factors influence the level of welfare hens experience and no housing system is perfect. Non-cage systems have many advantages, but also drawbacks. In complex environments that involve litter such as non-cage systems, cleaning the system will be more difficult and opportunities for disease and parasites to spread are more likely. Perches in non-cage systems can have the added benefit of an increase in natural behaviors such as flying and perching and can improve bone strength; however, landing failures can occur when jumping to and from perches, resulting in bone damage (Lay et al. 2011). Petrik et al. (2015) found an increase in keel bone fractures in a single-layer floor (barn) system. Regmi et al. (2016) conducted a study involving a comparison of bone properties and keel deformities between three layer strains and three housing systems – conventional cage, cage-free, and free-range. This study reported that each housing system had a high prevalence (greater than 90%) of keel deformities, but that the housing system and the genotype of the hen did influence the keel deformities.

Management of the littered floor can have an effect on air quality in a system – adequate ventilation and replacement of the litter with fresh bedding can help combat high ammonia levels that can occur in these types of systems. The presence of litter tends to lead to higher dust concentrations and emissions (Xin et al. 2011). In non-cage systems, floor-laid eggs can also become a problem, which can trigger broodiness in some hens and can also lower egg quality (Lay et al. 2011). Holt et al. (2011) wrote that moving from a conventional cage system to an enriched colony cage or non-cage system may affect egg quality and safety and Jones et al. (2015) examined the microbiological impact of three commercial laying hen housing systems – conventional cage, enriched colony cage, and cage-free aviary. Jones et al. (2015) came to the conclusion that floor eggs can be exposed to high levels of microorganisms and pathogens, posing a risk for improper egg safety and quality. Management of housing systems is important, and reduction of the occurrence of floor-laid eggs is ideal because nest box usage by hens can enhance egg safety by separating eggs from feces. Hen strain can also impact egg microbiology and safety in different housing systems, and Jones and Anderson (2013) reported that egg safety should be considered when making a selection on hen strain for each housing system, whether it be cage or non-cage.

In the end, there are multiple housing systems used by the U.S. egg production industry, all with different costs and impacts on hens, workers, and the environment (Ochs et al. 2018) and, while cage-free production has many benefits, there are also challenges that need to be addressed (Oliveira et al. 2019).

1.4 Cannibalism and Beak Trimming in Cage-Free Systems

While cage-free systems provide hens with more space to roam and exhibit natural behaviors, these systems can also encourage negative behaviors such as cannibalism and feather pecking (Lay et al. 2011). Cannibalism is one of the largest problems in alternative housing systems and poses a significant welfare issue for laying hens as it causes fear, stress, and an increased risk of hen mortality (Mertens et al. 2009, Heerkens et al. 2015, Louton et al. 2017, Brantsaeter et al. 2018). Fear and stress due to feather pecking and cannibalism can lead to decreased egg production (Mertens et al. 2009, Sun et al. 2014). Hens housed in non-cage systems are at an increased risk for mortality and feather pecking when compared to cage systems because outbreaks of these negative behaviors are difficult to stop, especially since it is difficult to identify the source hen (Heerkens et al. 2015, Cronin et al. 2018).

Cannibalism can lead to death from tissue trauma and hemorrhage inflicted by cage mates or pen mates and is often a learned behavior via visual contact or observational learning (Tablante et al. 2000, Cronin et al. 2018). Feather pecking is defined as pecking at and pulling out feathers of other birds, and both feather pecking and cannibalistic behavior damage the skin and underlying tissue of the hen (Sun et al. 2014, Heerkens et al. 2015). Vent-pecking, or cloacal cannibalism, is different from feather pecking and occurs due to prolapses or tearing of the vaginal mucosa during oviposition that exposes the cloacal mucous membranes, which may attract other hens to peck at the vent (Tablante et al. 2000, Louton et al. 2017). The continuous irritation of the exposed mucous membranes may lead to further prolapse of the oviduct, which may in turn lead to more pecking that often leads to death (Tablante et al. 2000). Severe feather pecking involves breaking off part or pulling out the entire feather of a hen, causing plumage damage and exposing bare skin, which attracts pecking that may lead to cannibalistic behavior and eventual death. This type of feather pecking typically begins around the base of the tail or the rump of the bird and is considered an abnormal behavior that can cause fear and pain for the recipient hen (Cronin et al. 2018).

Cannibalism and feather pecking tend to be exacerbated if hens' beaks are left intact, probably due to the large flock sizes and rapid spread of the behavior through observational learning (Lay et al. 2011). According to Craig and Swanson (1994), cannibalism and feather pecking without the presence of beak trimming can become a major problem in floor pen flocks. This feather pecking behavior is believed to be redirected ground pecking (Heerkens et al. 2015); hens' ability to forage or search for food is dependent on the housing system, and in commercial egg production the hen group sizes are much larger than the social groups in the wild, so the two may be linked (Brantsaeter et al. 2018). Louton et al. (2017) wrote that higher mortality due to cannibalism occurs in larger groups. Many factors affect the instance of feather pecking: some strains are more genetically predisposed to it and lighting, nutrition, enrichment, or management during hatching or rearing can be factors as well, and eventually feather pecking will lead to cannibalism (Mertens et al. 2009, Louton et al. 2017, Brantsaeter et al. 2018).

Beak trimming is a common practice to prevent feather pecking and cannibalism and has long been used (Cunningham 1992, Louton et al. 2017, Cronin et al. 2018). While beak trimming cannot fully prevent these behaviors (Louton et al. 2017), Tablante et al. (2000) writes that cannibalism often becomes severe unless the birds have been properly beak trimmed. Craig et al. (1992) writes that if properly done, beak trimming can provide insurance against these behaviors, which are likely to happen otherwise. Mertens et al. (2009) and Sun et al. (2014) found that survival of hens that were beak trimmed was much higher than those that had not been beak trimmed. Beak trimming can also improve feed efficiency, because birds with intact beaks seem more likely to flick feed out of the trough (Cunningham 1992). Management strategies can be used to prevent feather pecking, cannibalism (Louton et al. 2017), and aggression, such as giving chicks access to perches as early as four weeks of age (Lay et al. 2011).

1.5 Summary

A northern fowl mite infestation can negatively impact egg producers and laying hen welfare through mites' direct blood-feeding on the birds. This negative impact is translated to economic losses as a result of reduced egg production and feed conversion efficiency, along with irritation to hens and personnel working with infested flocks (Loomis et al. 1970, Mullens et al. 2009). These ectoparasites can travel between flocks via infested personnel, equipment, or can persist in housing systems between flocks (Mullens et al. 2001, Mullens et al. 2009).

As the U.S. egg production industry makes the shift towards alternative, non-cage housing systems such as a cage-free aviary or cage-free barn setting, the impact on animal welfare, egg production, and ease of management of the system as a result of this change needs to be considered. Non-cage systems have many advantages, such as providing the opportunity for exhibition of natural behaviors or load-bearing exercises that can prevent the onset of osteoporosis, as is an issue in caged laying hens (Lay et al. 2011, Heerkens et al. 2015, Regmi et al. 2016). These non-cage systems also have drawbacks, such as landing failures that can result in bone damage (Lay et al. 2011, Petrik et al. 2015) or difficulty cleaning the system that may result in additional opportunities for disease and ectoparasites (such as the northern fowl mite) to spread (Lay et al. 2011). In addition, cannibalism is an abnormal behavior that occurs both in conventional cages and cage-free systems; however, it is more difficult to stop in a cage-free system because the source hen is more difficult to identify and it is a learned behavior that can spread over time (Tablante et al. 2000, Heerkens et al. 2015, Cronin et al. 2018).

While there is information regarding the impact of the northern fowl mite on hen performance and welfare, a better understanding of NFM impact in a cage-free setting is necessary as the current shift away from conventional cage laying hen systems is occurring. This master's thesis aims to investigate the impact of the northern fowl mite on laying hen performance and welfare in a cage-free housing system and involves flock challenges with cannibalism.

CHAPTER 2. METHODS

2.1 Study Information and Hen Management

2.1.1 Management

This study involved the use of four cage-free rooms at the Purdue University Poultry Unit (Animal Sciences Research and Education Center, West Lafayette, Indiana) to house 200 Tetra Brown (TETRA Americana, Lexington, Georgia) laying hens per room (for a total of 800 hens). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC Protocol Number: 1706001582). Two of the four cage-free rooms (rooms 3 and 4) served as a control and two rooms (rooms 1 and 2) served as the treatment, or northern fowl mite infested rooms. The four rooms were located at the end of one wing of the layer building at the Purdue University Poultry Unit so that one would move in descending order from Room 4 to Room 1 and would exit the building at the end of the wing after Room 1. This allowed for proper biosecurity and prevented the spread of a northern fowl mite infestation to the control rooms or elsewhere in the building. Two flocks were examined as part of this project as Trials 1 and 2 and will be elaborated on below.

Water was supplied to the hens *ad libitum* and hens were hand-fed daily according to the Tetra Management Guide (Tetra Americana, 2017). Body weights were taken every 28 days and were used to adjust feed supplied to maintain body weights similar to what is found in the Tetra Management Guide.

Egg production and mortality were recorded daily and case weights were recorded weekly. Body weights, egg components, and Welfare Quality® assessments (WQ® Consortium, 2009) were taken for each period (every 28 days). In addition, fresh fecal pools, environmental samples, and egg samples were shipped to the Egg Safety and Quality Research Unit (ESQRU; USDA Agricultural Research Service, Athens, Georgia) for microbial analysis during each period. Every 12 to 14 weeks after 22 weeks of age, 54 eggs from the nest were shipped to the ESQRU for egg quality evaluation.

2.1.2 Laying Hen Room Information

Rooms 1 through 4 at the Purdue University Poultry Unit were fitted with the cage-free Big Dutchman Colony 2+ System (Big Dutchman USA, Holland MI). This system is a single-tier aviary system (or non-cage barn system) with 10 automated nest boxes, litter space with 12 perches, and a slatted floor area. Over the slatted area there were six hanging tube feeders and two bell drinkers. Eggs were able to roll from nest boxes onto a sedentary egg belt for daily collection. A table outlining the amount of resource per hen in each room (Table 2.1), lighting schedule during the laying period (Table 2.2), and a schematic of the room (Figure 2.1) are shown below.

Table 2.1. Amount of resource per hen in each cage-free room of the Colony 2+ System.

Item	Area	Number	Total[1]	Per hen
Area	27.8 m^2 (43,200 in^2)	1	27.8 m^2 (43,200 in^2)	1,393 cm^2 (216 in^2)
Feeder space	127 cm (50 in)	6	762 cm (300 in)	3.8 cm (1.5 in)
Bell drinker	109 cm (43 in)	2	218 cm (86 in)	1.0 cm (0.4 in)
Nest area	0.55 m^2 (864 in^2)	10	5.57 m^2 (8,640 in^2)	278.7 cm^2 (43.2 in^2)
Perch space			3,048 cm (1,200 in)	15.2 cm (6 in)

[1]Total space of each item (area of the item multiplied by number of items)

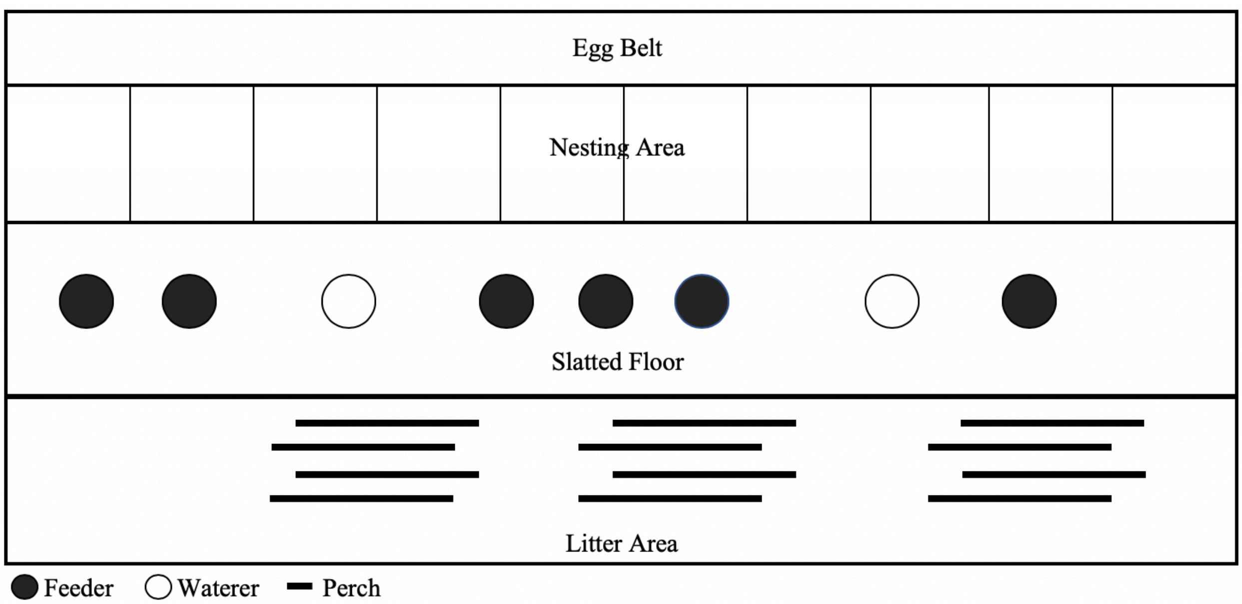

Figure 2.1. Schematic of the Colony 2+ cage-free room used during infestation of Tetra Brown laying hens with Northern Fowl Mites.

Table 2.2 Lighting schedule in each cage-free room from 17 weeks of age to termination of the project.

Age	Light Intensity	Hours[1]
17 weeks	10 to 5 lux	10
18 weeks	10 to 5 lux	11
19 weeks	10 to 5 lux	12
20 weeks	10 to 5 lux	13
21 weeks	10 to 5 lux	13.5
22 weeks	44 lux	14
23 weeks	44 lux	14.25
24 weeks	44 lux	14.5
25 weeks	44 lux	14.75
26 weeks	44 lux	15
27 weeks	44 lux	15.25
28 weeks	44 lux	15.5
29 weeks	44 lux	15.75
30 weeks to termination	44 lux	16

[1]Hours of light in the photoperiod.

2.1.3 Mite Infestation

Northern fowl mite infestation took place at 24 weeks of age for both flocks in Trials 1 and 2 in the present study. Northern fowl mites were sourced from commercial laying hen farms in Ohio and from a research facility at the University of California Riverside in Riverside, California. When sourced, northern fowl mites were kept in 50 mL conical tubes or were shipped from California in glass pipettes.

Initial northern fowl mite infestation occurred on 2% of hens (4 hens per NFM treatment room or 8 hens total) at 24 weeks of age for Trials 1 and 2. During infestation, hens were chosen at random from each of the two NFM treatment rooms (Rooms 1 and 2) for mite infestation. Each hen was captured and inverted to expose the vent area. Approximately 30 mites were shaken onto the vent area from either the glass pipettes or 50 mL conical tubes onto the vent area of each hen. The infested hen remained upside down for approximately ten seconds to allow the mites to crawl from the feathers to the vent skin. This process was repeated for each of the eight hens and is adapted from Martin and Mullens (2012).

2.1.4 Trial 1

The flock in Trial 1 was reared at the Purdue University Poultry Unit in the pullet barn according to the Tetra Management Guide (Tetra Americana, 2017). One thousand two hundred and five chicks were distributed among rooms 9 through 11 in the pullet barn for 400, 403, and

402 chicks per room, respectively. Chick placement was determined by average starting chick weight, which was derived from weighing chicks as a pen prior to placement.

Table 2.3 Amount of resource per hen in each pullet rearing room.

Item	Area	Number	Total[1]	Per pullet		
				Room 9	Room 10	Room 11
Area	37.7 m^2 (58,449 in^2)	1	37.7 m^2 (58,449 in^2)	941 cm^2 (146 in^2)	935 cm^2 (145 in^2)	937 cm^2 (146 in^2)
Feeder space	127 cm (50 in)	8	1,016 cm (400 in)	2.5 cm (1 in)	2.5 cm (1 in)	2.5 cm (1 in)
Bell drinker	109 cm (43 in)	4	436 cm (172 in)	1 cm (0.4 in)	1 cm (0.4 in)	1 cm (0.4 in)

[1]Total space of each item (area of the item multiplied by number of items).

Chicks were brooded on raised plastic platforms in the middle of the room and were given floor access at three weeks of age. Pullets were moved into rooms 1 through 4 in the layer barn at 17 weeks of age where they were maintained until 48 weeks of age. This flock arrived in March 2018 and was terminated in February 2019. Beak treatment did not occur at day of age resulting in cannibalism, so beak-modification occurred using a Dremel (Dremel 4000 Series, Dremel®, Racine, WI) at 20 weeks of age. A total of 25 replacement pullets, sisters of the original flock, were added to each of the four rooms at 21 weeks of age to offset mortality from cannibalism. One pullet was added to Room 4, nine pullets were added to Room 3, fifteen pullets were added to Room 2, and no replacement pullets were added to Room 1. Initial northern fowl mite infestation occurred on 2% of hens (4 hens per NFM treatment room or 8 hens total) at 24 weeks of age using infestation methods adapted from Martin and Mullens (2012) that are outlined in section 2.1.3. Mite populations remained low, so a second mite infestation took place on 2% of hens at 35 weeks of age and a final attempt to infest, to increase mite prevalence, occurred on all hens at 41 weeks of age. The study concluded at 47 weeks of age.

2.1.5 Trial 2

The flock in Trial 2 was purchased as started pullets from Herbruck's Poultry Ranch (Saranac, MI) at 15 weeks of age. This flock was beak-modified at the hatchery. Hens were transported to the four cage-free rooms in the layer building at the Purdue University Poultry Unit (n=200 hens per room). Data was collected during the laying period in this flock from 17 to 49 weeks of age. Trial 2 was carried out two weeks later in age than Trial 1 due to sampling dates and times in partnership with the USDA Egg Safety and Quality Research Unit (Athens, GA). The flock arrived in February 2019 and was carried out until October 2019. Initial northern fowl mite infestation took place on 2% of hens (4 hens per NFM treatment room or 8 hens total) at 24 weeks of age (outlined in section 2.1.3). A second mite infestation occurred on 2% of hens at 30 weeks of age to boost the mite population.

2.2 Data Collection

All data collected (as well as time points, i.e., daily, weekly, or monthly data collections) were the same for Trial 1 and Trial 2.

2.2.1 Egg Collection

Egg production for each of the four rooms was recorded daily into electronic databases (FileMaker Pro 18, Claris International, Inc., Santa Clara, CA) developed for this project. Eggs in each room were collected daily with floor and nest eggs included in the total egg count for each room per day.

2.2.2 Case Weights

Case weights for each of the four rooms were conducted every Wednesday beginning at 19 weeks of age through project termination. Due to biosecurity practices involved to prevent the spread of the northern fowl mite infestation, case weights in control rooms (4 and 3) were conducted prior to mite infected rooms (2 and 1).

Case weight data were entered into electronic databases (FileMaker Pro 18, Claris International, Inc., Santa Clara, CA) using iPads that remained outside of the four rooms. Tare weight of the pulp egg flats and total weight for the egg flats plus the eggs were recorded into the electronic database. The difference allowed for generation of the case weight (weight of the eggs minus tare weight of the egg flats) and average egg weights. When eggs were collected in a room, the stack of flats was placed on the scale (CPWplus 35, Adam Equipment Inc., Oxford, CT). This process was repeated for each room.

2.2.3 Mortality

Hen mortality (including culling when necessary) was recorded daily. Mortality data were documented in the databases and were recorded on the production data sheet placed outside each of the four rooms.

2.2.4 Mite Checks

From the time of northern fowl mite administration to 28 days after, 50 hens from each of the four rooms were selected at random throughout the entire room and examined weekly (n = 4 times) in order to investigate the spread and presence of mites. Mite populations were not quantified in this procedure.

Mite checks were conducted after lights turned off in each of the four rooms to facilitate catching hens. Four to five individuals inspecting birds spread out throughout the room and captured birds individually at random for assessment. Gloves were changed or washed between inspection of each bird in order to prevent the spread of mites through human contact. Examination began at the vent skin and around the cloacal area. The thigh area and the tip of the keel were also inspected. If at least one mite was found, the bird was deemed a "yes" (Y) and the hen was marked with a black livestock marker across the back and wings in a cross fashion. If no mites were found, the bird was deemed a "no" (N) and the hen was marked with a straight black line down the back. This process was repeated for each of the four rooms.

2.2.5 Mite Counts

After mite checks were completed once a week for 28 days post-infection with northern fowl mites, the first northern fowl mite count on all hens in all rooms was conducted. Mite counts continued every period (every 28 days) at the same time as Welfare Quality® assessments. This involved counting mite populations on individual hens using a scoring system adapted from Arthur and Axtell (1982) and Owen et al. (2009), shown in Table 2. Along with mite counts, welfare quality assessments and body weights were taken in all four rooms.

Table 2.4. Scoring system used for mite counts adapted from Arthur and Axtell (1982) and Owen et al. (2009).

Score[1]	Population[2]
0	0 mites
1	1-10 mites
2	11-50 mites
3	51-100 mites
4	101-500 mites
5	501-1,000 mites
6	1,001-10,000 mites
7	Greater than 10,000 mites

[1]Mite count score.
[2]Mite population level related to score.

In each room, birds were corralled to one side using an adjustable fence. As birds were inspected, weighed, and assessed, they were marked (using a black livestock marker) according to presence or absence of mites and were placed outside the fence. Hens without mites were marked with a black line down the back; hens with mites were marked with a black cross along the back and wings. This was done in order to avoid counting mites on an individual hen more than once. Fence size was adjusted as the number of birds left to sample decreased. Each hen in all four rooms was examined and mites were counted around the cloaca and vent, thighs, and tip of the keel, just as in mite checks. Mites were counted and an estimation score was given using the scoring system provided above. Hands were washed between handling of each hen in order to prevent the spread of Northern Fowl Mites through human contact. Two or three buckets of water mixed with dish soap were placed in each room for this purpose.

2.2.6 Welfare Quality Assessments

Every period, Welfare Quality® assessments (WQ® Consortium, 2009) were conducted in a similar manner to Regmi et al. (2018) and Weimer et al. (2019). All hens in rooms 1 and 4 were assessed and 20% of hens in rooms 2 and 3 were assessed. The reason for assessing all hens in rooms 1 and 4 was because of thermal images taken on hens in those two rooms for a separate project. Hens were assessed and body weights were recorded as mite counts were conducted. During WQ® assessments, each hen was examined for feather damage, comb abnormalities, footpad condition and toe damage, keel deformities, beak condition, skin condition, or abnormal conditions such as panting, enlarged crop, enteritis, or parasites. Data for each hen assessed was recorded into electronic databases (FileMaker Pro 18, Claris International, Inc., Santa Clara, CA) using iPads.

2.2.7 Environmental and Egg Sampling and Fecal Pools

Every 28 days (after start of lay), nine environmental swabs were taken per room and four six-egg pools were taken per room and shipped to the Egg Safety and Quality Research Unit (ESQRU; USDA-ARS, Athens, Georgia). For each room, four swabs from the nest boxes, four from the slatted floor, and one drag swab from the litter were taken. Twenty-four eggs taken from the egg belt were collected per room, pooled, and were sent for analysis with environmental swabs.

Fecal pools were collected from each of the four rooms every period and were shipped to ESQRU with environmental and egg pool samples. Four clean trays were placed in four grates directly underneath the slatted flooring in each of the four rooms the night before fecal pools were taken. Four 50 mL conical tubes (n = 1 conical tube per tray) were filled with fecal matter per room and were sent for analysis.

2.2.8 Egg Collection and Shipping for Egg Quality Measures

Beginning at 22 weeks of age, 54 eggs collected from the nest in each of the four rooms were sent to the USDA – ARS Egg Safety and Quality Research Unit in Athens, Georgia. Eggs were sent every 12 to 14 weeks. Further information and results pertaining to egg quality for this study can be found in Hull (2020).

2.2.9 Egg Components

Thirty nest-run eggs were collected each period from each of the four rooms for egg components. After egg collection, eggs were placed in the cooler in Creighton Hall of Animal Sciences. Once eggs had chilled for 24 hours, they were labeled with numbers 1 to 30 for each of the four rooms (n = 120 eggs per sampling) and individual egg weights were recorded.

Upon completion of labeling and egg weights, each egg was broken onto a break-out table. Egg components were conducted in the same manner as in Karcher et al. (2019). Each yolk was separated from the albumen and rolled in a paper towel to remove any remaining albumen. Each yolk was weighed immediately after rolling and weights were recorded. Weigh boats were labeled for each respective egg and shells were placed into the weigh boats as eggs were broken. Shells were washed in cold water to clean out any remnants of albumen. Once shells had dried in open air for a minimum of 24 hours, each shell was weighed and recorded. Three measurements of shell thickness were taken for each egg using a micrometer (Ames Series 25 Micrometer, B.C. Ames Inc., Framingham, MA). Three pieces were broken from each eggshell around the equator, approximately 120 degrees separate from one another. One measurement was taken per piece of shell and recorded. Albumen weight was recorded via subtraction of yolk and shell weight from whole egg weight.

2.3 Statistical Analysis

Two common metrics used by the poultry industry, hen-day production percentage and eggs per hen housed, were calculated from equations found in Anderson (2015). Hen-day production percentage was calculated by dividing the average number of eggs produced in a week by the number of hens present during that week for each room. Eggs per hen housed was calculated by dividing the total eggs produced in a week by the number of hens placed at the beginning of the study (n = 200 hens per room) and was calculated for each room. Values for the Tetra Brown management guide were added to graphs for hen-day percentage, eggs per hen housed, and percent livability in order to provide a baseline for comparison of flock production levels; however, values from the Tetra Brown management guide were not included in statistical analysis or post-hoc comparisons. Results were analyzed using the statistical programs SAS® University Edition and R (Version 3.5.1) was used to check outliers using Cook's Distance.

The GLM procedure of SAS® was used to detect effects from treatment, period, proportion of birds infested, or their interaction effects on each response variable. Within the GLM procedure of SAS®, an ANCOVA and Type III SS was used in this analysis, with proportion of birds infested with mites being the covariate. This covariate was the proportion of infested birds to non-infested birds recorded during mite counts at each period (every 28 days). The covariate was included to account for changes in spread of the mite infestation and to investigate changes in hen welfare and production as a result of changes in the mite infestation. Hen-day percentage, eggs per hen housed, percent livability, percent egg components, shell thickness, and Welfare Quality® parameters such as feather score, skin lesions, and presence of keel bone deformations were treated as individual response variables, while period (every 28 days), treatment (northern fowl mite or control), and proportion of birds infested with mites were treated as predictors and were analyzed simultaneously for each response variable. Least squared means were used to detect differences between treatment means and a post hoc test using the Bonferroni procedure was used to detect differences between group means. For Welfare Quality® data, feather damage was analyzed individually for the back, rump, keel, neck, head, crop, and belly, and abnormal conditions such as foot and toe damage, enteritis, and comb wounds were analyzed individually as well.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Trial 1 Results

3.1.1 Trial 1: Hen-Day Production

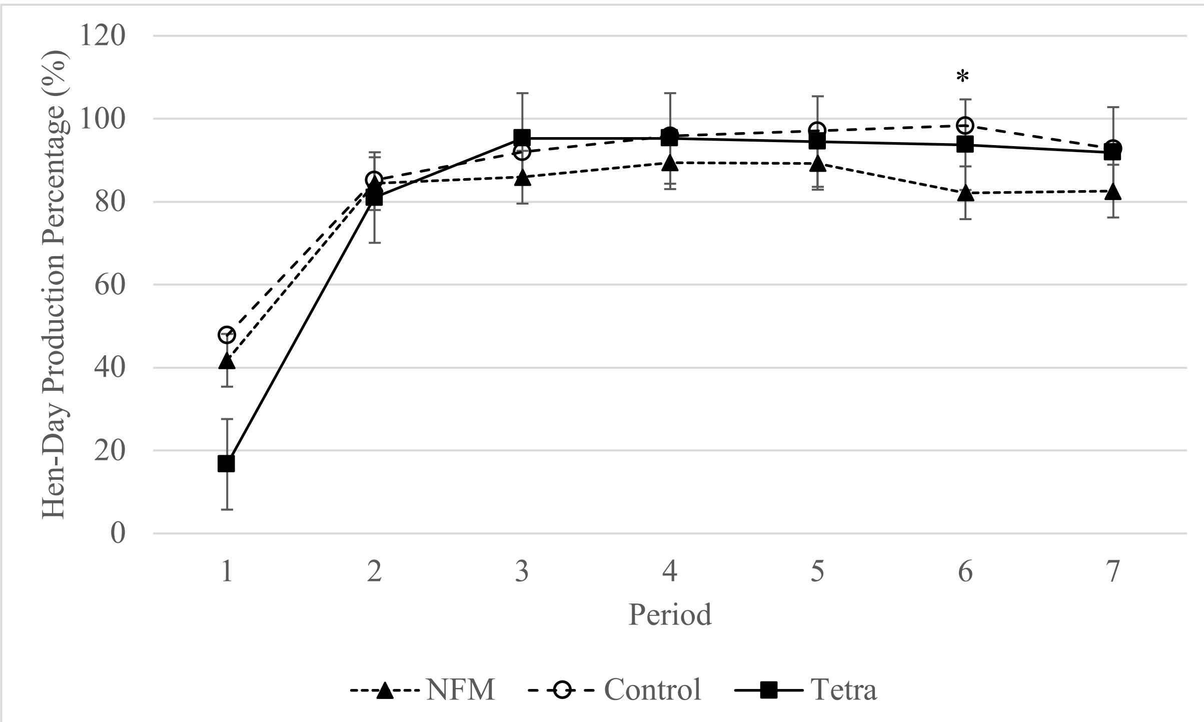

Figure 3.1. Hen-day percentage egg production for Tetra Brown hens from 18-47 weeks of age, or periods 1-7. The hen-day production percentage (HD) from the Tetra Brown management guide is included for baseline but was not included in the statistical analysis. Hens were either control or northern fowl mite (NFM) infested. Effects from period ($P < 0.0001$) and treatment ($P = 0.007$) were detected. *Difference between treatment means.

Hen-day production percentage (HD) was calculated weekly for each of the four rooms during the study. Hen-day production percentage was analyzed for differences between treatments and to investigate the effects of proportion of birds infested with mites on egg production as reported in Figure 3.1. The Tetra Brown management guide values are presented for comparison but were not included in the statistical analysis. The northern fowl mite (NFM) treatment was approximately 2% lower than the Tetra management guide overall, while the control group was approximately 7% higher than the Tetra management guide overall (Figure 3.1). The control treatment was approximately 10% higher than the NFM treatment overall. Period ($P < 0.0001$) and treatment ($P = 0.007$) had an effect on HD. A difference between treatment means was detected at period 6 (P

= 0.01). Proportion of birds infested with mites did not have an effect on hen-day production percentage (P > 0.05).

3.1.2 Trial 1: Eggs per Hen Housed

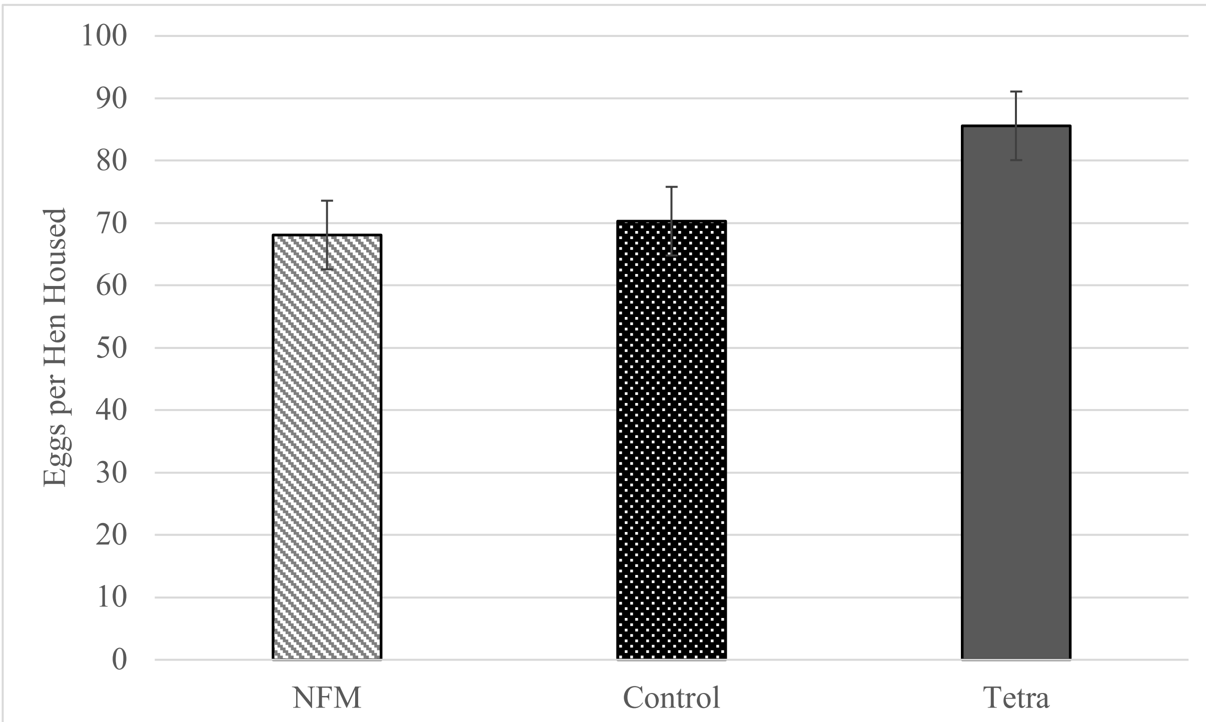

Figure 3.2. Eggs per hen housed summed over time for Tetra Brown hens from 18-47 weeks of age, or periods 1-7. Eggs per hen housed from the Tetra Brown management guide is included for baseline but was not included in the statistical analysis. Hens were either control or northern fowl mite (NFM) infested. A period effect was detected (P < 0.0001).

Eggs per hen housed were calculated for each treatment and overall values are presented in Figure 3.2. The Tetra Brown management guide values are presented for comparison but were not included in the statistical analysis. Eggs per hen housed were approximately 21% and 14.7% lower than the Tetra management guide for the NFM and control treatments, respectively. A period effect was detected (P < 0.0001), with pairwise differences found between period 4 and periods 6 and 7 for both groups, as well as between period 5 and 7 for the control group period 5 and periods 6 and 7 for both groups. No treatment effect or impact of proportion of birds infested with mites were found. A difference between treatment means was detected at period 7 (P = 0.03). As birds aged, eggs per hen housed increased (P < 0.05).

3.1.3 Trial 1: Mortality

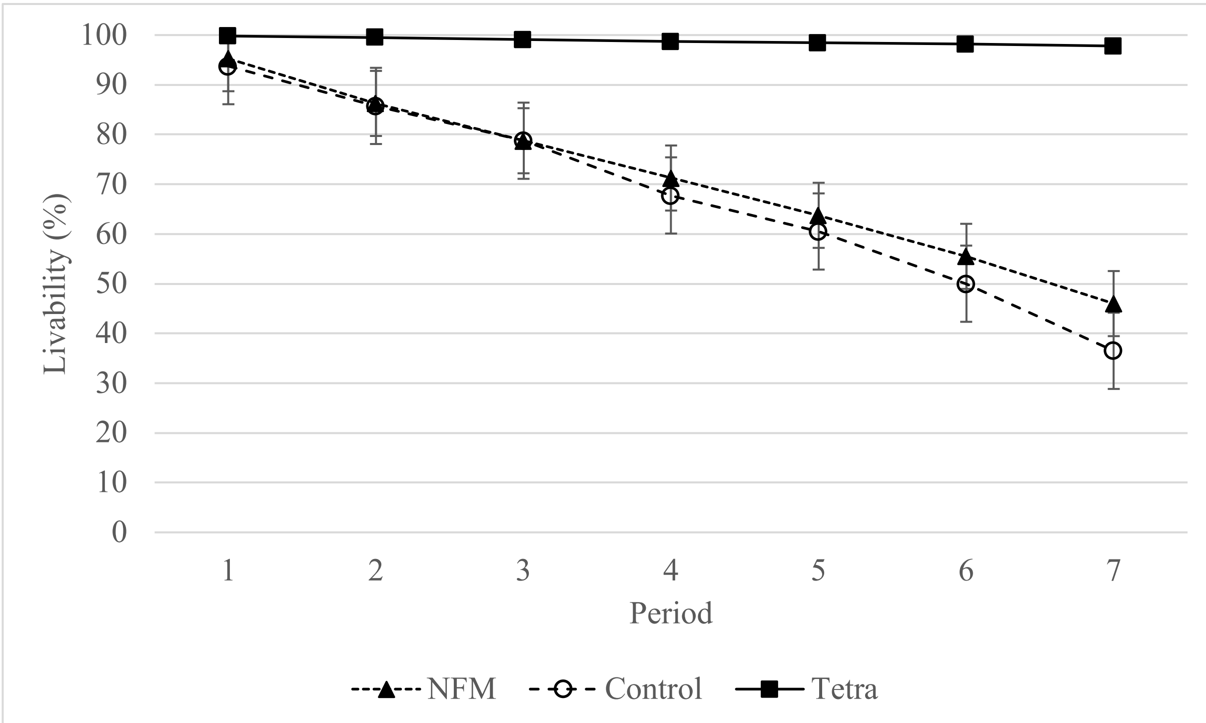

Figure 3.3. Percent livability for Tetra Brown hens from 18-47 weeks of age, or periods 1-7. The percent livability from the Tetra Brown management guide is included for baseline but was not included in the statistical analysis. Hens were either control or northern fowl mite (NFM) infested. A period effect was detected ($P = 0.0003$) with no post hoc pairwise differences revealed, as well as an interaction effect between proportion of birds infested with mites and period ($P = 0.01$).

Mortality was recorded daily in each of the four rooms. Mortality data were used to calculate percent livability (reported in Figure 3.3). The Tetra Brown management guide values are presented for comparison but were not included in the statistical analysis. Percent livability for each of the two treatments did not follow the Tetra Brown management guide and decreased over the experiment, with the control group ending at 36.5% and the NFM group ending at 46%. Effects of period ($P = 0.0003$) and an interaction effect between proportion of birds infested with mites and period ($P = 0.01$) were detected. Post hoc tests did not reveal pairwise differences between groups pertaining to period. Treatment and the proportion of birds infested with mites did not have an effect on percent livability ($P > 0.05$).

3.1.4 Trial 1: Case Weights

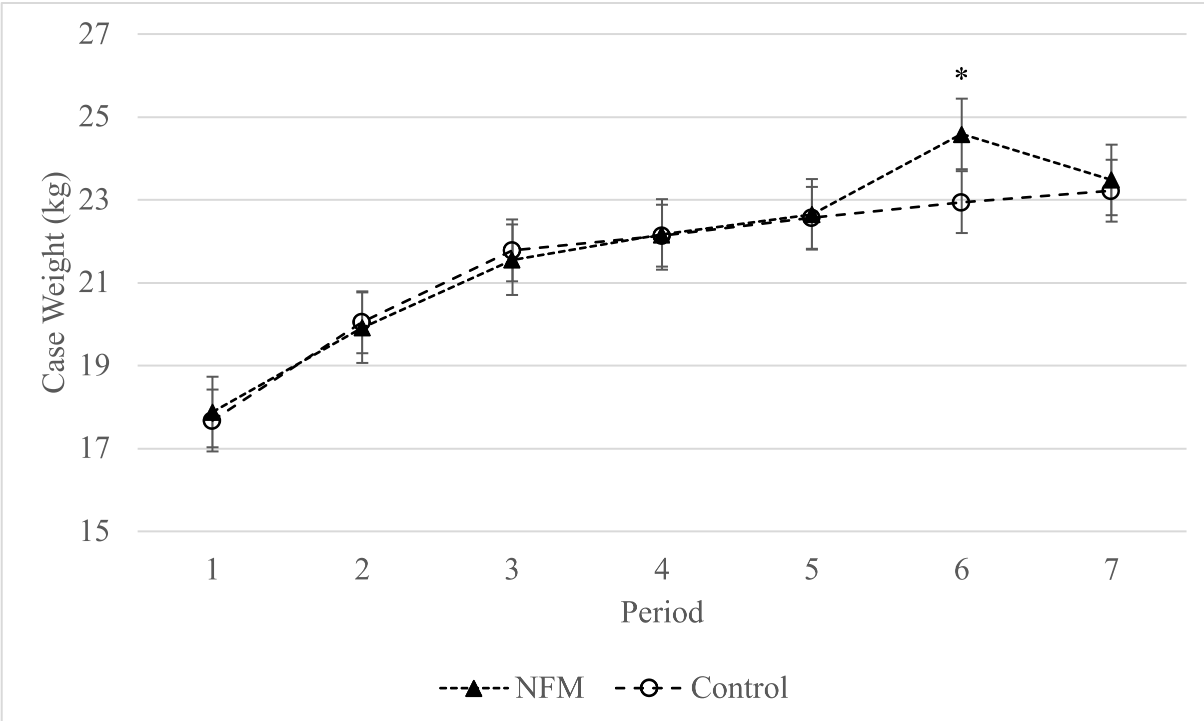

Figure 3.4. Weekly case weight values for Tetra Brown hens from 18-47 weeks of age, or periods 1-7. Hens were either control or northern fowl mite (NFM) infested. Effects of period (P < 0.0001), treatment (P = 0.03), and their interaction (P = 0.0006) were detected. *Difference between treatment means.

Case weights were recorded weekly for each of the four rooms. Case weight results are presented in Figure 3.4. Case weights steadily increased over time (P < 0.05) with post hoc comparisons revealing an increase in case weight value (kg) for the NFM group at period 6 compared to the control group (Figure 3.4). This value was not considered statistical outlier by using Cook's Distance. Effects of period (P < 0.0001), treatment (P = 0.03), and their interaction (P = 0.0006) were detected. Proportion of birds infested with mites did not have an effect on case weights (P > 0.05).

3.1.5 Trial 1: Egg Components

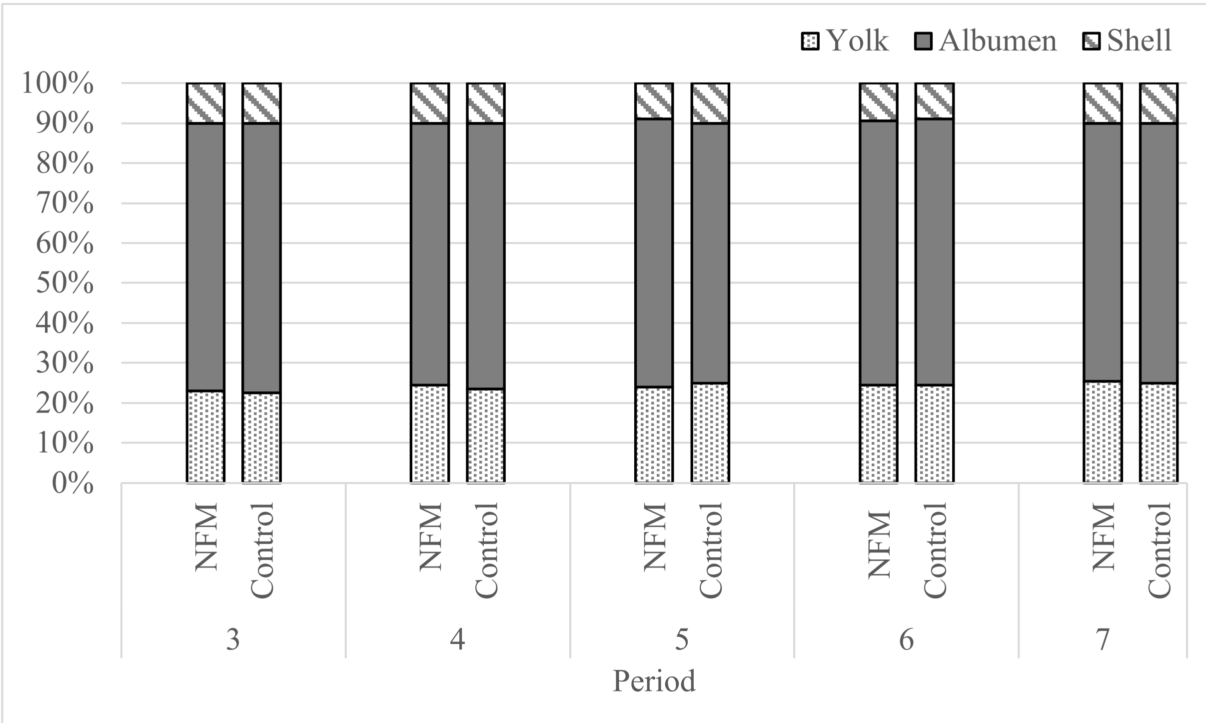

Figure 3.5. Yolk, albumen, and shell weights expressed as a percentage of whole egg weights for Tetra Brown hens for each period between 18-47 weeks of age, or periods 1-7. Egg components were taken between periods 3-7. Hens were either control or northern fowl mite (NFM) infested. No effects were detected for percent yolk or percent albumen. Effects of period, proportion of birds infested with mites, the interaction between period and proportion of birds infested with mites, and the interaction between period and treatment ($P < 0.0001$) were detected for percent shell.

Yolk, albumen, and shell weights were recorded each period for the duration of the study and are expressed as percentages of whole egg weight in Figure 3.5. Percent yolk increased slightly over time, while percent albumen slightly decreased over time. No effects were detected for percent yolk or percent albumen. Effects of period, proportion of birds infested with mites, the interaction between period and proportion of birds infested with mites, and the interaction between period and treatment ($P < 0.0001$) were detected for percent shell. A difference between treatment means was detected for percent shell at periods 5 and 6 ($P < 0.0001$). As birds aged, percent shell was variable ($P < 0.05$), ranging between 9-10% of the whole egg. Treatment did not affect percent shell ($P > 0.05$).

Trial 1: Shell Thickness

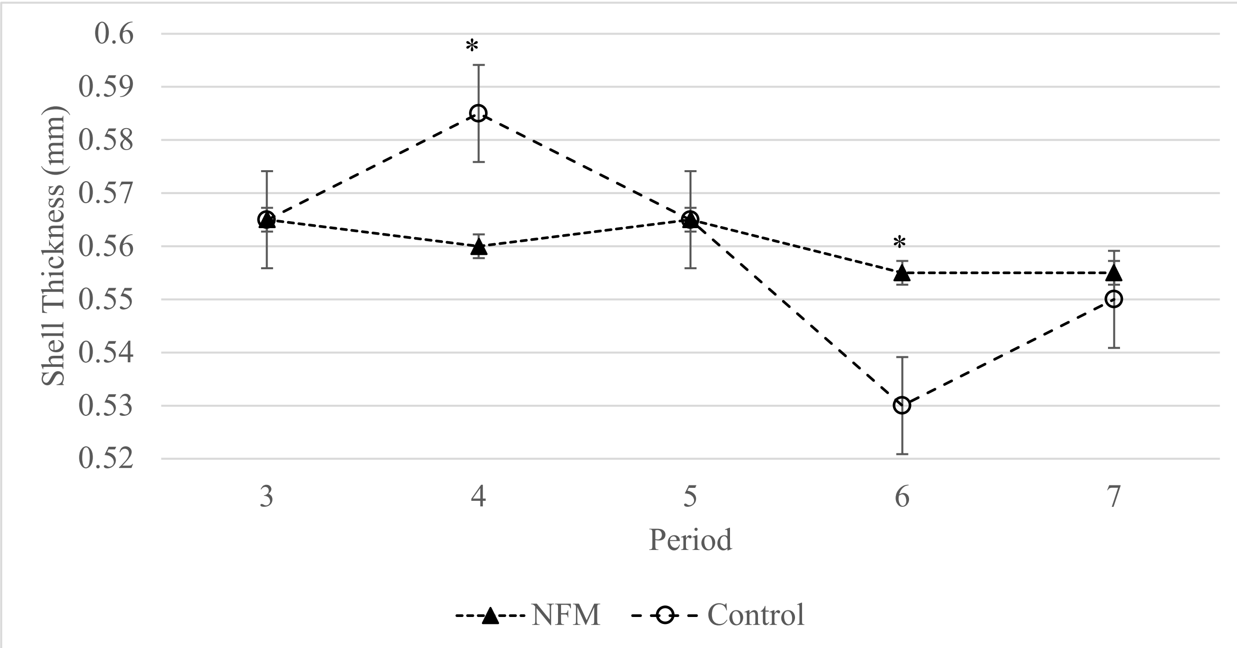

Figure 3.6. Shell thickness (mm) for Tetra Brown hens for each period between 18-47 weeks of age, or periods 1-7. Egg components were taken between periods 3-7. Hens were either control or northern fowl mite (NFM) infested. A period effect ($P < 0.0001$) and an interaction effect between treatment and period ($P = 0.02$) were detected. No treatment effect was detected. *Difference between treatment means.

Shell thickness slightly increased for both the NFM and control treatments, decreased, and increased again by period 7. There was a decrease in shell thickness at period 6, especially for the control group ($P < 0.05$). A period effect ($P < 0.0001$) and an interaction effect between period and treatment ($P = 0.02$) were detected. A difference between treatment means was detected at periods 4 and 6 ($P = 0.04$ and $P = 0.005$, respectively; Figure 3.6). Treatment and the proportion of birds infested with mites did not have an effect on shell thickness ($P > 0.05$).

3.1.6 Trial 1: Mite Counts

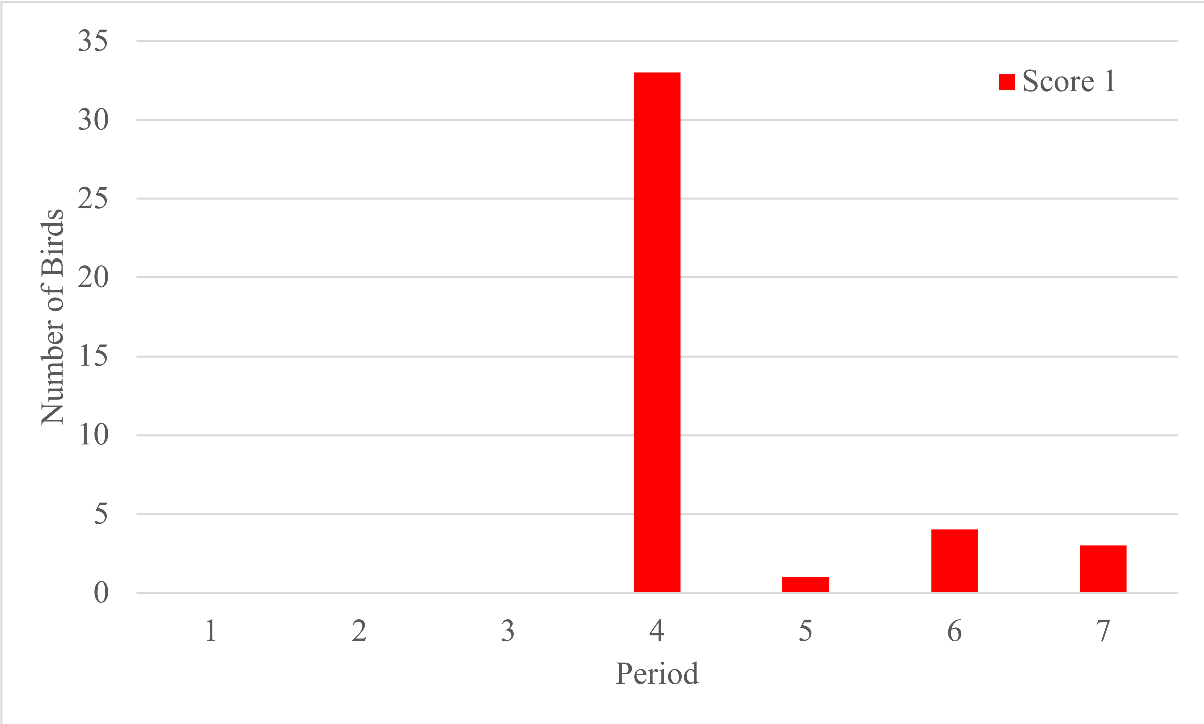

Figure 3.7. Frequency of scores reported during mite count data collection on Tetra Brown hens for each period between 18-47 weeks of age, or periods 1-7. Scores were based on a scoring system (Arthur and Axtell, 1982 and Owen et al., 2009). Only a score of 1 (1-10 mites) was detected throughout Trial 1.

Monthly mite counts were scored on 100% of birds in each of the four rooms (Table 2.4). An initial northern fowl mite infestation occurred at 24 weeks of age on 2% of hens in the two NFM treated rooms (8 hens total). A second mite infestation occurred at 35 weeks on 2% of hens, and a final attempt to infest occurred at 41 weeks on 100% of hens in the two NFM treated rooms. No score above 1 (1-10 mites) was detected throughout the study, and mite infestations remained low in each of the two NFM rooms (Figure 3.7).

3.1.7 Trial 1: Body Weights

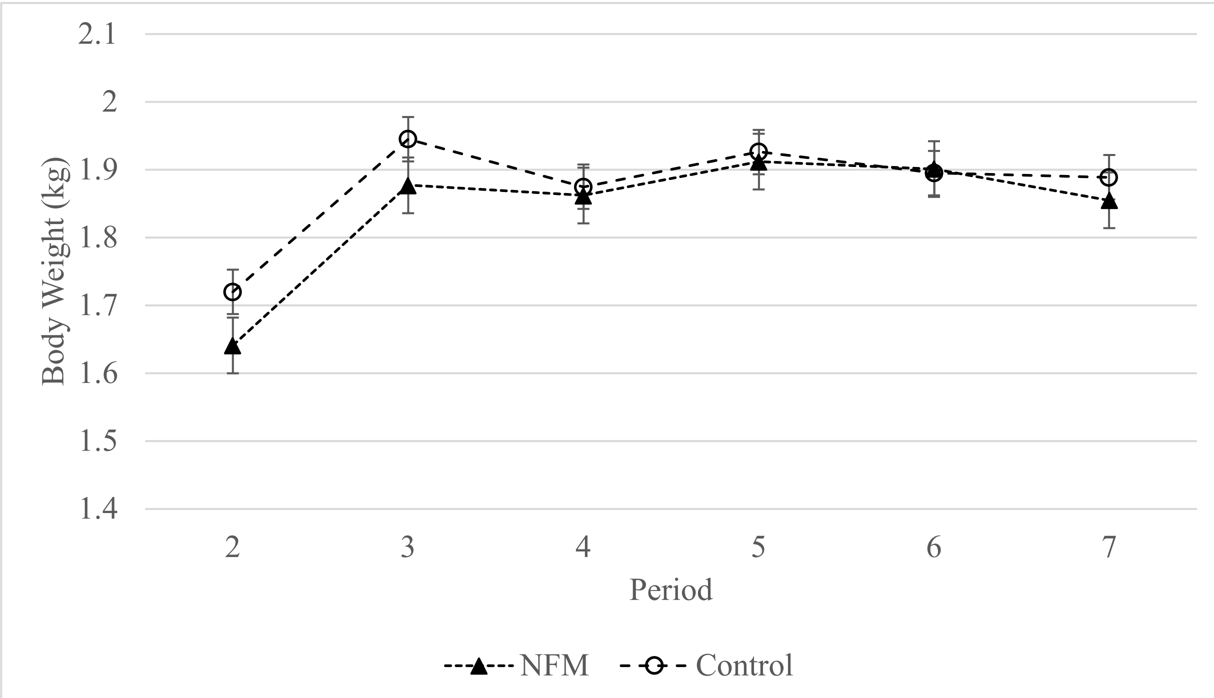

Figure 3.8. Body weights (kg) for Tetra Brown hens for each period between 18-47 weeks of age, or periods 1-7. Hens were either Control or northern fowl mite (NFM) infested. Period effects ($P < 0.0001$) were detected with no pairwise differences revealed using post hoc tests. No treatment effects were found.

Body weights increased between periods 2 and 3 and remained steady until period 7 (Figure 3.8). A period effect ($P < 0.0001$) was detected, with post hoc tests revealing no pairwise differences. No treatment effects or effects as a result of proportion of birds infested with mites were found ($P > 0.05$). Feed was not adjusted for mortality in the flock.

3.1.8 Trial 1: Welfare Quality

During Welfare Quality® Assessments (taken every period, or every 28 d), hens were assessed for feather damage and abnormal conditions such as enteritis, enlarged crop, skin lesions, respiratory issues, and toe and foot damage. No hens were found to have comb abnormalities, eye discharge, panting, and respiratory discharge. One hen had an enlarged crop in room 4 at period 4. No effects were detected for enteritis, comb wounds, keel bone deformities and deformations, tip fractures on the keel bone, skin lesions, toe damage, or feather damage on the rump; therefore, results for those parameters will not be presented in detail. Feather damage on the rump is highlighted below (Figure 3.11) as an example of the severity of feather loss in that area as a result of feather pecking behavior in Trial 1.

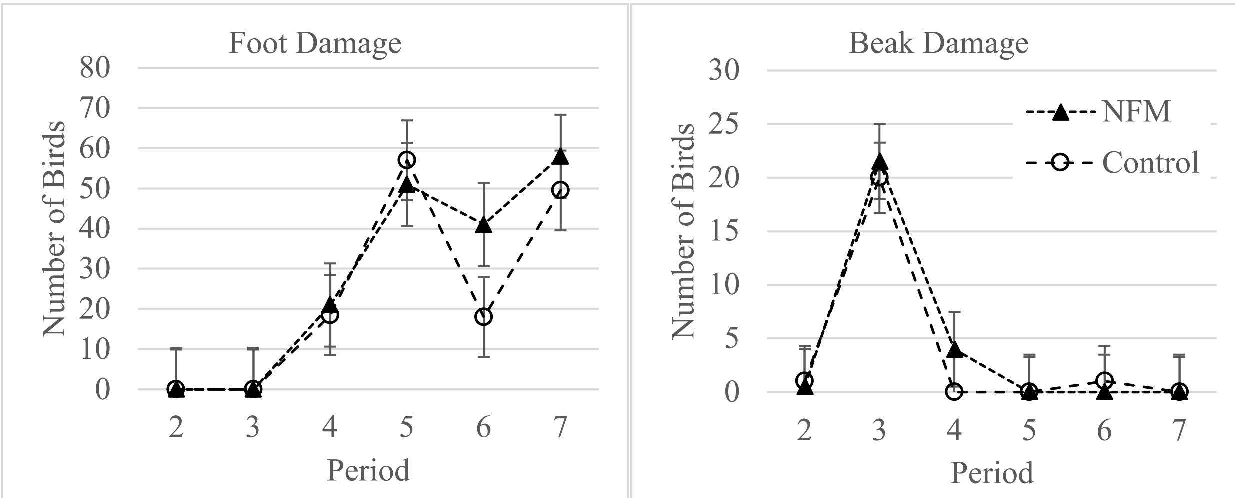

Figure 3.9. Number of Tetra Brown hens with foot damage (left), and beak damage (right) between 18-47 weeks of age, or periods 1-7 during Welfare Quality(R) Assessments. Welfare Quality® Assessments were taken between periods 2-7. Hens were either northern fowl mite (NFM) infested or control. Period effects were detected for foot damage ($P = 0.01$) and beak damage ($P = 0.0009$); however post hoc tests did not reveal pairwise comparisons for either. An interaction effect between proportion of birds infested with mites and period ($P = 0.08$) was found for foot damage.

Foot damage and beak damage were scored on a 0 or 1 scale. 0 meant that no damage was present and 1 indicated presence of damage. Foot damage involved any lesions on the foot, footpad dermatitis, or swelling of the feet. Beak damage indicated any external damage to the beak, including damage inflicted by beak modification (such as the modification via a Dremel tool that occurred at 21 weeks of age).

Presence of foot damage increased after period 3, with an improvement in foot damage at period 6 in both the northern fowl mite and control groups; however, foot damage worsened until the end of the study at period 7 (Figure 3.9). An improvement in foot condition could be attributed to replacement of litter with new wood shavings at that time point. Period effects were detected for foot damage ($P = 0.01$), as well as an interaction effect between proportion of birds infested with mites and period ($P = 0.08$). Hens in Trial 1 were not beak trimmed; however, they were modified at 21 weeks of age (Period 2). An increase in the number of birds found with beak damage was found at period 3 in both groups, perhaps due to a delayed effect of beak modification on beak condition (Figure 3.9). A period effect was detected for beak damage ($P = 0.0009$).

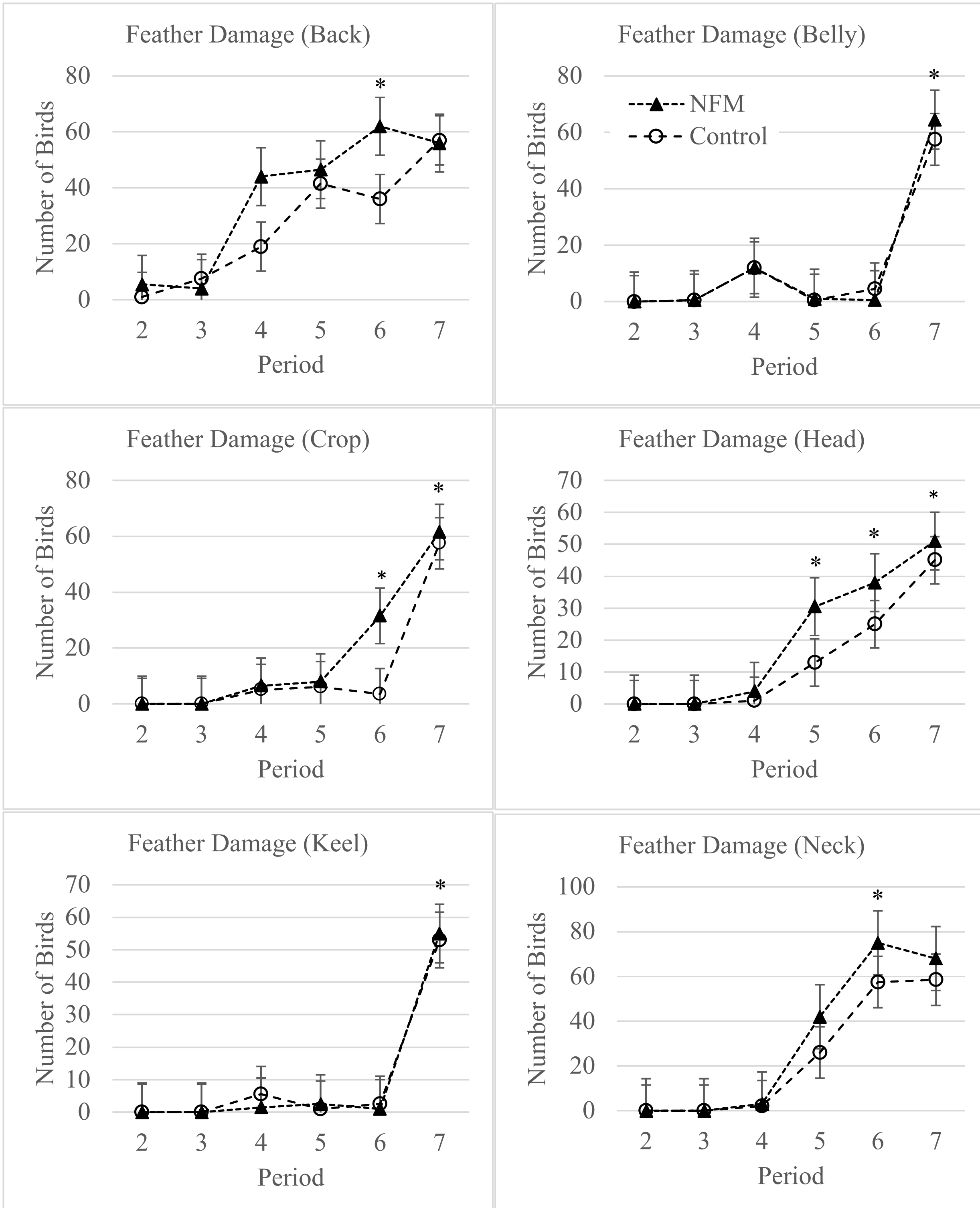

Figure 3.10. Number of Tetra Brown hens with feather damage on the back (top left), belly (top right), crop (middle left), head (middle right), keel (bottom left), and neck (bottom right) between 18-47 weeks of age, or periods 1-7 during Welfare Quality® Assessments. Welfare Quality® Assessments were taken between periods 2-7. Hens were either northern fowl mite (NFM) infested or control. A period effect was detected for feather damage on the back ($P = 0.01$), head ($P = 0.001$), and neck ($P = 0.004$). Treatment ($P = 0.03$) and mite population ($P = 0.02$) had an effect on feather damage on the belly. An interaction effect between proportion of birds infested with mites and period was

found for feather damage on the back (P = 0.02), belly (P = 0.03), crop (P = 0.02), head (P = 0.0008), and neck (P = 0.03). An interaction effect between treatment and period was detected for feather damage on the crop (P = 0.04) and head (P = 0.01). No effects were detected for feather damage on the keel (P > 0.05). *Interaction between period and treatment.

Feather damage found during Welfare Quality® Assessments is presented in Figure 3.10. A hen was determined to have feather damage if she were missing feathers in at least one spot greater than or equal to 2 cm on an area of the body, which was adapted from the Welfare Quality® assessment protocol for poultry (WQ® Consortium, 2009).

A period effect was detected for feather damage on the back (P = 0.01), head (P = 0.001), and neck (P = 0.004) (Figure 3.10). Treatment (P = 0.03) and mite population (P = 0.02) affected feather damage on the belly. An interaction effect between proportion of birds infested with mites and period was found for feather damage on the back (P = 0.02), belly (P = 0.03), crop (P = 0.02), head (P = 0.0008), and neck (P = 0.03). An interaction effect between treatment and period was detected for feather damage on the crop (P = 0.04) and head (P = 0.01). No effects were detected for feather damage on the keel (P > 0.05). A difference between treatment means was detected for the back at period 6 (P = 0.03); for the belly at period 7 (P = 0.01); for the crop at periods 6 and 7 (P = 0.01); for the head at periods 5, 6, and 7 (P < 0.008); for the keel at period 7 (P = 0.02); and for the neck at period 6 (P = 0.04).

Post hoc analysis showed that feather damage on the belly worsened sharply at period 7, with a difference between treatment means at period 7 (P < 0.05). Feather damage on the crop, head, and keel worsened sharply by period 7 (P < 0.05).

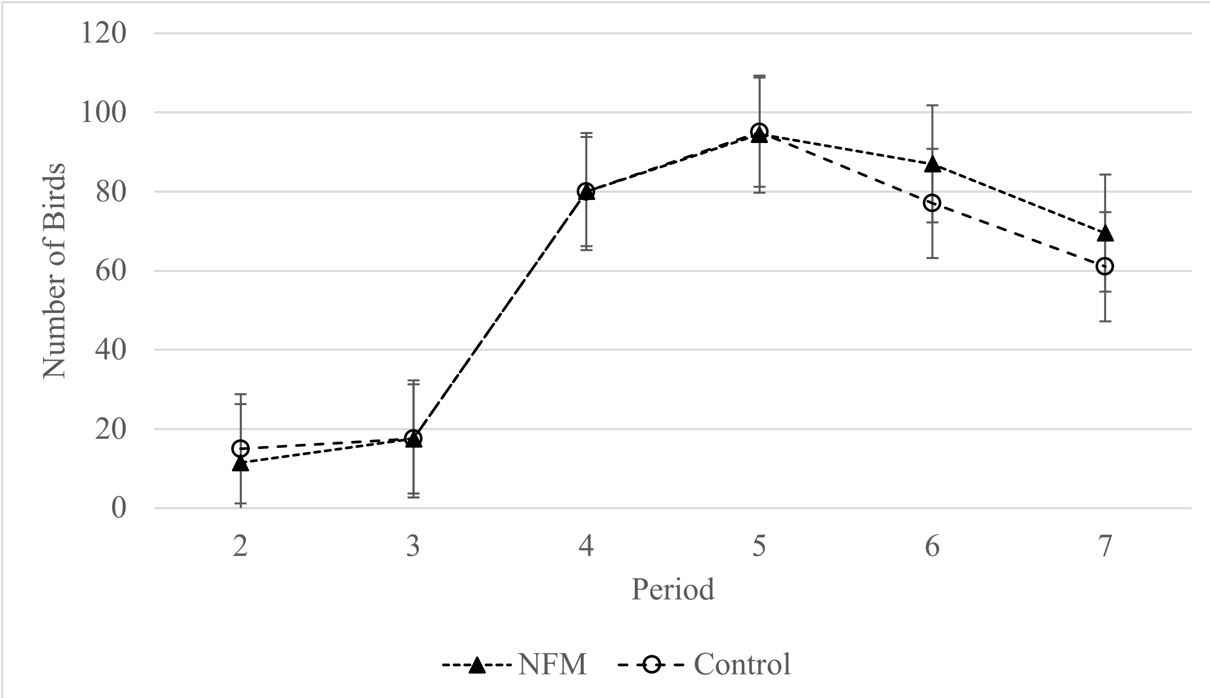

Figure 3.11. Number of Tetra Brown hens with feather damage on the rump area between 18-47 weeks of age, or periods 1-7 during Welfare Quality® Assessments. Welfare Quality® Assessments were taken between periods 2-7. No effects were detected for feather damage on the rump ($P > 0.05$).

Rump feather damage worsened over time because of feather pecking and cannibalism issues in the Trial 1 flock (Figure 3.11). No effects were detected on rump feather damage ($P > 0.05$).

3.2 Trial 2 Results

3.2.1 Trial 2: Hen-Day Production

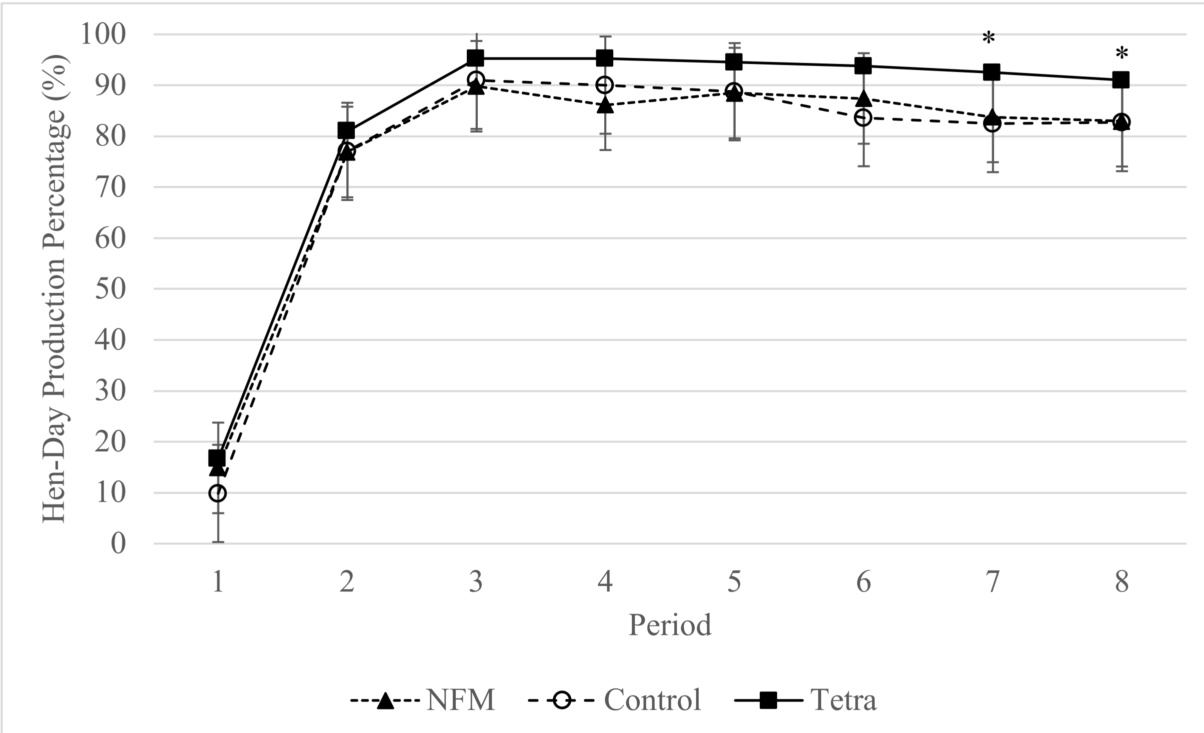

Figure 3.12. Hen-day percentage egg production for Tetra Brown hens from 18-49 weeks of age, or periods 1-8. The hen-day percentage (HD) from the Tetra Brown management guide is included for baseline but was not included in the statistical analysis. Hens were either control or northern fowl mite (NFM) infested. A period effect was detected ($P < 0.0001$) as well as an interaction effect between period and the proportion of birds infested with mites ($P = 0.04$). *Difference between treatment means.

Hen-day production percentage (HD) was calculated weekly for each of the four rooms during the study. Hen-day production percentage was analyzed for differences between treatments, as reported in Figure 3.12. The Tetra Brown management guide values are presented for comparison but were not included in the statistical analysis. Hen-day production percentage for the northern fowl mite (NFM) group was approximately 8% lower overall than the Tetra Brown management guide, while the control group was approximately 9% lower than the Tetra Brown management guide. Overall, hen-day production percentage for the NFM group was approximately 1% higher than the control group (Figure 3.12). A period effect was detected for hen-day production percentage ($P < 0.0001$, periods 7 and 8) as well as an interaction effect between period and the proportion of birds infested with mites ($P = 0.04$). Treatment and the proportion of birds infested with mites did not have an effect on hen-day production percentage ($P > 0.05$)

3.2.2 Trial 2: Eggs per Hen Housed

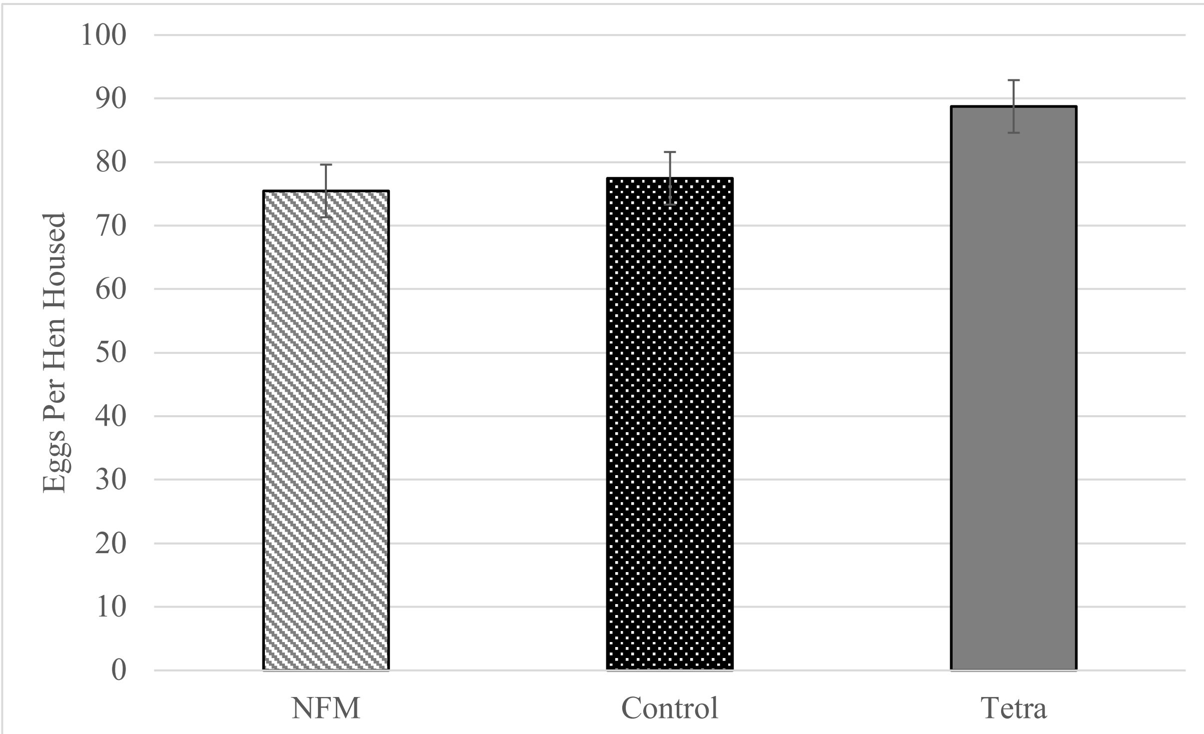

Figure 3.13. Eggs per hen housed for Tetra Brown hens from 18-49 weeks of age, or periods 1-8. Eggs per hen housed from the Tetra Brown management guide is included for baseline but was not included in the statistical analysis. Hens were either control or northern fowl mite (NFM) infested. Effects of period ($P < 0.0001$) and treatment ($P = 0.004$) were detected, as well as interaction effects between period and treatment ($P < 0.0001$) and between period and the proportion of birds infested with mites ($P < 0.0001$).

Eggs per hen housed were calculated and overall values are presented in Figure 3.13. Eggs per hen housed were 14.4% and 11.5% lower than the Tetra Brown management guide for the northern fowl mite and control groups, respectively (Figure 3.13). A period effect ($P < 0.0001$) was detected, with pairwise differences between all periods 5-8 for both groups ($P < 0.05$). Treatment ($P = 0.004$) effects were detected, as well as interaction effects between period and treatment ($P < 0.0001$) and between period and the proportion of birds infested with mites ($P < 0.0001$). Differences between treatment means were found at periods 4, 5, 6, 7, and 8 ($P < 0.05$).).

3.2.3 Trial 2: Mortality

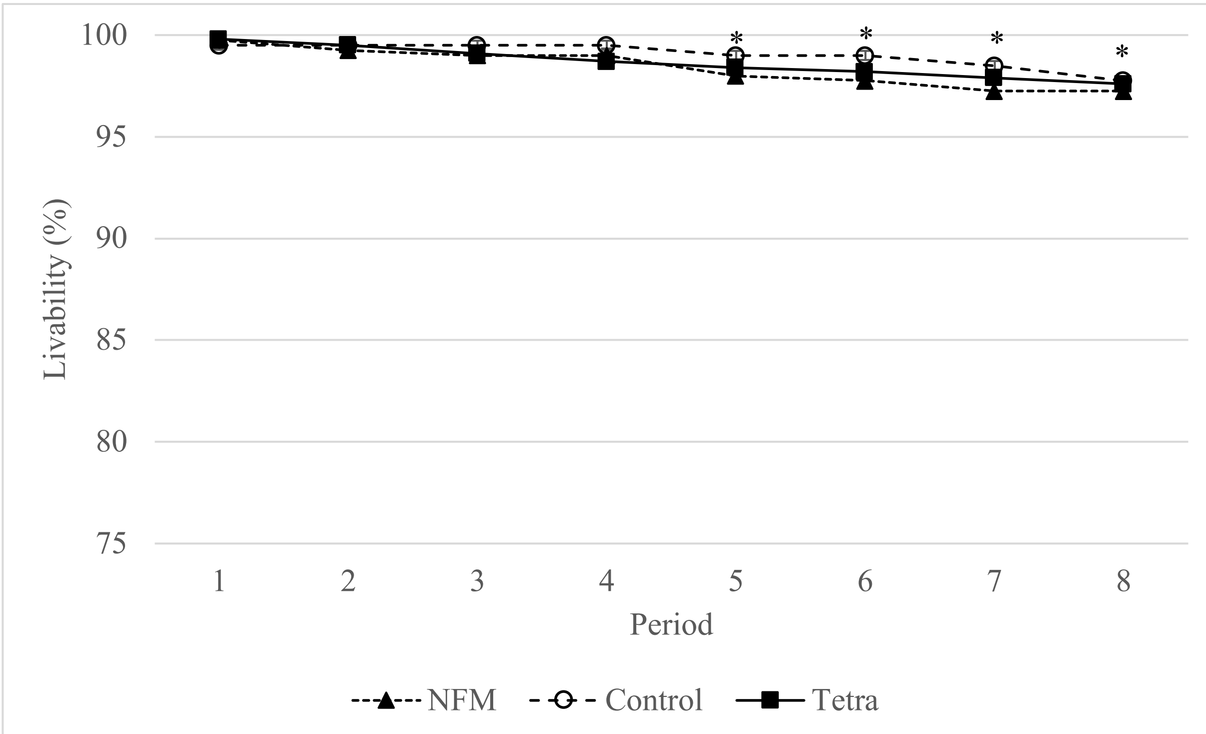

Figure 3.14. Percent livability for Tetra Brown hens from 18-49 weeks of age or periods 1-8. The percent livability from the Tetra Brown management guide is included for baseline but was not included in the statistical analysis. Hens were either control or northern fowl mite (NFM) infested. Effects of period (P = 0.001) and treatment (P = 0.03) were detected, as well as interaction effects between period and treatment (P = 0.01) and period and the proportion of birds infested with mites (P = 0.02). *Differences between treatment means.

Mortality was recorded daily in each of the four rooms. Mortality data were used to calculate percent livability. Percent livability is reported in Figure 3.14 with values from the Tetra management guide added for comparison. Percent livability for the control treatment remained approximately in-line with the Tetra Brown management guide, ending at 97.75% by the conclusion of Trial 2, while the Tetra Brown management guide reports a percent livability of 97.6% at that point (Figure 3.14). Percent livability for the NFM treatment remained slightly lower than the Tetra Brown management guide (except for at period 4), ending at 97.25% by the conclusion of Trial 2. Effects of period (P = 0.001) and treatment (P = 0.03) were detected, as well as interaction effects between period and treatment (P = 0.01) and period and the proportion of birds infested with mites (P = 0.02). Differences between treatment means were found at periods 5, 6, 7, and 8 (P < 0.05).

3.2.4 Case Weights

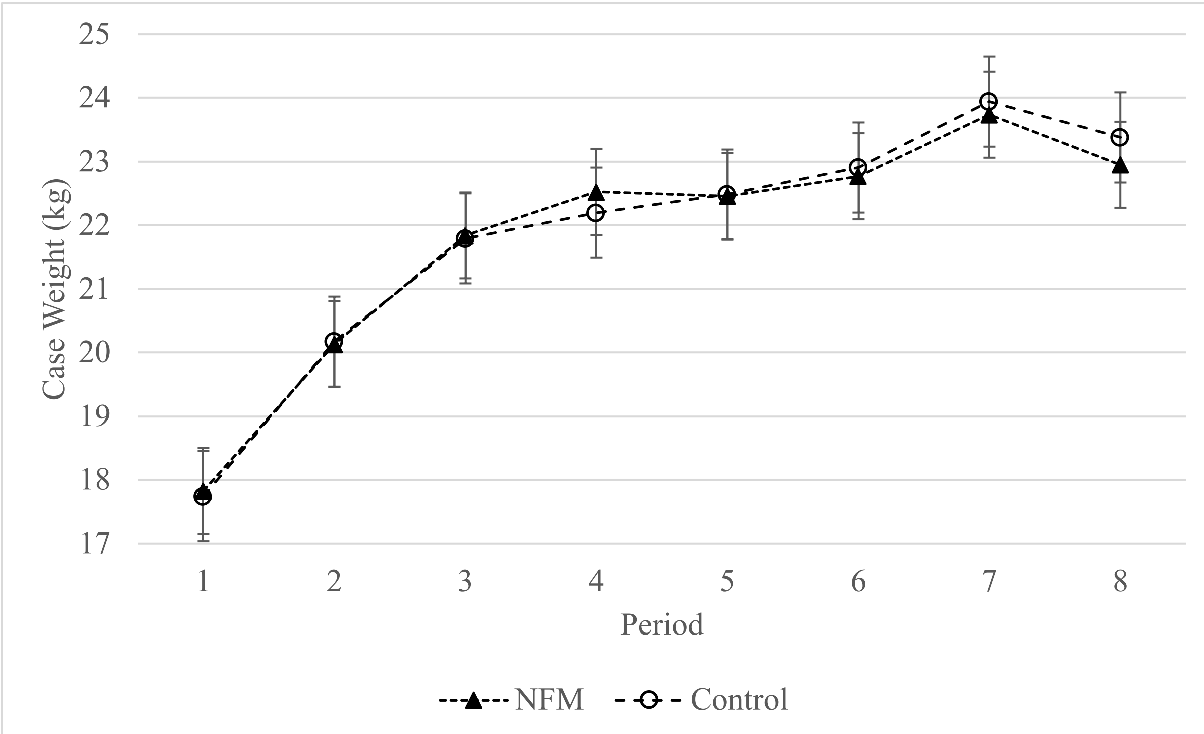

Figure 3.15. Weekly case weight values for Tetra Brown hens from 18-49 weeks of age or periods 1-8. Hens were either control or northern fowl mite (NFM) infested. Period had an effect on case weights ($P < 0.0001$) and post hoc pairwise differences were found between the NFM group at period 7 and the control group at period 5, as well as between the NFM group at period 7 and both groups at period 4 ($P < 0.05$).

Case weights were recorded weekly for each of the four rooms and data were analyzed for differences between treatments. Case weights steadily increased over time with an increase in case weight value (kg) for both the NFM and control groups (Figure 3.15). A period effect ($P < 0.0001$) was detected and no effects from treatment or proportion of birds infested with mites were detected ($P > 0.05$). According to post hoc analysis, case weights increased significantly between periods 4 and 7 and 5 and 7 ($P < 0.05$).

3.2.5 Egg Components

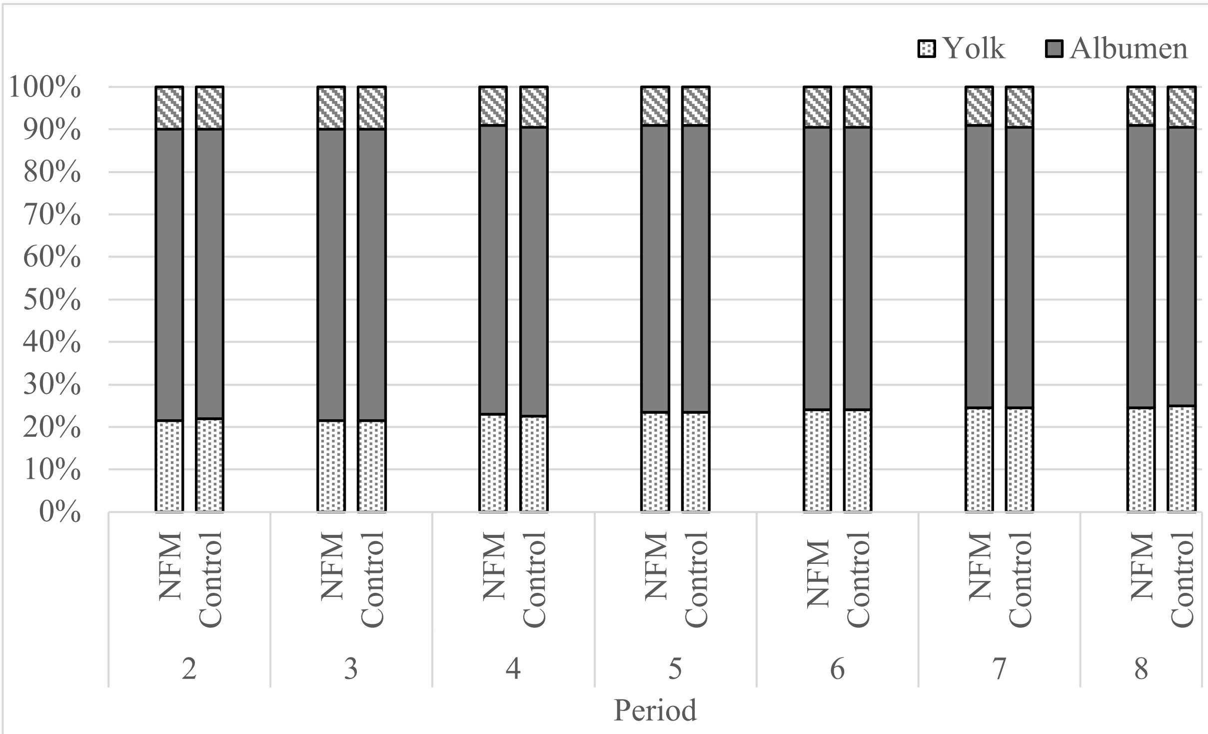

Figure 3.16. Yolk, albumen, and shell weights expressed as a percentage of whole egg weights for Tetra Brown hens for each period between 18-49 weeks of age or periods 1-8. Egg components were taken between periods 2-8. Hens were either control or northern fowl mite (NFM) infested. A period effect was detected for percent yolk and percent albumen (P = 0.003 and P = 0.03, respectively). No effects were found for percent shell.

Yolk, albumen, and shell weights were recorded each period for the duration of the study and are expressed as percentages of whole egg weight in Figure 3.16. Percent yolk increased slightly over time. A period effect was detected for percent shell and percent albumen (P = 0.003 and P = 0.03, respectively). No effects were found for percent shell. Treatment and proportion of birds infested with mites did not have an effect on percent yolk, percent albumen, or percent shell (P > 0.05; Figure 3.16).

Shell Thickness

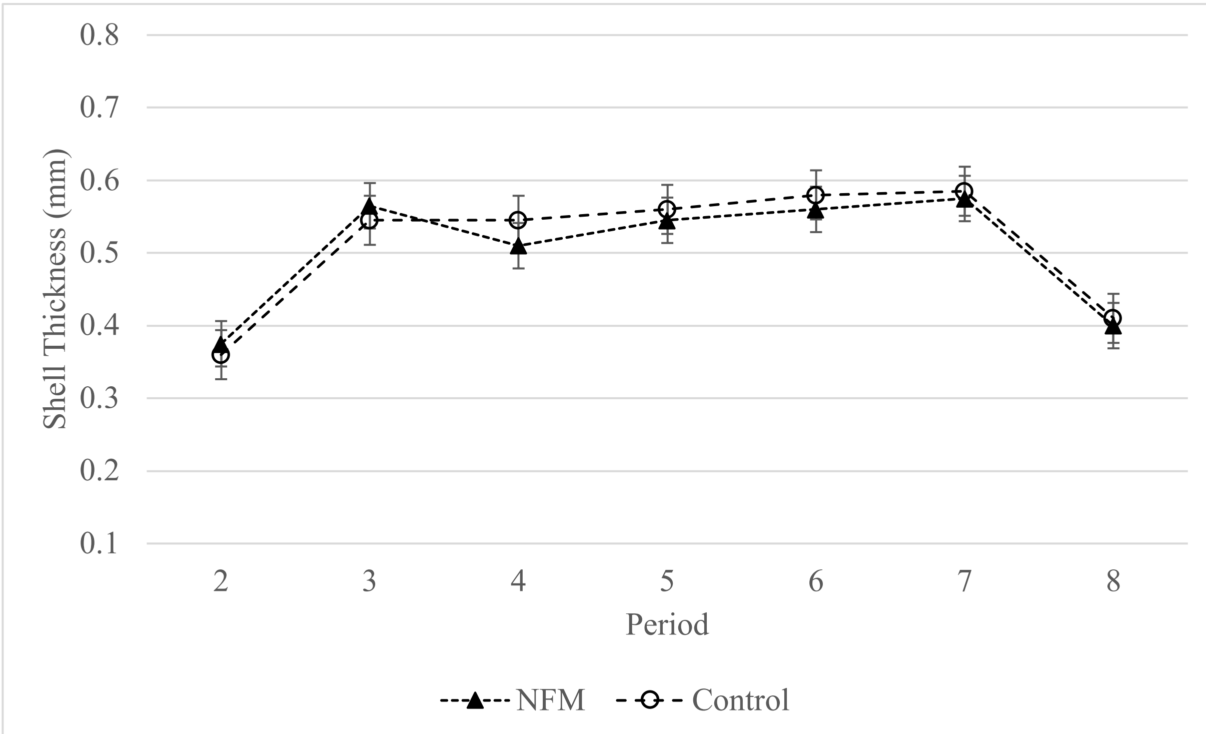

Figure 3.17. Shell thickness (mm) for Tetra Brown hens for each period between 18-49 weeks of age or periods 1-8. Shell thickness was measured between periods 2-8. Hens were either control or northern fowl mite (NFM) infested. A period effect was found for shell thickness ($P < 0.0001$).

Post hoc analysis showed that shell thickness increased slightly between periods 4 and 7, then dropped at period 8 ($P < 0.05$; Figure 3.17). Period had an effect on shell thickness ($P < 0.0001$), and no effects from treatment or the proportion of birds infested with mites were found ($P > 0.05$).

3.2.6 Mite Counts

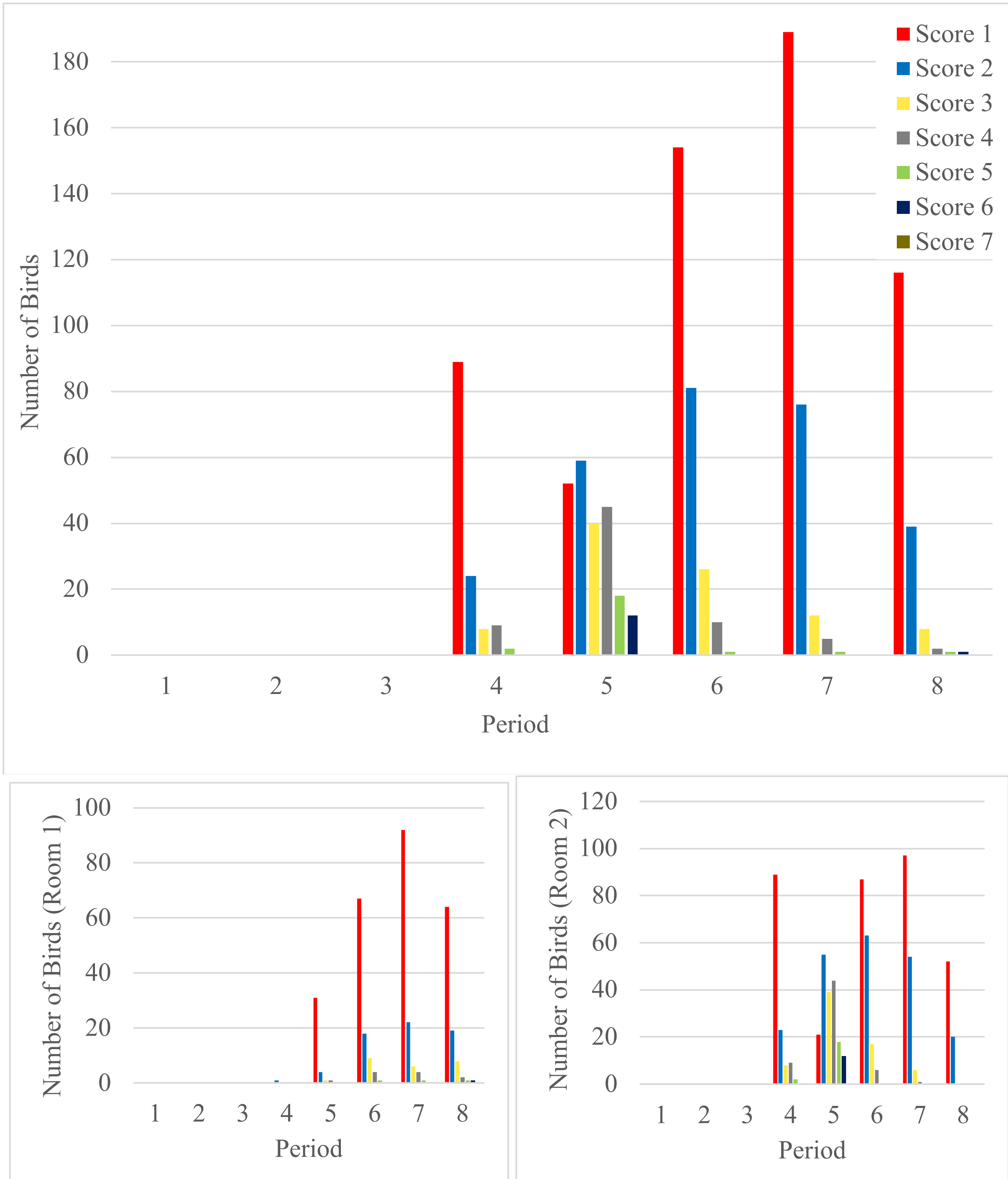

Figure 3.18. Frequency of scores collected during mite count data collection based on a scoring system (Arthur and Axtell, 1982 and Owen et al., 2009). Overall mite scores collected is shown in the top graph, while the bottom two graphs break down frequency of scores collected in each of the two northern fowl mite rooms (Rooms 1 and 2). Mite counts were conducted between periods 4-8, after introduction of mites had occurred at period 3.

Monthly mite counts were scored on 100% of birds in each of the four rooms (Table 2.4). An initial NFM infestation occurred at 24 weeks of age on 2% of hens in the two NFM treated rooms (8 hens total). A second NFM infestation occurred at 30 weeks on 2% of hens in room 1. No score above 7 (> 10,000 mites) was detected, and a score of 1 (1-10 mites) was most frequently detected in each of the two NFM treated rooms (Figure 3.18). An increase in the number of birds with a mite infestation occurred until period 7, with mite populations decreasing by period 8. Higher mite count scores (meaning higher infestation levels) were more prevalent at period 5, with scores decreasing to 1 or 2 (1-10 mites or 11-50 mites, respectively) as the study carried on (Figure 3.18).

3.2.7 Body Weights

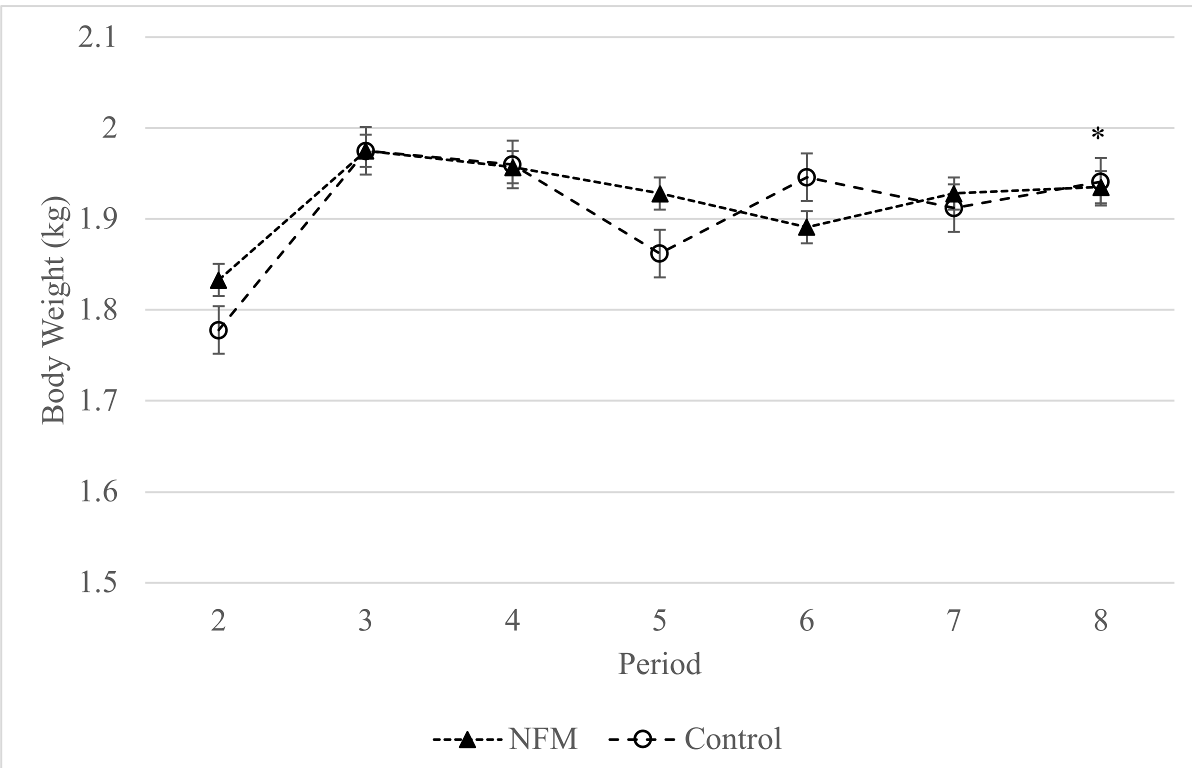

Figure 3.19. Body weights (kg) for Tetra Brown hens for each period between 18-49 weeks of age or periods 1-8. Body weights were taken between periods 2-8. Hens were either control or northern fowl mite (NFM) infested. A period effect was detected for body weight (P = 0.003). *Difference between treatment means.

Body weights remained steady over time after period 3 (Figure 3.19). A period effect (P = 0.003) on body weight was detected. A difference between treatment means was detected at period 8 (P = 0.03). Feed was adjusted based on body weight in Trial 2.

3.2.8 Welfare Quality

During Welfare Quality® Assessments (taken every period, or every 28 d), hens were assessed for feather damage and abnormal conditions such as enteritis, enlarged crop, skin lesions, respiratory issues, and toe and foot damage. No hens were found to have comb abnormalities, panting, or respiratory discharge. One hen had eye discharge in room 1 at period 5 and another hen had eye discharge in room 2 at period 6. No effects were detected for enteritis, comb wounds, foot damage, keel bone deformities and deformations, tip fractures on the keel bone, or feather damage on the belly; therefore, results for those parameters will not be presented in detail.

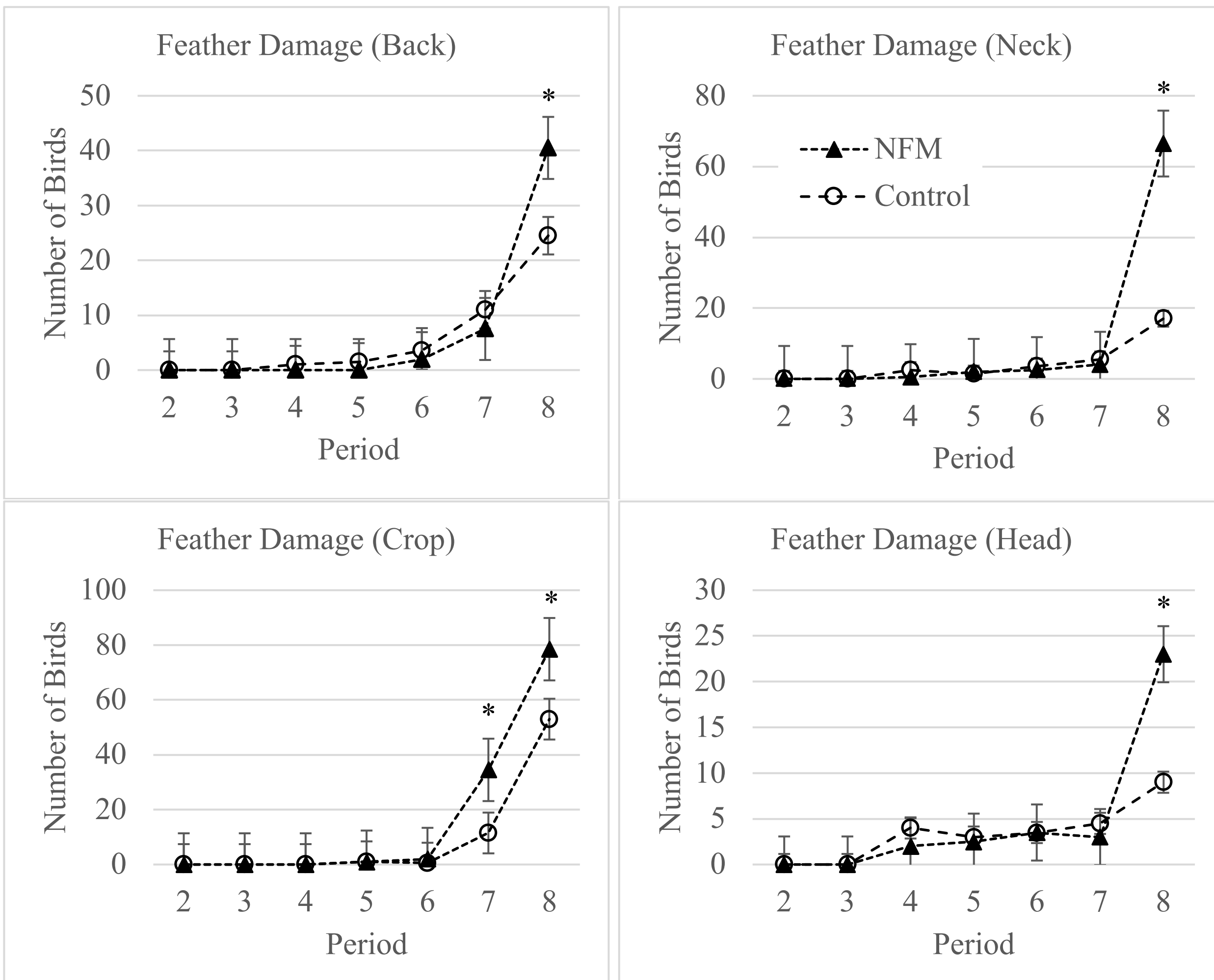

Figure 3.20. Number of Tetra Brown hens with feather damage on the back (top left), neck (top right), crop (bottom left), and head (bottom right) between 18-49 weeks of age, or periods 1-8 during Welfare Quality® Assessments. Welfare Quality® Assessments were taken between periods 2-8. Hens were either control or northern fowl mite (NFM) infested. A period effect was detected for feather damage on the crop, head, and neck ($P < 0.05$). Treatment had an effect on feather damage on the head and neck ($P < 0.05$). Feather damage on the back, crop, head, and neck was

affected by proportion of birds infested with mites, the interaction between period and treatment, and the interaction between period and proportion of birds infested with mites (P < 0.05). *Differences between treatment means.

Feather damage found during Welfare Quality® Assessments is presented in Figure 3.20. A hen was determined to have feather damage if she were missing feathers in at least one spot greater than or equal to 2 cm on an area of the body, which was adapted from the Welfare Quality® assessment protocol for poultry (WQ® Consortium, 2009).

A period effect was detected for feather damage on the crop, head, and neck (P < 0.05). Treatment had an effect on feather damage on the head and neck (P < 0.05). Feather damage on the back, crop, head, and neck was affected by proportion of birds infested with mites, the interaction between period and treatment, and the interaction between period and proportion of birds infested with mites (P < 0.05). A difference between treatment means was detected for the back at period 8 (P = 0.0005); for the crop at periods 7 and 8 (P = 0.03 and P = 0.003, respectively); for the head at period 8 (P < 0.0001); and for the neck at period 8 (P < 0.0001).

Post hoc analysis showed that feather damage on the belly worsened sharply at period 8 for the back, crop, head, and neck (P < 0.05), as shown in Figure 3.20.

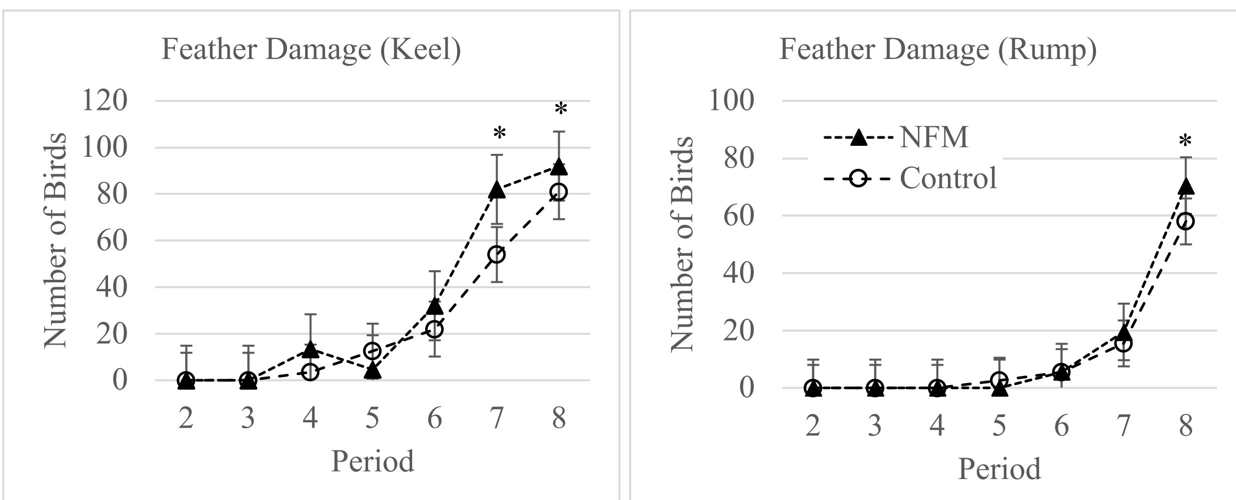

Figure 3.21. Number of Tetra Brown hens with feather damage on the keel (left) and rump (right) between 18-49 weeks of age, or periods 1-8 during Welfare Quality® Assessments. Welfare Quality® Assessments were taken between periods 2-8. Hens were either control or northern fowl mite (NFM) infested. An interaction effect between period and the proportion of birds infested with mites was detected for feather damage on the keel (P = 0.03) and rump (P = 0.04). *Differences between treatment means.

Figure 3.21 shows the number of birds with feather damage on the keel and rump areas for each period during Welfare Quality® Assessments. No treatment effects or effects from the proportion of birds infested with mites was found (P > 0.05). Interaction effects between period and the proportion of birds infested with mites was found for feather damage on the keel and rump (P = 0.03 and P = 0.04, respectively. Differences between treatment means were found at periods 7 and 8 (P = 0.008 and P = 0.03, respectively) for feather damage on the keel and at period 8 (P = 0.008) for feather damage on the rump. Feather damage on the keel and rump areas worsened by period 8 (Figure 3.21).

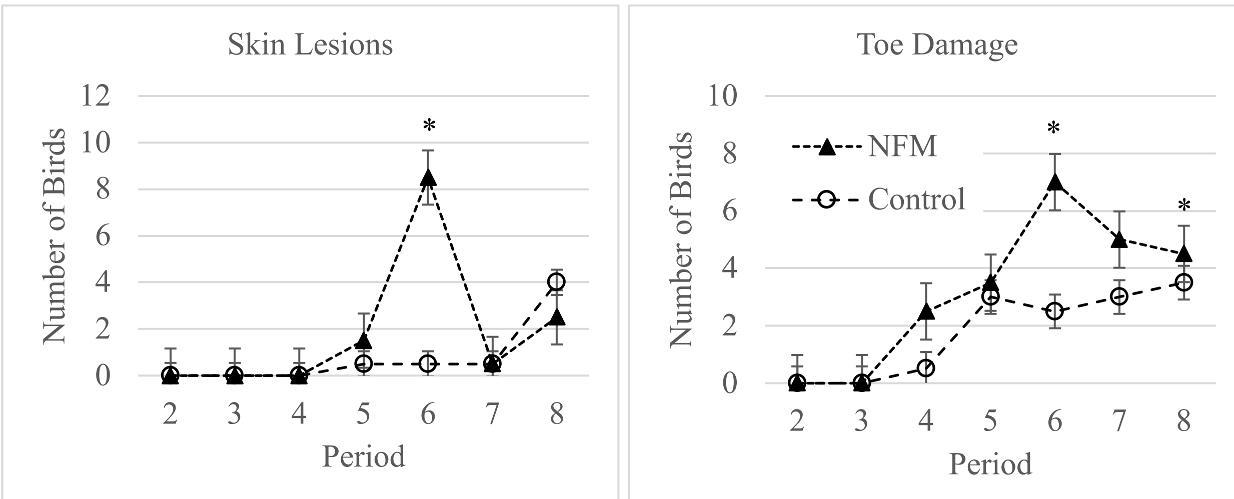

Figure 3.22. Number of Tetra Brown hens with skin lesions (left) and toe damage (right) between 18-49 weeks of age, or periods 1-8 during Welfare Quality® Assessments. Welfare Quality® Assessments were taken between periods 2-8. Hens were either control or northern fowl mite (NFM) infested. A period effect was detected for skin lesions and toe damage (P = 0.0003 and P = 0.01, respectively). Treatment had an effect on skin lesions (P = 0.01). Interaction effects between period and treatment and between period and the proportion of birds infested with mites had an effect on skin lesions and toe damage (P < 0.05). *Differences between treatment means.

The number of birds with skin lesions and toe damage at each period during Welfare Quality® Assessments are shown in Figure 3.22. Treatment had an effect on skin lesions (P = 0.01). Interaction effects between period and treatment and between period and the proportion of birds infested with mites had an effect on skin lesions and toe damage (P < 0.05). Differences between treatment means were found at period 6 for skin lesions (P < 0.0001) and at periods 6 and 8 (P = 0.01 and P = 0.02, respectively) for toe damage (Figure 3.22).

3.3 Discussion

3.3.1 Trial 1

Because of the large cannibalism issue in Trial 1, approximately 60% of the flock was lost by the conclusion of the production cycle (47 weeks of age). Percent livability was as low as 46% and

36.5% for the northern fowl mite and control treatments, respectively (Figure 3.3), while the Tetra Brown management guide predicts a livability of 97.7% by 47 weeks of age (Tetra Americana, 2017). This high mortality affected hen-day production and eggs per hen housed and may have had an effect on case weights and egg components. Because cannibalism began early on in Trial 1 and to offset early mortality, replacement pullets were added at 21 weeks of age to Rooms 2, 3, and 4 that were sisters of the original flock. Hens in Trial 1 were not beak treated, but beaks were modified using a Dremel tool at 20 weeks of age. The intact beaks may have contributed to the exacerbation of cannibalism and large amounts of feather loss (Lay et al. 2011, Tablante et al. 2000, Heerkens et al. 2015), contributing to the low northern fowl mite populations. Northern fowl mites prefer the down-like feathering in the vent region of hens (DeVaney and Augustine 1987, Vezzoli et al. 2016), and excessive feather loss on hens in Trial 1 may have contributed to low mite populations in the flock. The intact beaks on hens in Trial 1 could have also caused low northern fowl mite populations. Chen et al. (2011) showed that birds with intact beaks are better able to groom themselves and control mite populations.

There was a period effect on eggs per hen housed, body weight, and beak damage in Trial 1 (Figures 3.2, 3.8, and 3.9, respectively) but the low northern fowl mite infestation or proportion of birds infested did not have an effect on those parameters. Period and treatment affected hen-day production percentage in Trial 1 (Figure 3.1). Period, treatment, and their interaction had an effect on case weights (Figure 3.4). Treatment and the proportion of birds infested with mites had an effect on feather damage on the belly, as well as an interaction effect between period and the proportion of birds infested with mites (Figure 3.10). Proportion of birds infested with mites, period, their interaction, and the interaction between treatment and period had an effect on percent shell (Figure 3.5). Foot damage, percent livability, and feather damage on the back and neck were affected by period and the interaction between period and proportion of birds infested with mites (Figures 3.9, 3.3, and 3.10). Period and the interaction between period and treatment had an effect on shell thickness (Figure 3.6). Feather damage on the crop and head were affected by the interaction between period and treatment and also by the interaction between period and proportion of birds infested with mites (Figure 3.10). Because northern fowl mite populations were so low throughout the production cycle in Trial 1 (Figure 3.7), treatment effects as well as effects from proportion of birds infested with mites could have been a result of depressed production parameters due to cannibalism stress in the four rooms or unforeseen management and environmental issues in the rooms such as humidity and litter moisture. The compounding effects of cannibalism and feather pecking, humidity, and litter moisture are likely what depressed aspects of production and welfare quality rather than presence of a mite infestation. According to Talukder et al. (2010), environmental stressors such as high air humidity can negatively impact performance. In addition, fear and stress from feather pecking and cannibalism can lead to decreased egg production (Mertens et al. 2009, Sun et al. 2014). The low percent livability in Trial 1 had an effect on hen-day production percentage, and fear and stress from the environment and feather pecking behaviors may have had an effect on case weight values. Because northern fowl mites typically reside in the vent area (DeVaney and Augustine 1987, Vezzoli et al. 2016) and because hens in Trial 1 had intact beaks, this may have been what led to feather damage in the belly area due to hens' increased ability to groom themselves, especially if irritation from introduction of northern fowl mites was occurring. Increased grooming combined with feather pecking behavior in the flock may have impacted feather damage in the belly area.

As shown in Figure 3.7, no mite count score above 1 was detected in Trial 1. After the initial northern fowl mite infestation (24 weeks of age), 33 hens were found to have a mite count score of 1 (1-10 mites per hen) during the data collection at 25 weeks of age. By 33 weeks of age, that number dropped to only 1 hen where mites could be detected. The second attempt to infest with NFM occurred at 35 weeks of age on 8 hens total between the two NFM rooms, and by the data collection at 36 weeks of age, NFM were only detected on 4 hens. The final attempt to infest with NFM occurred at 41 weeks of age (8 hens), and by the data collection at 47 weeks of age, mites could be detected on only 3 hens. The issue with cannibalism and mortality as well as management and environmental issues with humidity in the rooms may have played a role in the low NFM populations. Chen and Mullens (2008) found that NFM survive well at higher humidities and moderate temperatures. Specifically, optimum survival (35 days off-host) occurs at 15 degrees Celsius and 85% relative humidity (RH). In a study conducted by Abasa (1969), oviposition of NFM was reduced between 81 and 86% RH and optimum preoviposition occurred between 73-96% RH. High mortality of NFM will occur at 98% RH because of drowning (Abasa 1969). Litter became quite damp, especially in Room 1, which may have played a role in the survival and oviposition of NFM; however, exact humidity was not recorded. Mullens et al. (2010) reported that beak trimming has a negative effect on NFM populations, meaning birds are able to groom themselves more efficiently with intact beaks and Lay et al. (2011) wrote that beak trimmed hens typically harbor 3 to 10 times more ectoparasites than that of birds with intact beaks. Therefore, the fact that hens in Trial 1 were not beak modified and a cannibalism and feather pecking issue was present contributed to low NFM populations. Because feather pecking behavior and cannibalism were occurring in the flock, hens may have been efficiently removing mites from one another while performing these behaviors, which may have impacted the mite population.

3.3.2 Trial 2

Trial 2 hens were purchased as started pullets and were beak modified. The percent livability was 97.75% and 97.25% for the control and northern fowl mite treatments, respectively by period 8 (Figure 3.14). Cannibalism and feather pecking behaviors were not an issue in Trial 2. Mortality was impacted by period, treatment, their interaction, and the interaction between period and the proportion of birds infested with mites. While the difference in percent livability was extremely small between the control and NFM groups (0.5% difference), the treatment effect indicates that a mite infestation does have a slight negative impact on percent livability. Hen-day production percentage for the control and NFM treatments remained similar to the Tetra management guide; however, the hen-day percentage never achieved the maximum listed in the Tetra management guide for either group (Figure 3.12). Period had an effect on hen-day production percentage, as well as the interaction between period and the proportion of birds infested with mites (Figure 3.12) Eggs per hen housed for the NFM treatment was approximately 2.6% lower than the control treatment overall, and as a whole Trial 2 eggs per hen housed were lower than the Tetra management guide. Egg-eating behavior was observed in both the NFM and control groups in Trial 2 throughout the duration of the study. Hens in Trial 2 had a preference for laying eggs in the first two nest boxes (closest to the front of the room), leading to an overflow of eggs that collected in the nest box once the egg belt had filled up. This provided access to the eggs for the hens, leading to egg-eating behavior. This egg-eating behavior in the Trial 2 flock was likely what led to lower egg numbers than what was expected in the Tetra management guide. A higher eggs per hen housed value for the Tetra management guide is presented for Flock 2 because Flock 2 was carried out two weeks longer than Flock 1, as outlined in Section 2.1.5. Period and treatment had an effect on eggs per hen housed, as well as their interaction and the interaction between period and the proportion of birds infested with mites (Figure 3.13).

There was a period effect on case weights, percent yolk and percent albumen, shell thickness, and body weight, but no differences between treatments and no interactions between age and treatment were detected (Figure 3.15, 3.16, 3.17, and 3.19). Silversides and Scott (2001) examined the effects of egg storage and layer age on egg quality in ISA-White and ISA-Brown laying hens. They reported that as ISA-Brown hens aged, yolk increased in size and albumen decreased. While the hens used in this study were Tetra Brown hens, the study by Silversides and Scott may give us some insight into the age effect detected on percent yolk and percent albumen. In Flock 2, percent yolk increased slightly over time and percent albumen slightly decreased over time, which is consistent with the findings in Silversides and Scott (2001), although reasoning behind this finding was not expanded on in the study. Case weights increased over time for both treatments (Figure 3.12). DeVaney (1981) reported that infested White Leghorn hens had thicker shells than non-infested hens; however, in the present study presence of a mite infestation did not have an effect on shell thickness (Figure 3.17).

Feather damage worsened over time and was impacted by the northern fowl mite infestation. Period, treatment, proportion of birds infested with mites, the interaction between period and treatment, and the interaction between period and proportion of birds infested with mites affected feather damage on the head and neck (Figure 3.20). Feather damage on the crop was impacted by period, proportion of birds infested with mites, the interaction between period and treatment, and the interaction between period and proportion of birds infested with mites (Figure 3.20. Feather damage on the back was impacted by proportion of birds infested with mites, the interaction between period and treatment, and the interaction between period and the proportion of birds infested with mites (Figure 3.20). Feather damage on the keel and rump were affected by the interaction between period and proportion of birds infested with mites (Figure 3.21). Presence of feather damage in the areas sampled during Welfare Quality® Assessments peaked in the later periods (7 and 8) and appeared to be worse at period 8 in the NFM group compared to the control group for the head, neck, crop, and back areas (Figure 3.20), suggesting that the mite infestation negatively impacted feather coverage. Toe damage and presence of skin lesions were impacted by the interaction between period and treatment and the interaction between period and proportion of birds infested with mites (Figure 3.22). Skin lesions were also impacted by treatment (Figure 3.22). While more skin lesions were present in the control group than in the NFM group at period 8, there was a spike in skin lesions in the NFM group at period 6. This is at the same time point when infestation levels were strong (Figure 3.18), indicating that the increased presence of skin lesions may be attributed to irritation and increased grooming in the vent area because of the mite infestation. Loomis et al. (1970) wrote that NFM cause inflammation and scabbing in the vent region, which aligns with the spike in presence of skin lesions in the NFM treatment at period 6.

No mite count score above a score of 7 (> 10,000 mites) was detected in Flock 2 and a score of 1 was most frequently detected (Figure 3.18). Room 1 had fewer birds detected with a northern fowl mite infestation than Room 2 over the course of the study. Mites were detected in Room 2 723 times (regardless of score), while mites were detected 357 times in Room 1. Some management issues such as damp litter in Room 1 may have played a role in the lower mite populations than in Room 2, and room had an effect on mite populations (Figure 3.18).

3.3.3 Summary

Overall, a northern fowl mite infestation played a role in differences in mortality, feather damage, eggs per hen housed, and presence of skin lesions in Trial 2. NFM may have impacted hen-day production percentage, case weights, percent shell, and feather damage in the belly region in Trial 1. Production levels in Trial 2 were better overall than in Trial 1 due to excessive mortality in Trial 1. Because of higher and more consistent infestation levels in Trial 2, treatment effects detected in Trial 2 may be more attributable to the NFM infestation than in Trial 1. The NFM infestation in Trial 2 depressed egg production in regard to eggs per hen housed as well as aspects of hen welfare (such as feather damage and presence of skin lesions). Because of management limitations in this study and cannibalism and mortality issues in Trial 1, it can be concluded that treatment effects on production and welfare in Trial 1 can be attributed to environmental stressors within the flock and in each of the four rooms. Trial 2 provides a more concrete example of how NFM can impact production in a cage-free environment because of the increased infestation levels when compared to Trial 1. The mite infestation in Trial 2 did not have an impact on production parameters such as hen-day production percentage; however, it did impact eggs per hen housed and percent livability. Feather damage was affected by the presence of NFM in the head, neck, crop, and back areas, with feather quality being poorer in the NFM treatment than in the control treatment at period 8, specifically. It can be concluded from this study that NFM impact production parameters such as percent livability and eggs per hen housed, and welfare quality parameters such as feather damage and presence of skin lesions in a cage-free setting.

CHAPTER 4. CONCLUSIONS

Traditional egg production systems, such as conventional caged housing, has undergone scrutiny in recent years as consumers grow more concerned about where their food comes from, how it is produced, and the effects that production may have on animal welfare and environmental sustainability (Lay et al. 2011, Ochs et al. 2018). This scrutiny has led to legal changes in the European Union and the U.S. and responses from retailers and restaurants and has catalyzed a shift from conventional caged housing to alternative housing systems (Ochs et al. 2018, Campbell et al. 2016, Petrik et al. 2015). Because of the shift in consumer and retailer preference and because of recent legislation, cage-free egg production is increasing – as of March 2019, 18.4% of the U.S. laying hen flock was housed in a cage-free setting (United Egg Producers 2019). According to United Egg Producers (2019), the USDA's Agricultural Marketing Service estimated that 71% of the U.S. commercial laying hen flock must be in cage-free production by 2026 in order to meet demand. Because of this shift and the immense pressure on the industry to quickly move into cage-free production, it is important to consider the impact of cage-free systems on animal welfare, egg production, and ease of management of the system.

Cage-free (or non-cage) systems have many advantages, such as providing the opportunity for exhibition of natural behaviors or load-bearing exercises (Lay et al. 2011, Heerkens et al. 2015). These non-cage systems also have their drawbacks – for example, cleaning these systems may be difficult, which may result in additional opportunities for disease and parasites, such as the northern fowl mite, to spread (Lay et al. 2011). A northern fowl mite infestation can negatively impact egg producers and hen welfare through the mite's direct blood-feeding on the birds. This negative impact translates to reduced egg production and feed efficiency, along with irritation to hens and personnel working with infested flocks (Mullens et al. 2009, Loomis et al. 1970). As the shift toward non-cage systems is occurring, a better understanding of the impact of the northern fowl mite on laying hen performance and welfare in a cage-free setting is necessary. This thesis aimed to investigate the impact of the northern fowl mite on laying hen performance and welfare in a cage-free barn or single-tier aviary system.

Trial 1 of this project dealt with a cannibalism and feather pecking issue that led to a loss of approximately 60% of the flock by the conclusion of the production cycle (47 weeks of age). High mortality affected hen-day production percentage and eggs per hen housed. Because of feather pecking behavior in Trial 1, excessive feather loss on hens occurred, leading to large spikes in presence of feather damage on the back, neck, head, rump, belly, crop, and keel areas by the end of the study (Figure 3.10). Mortality in Trial 1 as a result of cannibalism and excessive feather loss due to feather pecking behavior resulted in low mite populations. Feather pecking behavior between hens could have also resulted in physical removal of mites on each hen. Treatment had an effect on hen-day production percentage, case weights, and feather damage on the belly (Figures 3.1, 3.4, and 3.10), and proportion of birds infested with mites had an effect on percent shell (Figure 3.5). Treatment effects may not be fully attributable to the northern fowl mite infestation because infestation remained low in Trial 1 (Figure 3.7). The compounding effects of cannibalism and feather pecking, humidity, and litter moisture are likely what depressed aspects of production and welfare quality rather than presence of a mite infestation. No mite count score above 1 was ever detected in Trial 1 (Figure 3.7) and three unsuccessful attempts to infect with mites occurred.

Percent livability in Trial 2 was much higher than in Trial 1, and by the conclusion of the production cycle (49 weeks of age) percent livability for the control and NFM groups was 97.75% and 97.25%, respectively (Figure 3.14). Hen-day percentage for Trial 2 remained similar to that of the Tetra management guide; however, eggs per hen housed for Trial 2 were lower than that of the Tetra management guide, due to lower egg production values than what is typical of Tetra Brown hens because of egg-eating behavior in both the NFM and control groups (Figures 3.12, 3.13). Eggs per hen housed for the NFM treatment was approximately 2.6% lower than that of the control treatment, and treatment had an effect on eggs per hen housed (Figure 3.13), indicating lower production values that resulted from the northern fowl mite infestation. The northern fowl mite infestation did not impact egg component parameters, but did impact percent livability, feather damage in the back, crop, head, and neck, and presence of skin lesions (Figures 3.16, 3.17, 3.14, and 3.20).

In Trial 2, hen-day production percentage between treatments remained similar (NFM hen-day production percentage was 1% higher than the control group), whereas in Trial 1, hen-day production percentage for the NFM treatment was 10% lower than that of the control treatment. Both flocks in Trials 1 and 2 underperformed what is found in the Tetra management guide. This can be attributed to stress and mortality as a result of cannibalism in Trial 1 and is a result of the northern fowl mite infestation and egg-eating behavior in both treatment groups in Trial 2. Values for eggs per hen housed in Trial 1 were lower than that of Trial 2 and percent livability in Trial 1 was much lower than that of Trial 2. Nearly 60% of the flock was lost due to mortality in Trial 1, whereas less than 3% of the flock was lost in Trial 2. Overall, the flock in Trial 2 outperformed the flock in Trial 1 in terms of egg production and livability. There were no influential changes in case weights, egg components, or body weights between flocks. Feather damage in Trial 1 was more pronounced than in Trial 2. In Trial 1, 50-60 hens per room had feather damage, and feather damage began earlier on in the flock, whereas presence of feather damage was lower in the control treatment in Trial 2, and feather damage for both treatment groups worsened in the later stages of the project (between periods 7 and 8). This difference can be attributed to the aggressive feather pecking behavior in Trial 1 that was absent in Trial 2. Because percent livability was much greater in Trial 2 than in Trial 1, more birds were sampled in each period in Trial 2.

Treatment effects and effects from proportion of birds infested with mites on production, percent shell, and feather damage on the belly in Trial 1 can be attributed to a result of environment and stressors within the flock and in each of the four rooms rather than a result of the low mite populations. The low mite populations are a result of feather loss on the hens due to feather pecking behavior, and this feather pecking behavior could have also resulted in hens removing mites from one another while performing this behavior. Percent livability in the control group was lower than in the NFM group (36.5% compared to 46%), suggesting that the presence of mites could have satisfied the cannibalism habits, whereas no mites were present in the control group. Environment could also play a large role in NFM population – while the complex environment in cage-free systems can be more difficult from an ectoparasite management standpoint (Lay et al. 2011), the presence of litter substrate where hens were able to dustbathe could have allowed for mechanical removal of mites during dustbathing behaviors. Multiple studies (Mullens et al. 2010, Martin and Mullens 2016, Murillo and Mullens 2016a) have shown that the presence of dust boxes and ability to dustbathe causes a decline in mite populations. In these studies, diatomaceous earth and sulfur were used as a dustbathing substrate; however, mechanical removal of northern fowl mites through dustbathing in shavings and litter in this study could have removed the mites. In addition, the amount of space hens had in the cage-free rooms used in this study could have impacted transmission of the infestation, making transmission inconsistent. Because hens had space to move around in this study and because stocking density was altered by rapid mortality in the flock, hens had the choice to spread out or to be in contact with one another, whereas in a conventional cage setting hens would be in continuous contact with one another.

Trial 2 can give more insight into the effect of NFM on production and welfare in a cage-free environment because of higher and more consistent infestation levels than in Trial 1. More consistent infestation levels were due to the lack of cannibalism and feather pecking behavior in the second flock, as well as the fact that hens were beak-modified, which did not allow for efficient grooming. NFM did impact egg production (eggs per hen housed), as well as percent livability and welfare quality parameters such as feather damage and presence of skin lesions. The NFM group was observed to have poorer feather coverage than in the control treatment by period 8 in the back, crop, head, and neck areas. Skin lesions were more prevalent in the NFM treatment at period 6, when the mite infestation was strong. The mite infestation dropped off after this, which is consistent with findings in Owen et al. (2009). Skin lesions in the vent region were likely due to irritation and scabbing from blood-sucking from mites and additional grooming behavior from the hens. This irritation on the skin is a host immune response to the northern fowl mite, and the inflammation increases the blood vessels under the skin beyond the length of NFM mouthparts. Because of this, mite populations will experience a decrease as mites move on to find another host (Owen et al. 2009).

We can conclude from this study that northern fowl mites impact eggs per hen housed, percent livability, presence of skin lesions, and feather damage (specifically in the back, crop, head, and neck regions). This is somewhat consistent with previous literature that outlines the negative impact of NFM on production parameters (Mullens et al. 2009); however, because no reduction in hen-day production percentage was found in the present study, more information and research are necessary to better understand the true impact of NFM on production parameters in a cage-free setting, as previous literature have found a reduction in hen-day production percentage of 2.1-4.0% in a conventional cage setting (Mullens et al. 2009).

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