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Measuring behaviors in agricultural animals using technological sensors

By

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ABSTRACT

The drive for producers and researchers to optimize productivity and sustainability in animals has created a new management system, precision livestock farming, that utilizes modern technology in order to access animal welfare. New technological innovations, such as sensors, have the ability to continuously monitor in real time individual agricultural species. They are affordable, reliable, easy-to-use, and can be easily applied to whatever parameter is being analyzed. Whether the sensors are wearable or close proximity to the animal, they provide precise measurements in a minimally invasive way. Two types of sensors, acoustic monitoring devices and accelerometers, have been used to examine the different components of animal welfare. The sound an animal makes contains vital information about its well-being. Acoustic devices such as microphones and sound-based monitoring systems have been used to study diseases, stress, foraging behaviors, and more. We proposed to utilize acoustic sensors to determine how animals use their environment, but that the study was cancelled due to the COVID-19 pandemic.

A common wearable device for animal monitoring is the accelerometer which measures changes in movement over time. In order to evaluate hen's behavior throughout the different stages of infestation of northern fowl mites, we fitted three-axis accelerometer sensors on 48 brown laying hens. Foraging, preening, dustbathing, and shaking were recorded during four phases throughout the study: Phase 1= prior to infestation, Phase 2= low levels of mites (early infestation), Phase 3= high levels of mites (peak infestation), Phase 4= treated (no mites). Hens spent significantly increasing amount of time preening and dustbathing as mite infestation levels increased ($p < 0.0001$) and significantly reduced the amount of time performing

these behaviors after they were treated with acaricide. Foraging significantly increased from Phase 1 to Phase 2 and stayed consistently high throughout the entire study, however the results were not significant. There was significant decrease in shaking behavior when hens went from no mites to low levels of mites, and then it significantly increased from low levels of mites to high levels of mites and from high levels of mites and to when the hens were treated. The treated birds perform shaking behaviors approximately the same amount of time as the baseline. These results indicate a possibility that accelerometer would be a useful tool for detecting behavioral changes during mite outbreaks on chickens. Technological sensors provide real-time information on individual animals which allows the potential for producers to make reformative decisions in management based on an individual's need or well-being.

CHAPTER 1

Acoustic monitoring to measure animal welfare in agricultural species

1. Introduction

Advanced technology coupled with precision engineering is increasingly allowing producers to gather real-time information on their animals. This new management system called Precision Livestock Farming (PLF) applies automatic monitoring to the animals to ensure optimal health and welfare (Exadaktylos et al., 2014; Li et al., 2019; Sassi et al., 2016). The market for PLF in 2015 was \$3.2 billion and is expected to increase to about \$7.9 billion by 2022 (Markets and Markets). Recent innovations in technological development have created devices that are affordable, durable, and easy-to-use (Berckmans, 2014). One such emerging technology, utilizing acoustic monitoring, shows promise as a method in commercial farming to monitor animal welfare in real-time.

Recent studies have shown an animal's vocalizations can provide information on their sex (Cordeiro et al., 2018; Pereira et al., 2015), age (Cordeiro et al., 2018), mother and offspring's relationship (Briefer & McElligott, 2011b; Padilla de la Torre et al., 2015), if their affective state is disrupted (Du et al., 2020; Lee et al., 2015; Marx et al., 2003; Meen et al., 2015; SEC et al., 2013), and growth rate (Fontana et al., 2015). For example, the method of using sound source localization from acoustic technology has allowed researchers to study abnormal night vocalizations of Hy-line brown chickens in laboratory and small-scale testing (Du et al., 2018). When applied to a large-scale commercial farm, acoustic monitoring has the potential to

allow producers to access abnormal behaviors in an automatic way in order to improve welfare protocols and facility layouts. In addition to detecting vocalizations, acoustic sensors can also monitor other sounds associated with an animal's health such as coughing (Ferrari et al., 2008). Understanding the animals' health and behaviors by using acoustic technology can let researchers and producers automatically classify and quantify behavioral events that may lead to early health detection without human contact.

Real-time objective measures of animal welfare using acoustic technology is a growing area of research. Acoustic devices provide researchers and producers continuous information on an individual level as compared to the whole group to help them understand the behaviors while removing the potential for observer effects. The aim of this review is to describe the different applications of acoustic monitoring on the welfare of agricultural species that investigate the various aspects of animal welfare. The limitations and future directions of acoustic monitoring devices are also discussed.

2. Early life decisions

It is critically important that any agricultural animal is carefully monitored at the beginning of their life. If neonates don't receive the correct nutrition and proper care, then this can lead to disruptions in their physiological growth and the development of social behaviors (Noy & Sklan, 1999; Rilling & Young, 2014). Vocalizations have been used as a tool for investigating maternal bonds and classifying sex and age in agricultural species (Briefer & McElligott, 2011; Reby & McComb, 2003; Watts & Stookey, 2000). Acoustic monitors have

also been used to optimize hatchability in the poultry industry (Bamelis et al., 2005). Using acoustic analysis during these early critical stages of production can give one insight on the welfare state of the animal which would be economically beneficial for producers.

2.1 Identifying sex

Researchers have observed sexual dimorphism and how animals communicate with one another in wild mammals by exploring variations in the structure of vocalizations as it relates to hormonal changes and body size (Charlton et al., 2009; Ey et al., 2007; Fischer et al., 2004; Khan et al., 2006). For example, Volodin et al. (2005) investigated the sex of adult whistling ducks using acoustic monitoring techniques. First, they figured out how female and male calls differ, and then they used that information to create an algorithm that would sort the calls. The researchers were able to determine that all twelve call parameters (i.e., initial frequency, end frequency, duration of first call, duration between second and third frequency maxima) that were used in the study showed females calls were higher in frequency compared to male. Further, they found that out of fifty-nine adult ducks their acoustic approach was 100% accurate in identifying the sex in comparison to DNA PCR sexing while cloacal sexing was only 89.8% accurate. This allowed researchers to establish a well-rounded and non-invasive sexing method in whistling ducks who are physically indistinguishable to humans (Volodin et al., 2009). Researchers have compartmentalized the acoustic structures in these animals' vocalizations to understand sexual differences using acoustic monitoring techniques. There has been a natural complement for this method in agricultural species in order to make the appropriate welfare decisions based on the sex of the animal.

There are limited studies that have looked to identify sex based on vocalizations in the livestock and poultry industry. In the egg industry, producers need to sex day-old chicks in order to separate out the males and females, but this can be challenging as chicks have no external sex organs (Cerit & Avanus, 2007). Available techniques for sexing avian species include laparoscopy sexing (Cerit & Avanus, 2007), cloacal inspection (Cerit & Avanus, 2007; Volodin et al., 2009), and DNA PCR-based methods (Aun & Kumaran, 2010; Cerit & Avanus, 2007; Volodin et al., 2009). Cloacal inspections require a trained handler to hold the bird upside down while everting the cloaca to observe the sex organs. Laparoscopy sexing entail making a small incision to examine the sex organs directly, and DNA PCR-based method requires DNA extraction from feathers or blood sample and the sex gene is amplified by PCR to determine the sex chromosome (Griffiths, 2000; Kaleta & Redmann, 2008). All of these techniques can be time-consuming and require some sort of handling which can lead to stress and impair their welfare. Sound analysis has a promising outcome in captive avian species. Pereira et al. (2015) investigated sex differences in day-old chicks from two different strains by recording the acoustic sounds produced by the chicks. They confirmed that the acoustic parameter of second formant (F2), a peak in the spectral sound, can determine the sex of the bird. Future studies should test this application in a commercial study in order to validate the use of acoustic monitoring to distinguish sex in a large scale. The potential use of acoustic monitoring as a way to differentiate male and female chicks in early age enables producers to make important management decisions based on the sex without capturing or manipulating them.

In the livestock industry, few studies have examined acoustic monitoring to determine the sex of individuals to help assess welfare. Producers may want to separate a large herd based on sex for their breeding or market programs and may also be easier to access the health and well-

being if males and females were separated. For example, a study recorded the vocalizations of male and female pigs throughout the farrowing, nursery, growing, and finishing stage while experiencing different distress conditions (e.g. cold stress, heat stress, water restrictions, and feed restrictions). After extracting ten acoustic attributes from the vocalizations, the results revealed that pitch was the only one of the ten attributes extracted that showed significant differences by sex. Male pigs had a lower pitch (194.5 Hz) compared to female pigs (218.2 Hz) (Cordeiro et al., 2018). Determining the sex of a pig based on their vocalizations would enable the researchers to evaluate the effects of the stressors more efficiently.

Separating animals early based on sex using acoustic technology has the benefit of saving time when sorting a large population and also eliminates close contact with the animal, which in turn reduces stress and has the potential to improve welfare. In addition, it would allow producers to apply breeding programs more successfully. The limited studies that identified sex using acoustic measures show potential in utilizing animals' vocalizations to accurately measure compared to already established methods. Since there are small number of studies that investigate sex identification using acoustic monitoring in agricultural species, this novel approach needs further examination on its validity before applying it on a farm.

2.2 Mother-offspring communication

Vocalizations are part of the communication line between mother and her offspring in order to assert the mother-young recognition. Mother discrimination by the young is important for correct maternal bonding in highly sociable animals and is essential for the survival of the progeny (Briefer & McElligott, 2011; von Keyserlingk & Weary, 2007).

Acoustic monitoring has allowed for more in-depth knowledge about vocal recognition in ruminant species. Studies have used playbacks of both the mother/offspring's vocalizations and alien mother/offspring's vocalizations to see how the animals respond back (Briefer & McElligott, 2011; Marchant-Forde et al., 2002; Sèbe et al., 2010). Youngs of ruminant species are able to individually recognize their mother's calls within a week of being born (Briefer & McElligott, 2011; Sèbe et al., 2010). For example, when one-week-old kids were played their mother's vocalizations and a familiar female's vocalizations on two loudspeakers, the one-week-old kids would vocalize more and respond more quickly to their mother's calls (Briefer & McElligott, 2011). This study highlights that offspring can recognize the uniqueness of their mother's voice at a young age. Playbacks of the smallest and slowest growing piglets versus the largest and fastest growing piglets in a litter showed that sows responded and faced the loudspeaker more to the slow-growing piglet than the fast-growing piglet (Weary et al., 1996). This study reveals that animals communicate with each other about their level of need. Producers can observe the conditions and characteristics of the young based on the communication between mother and offspring and adjust protocols if need to optimize piglets' welfare. For example, differences in the number of calls between mother and offsprings can help producers determine if the offsprings are thriving or not and create a feeding plan to help meet the offsprings' needs. In free-range conditions, researchers were able to study contact calls in cows and their calves by acoustic technology. The results revealed that cows will produce high frequency calls when their calf is separated, and they will make low frequency calls when the calf is nearby (Padilla de la Torre et al., 2015). Acoustic monitoring allows researchers to understand how a parent's vocalizations to offspring produce important information on the well-being and location of the individual young (Padilla de la Torre et al., 2015; Weary et al., 1996). This kind of information

can be advantageous to producers who raise large numbers or free-ranging animals as they might not observe mothers and their offspring(s) constantly.

Frequency and the number of vocalizations offers insight about the affective state between a mother and her progeny. These studies illustrate the important steps in our comprehension about mother/young communication, which is essential for creating robust vocal welfare measures in the livestock industries.

2.3 Incubation time in chicks

In incubation stage, poultry managers and producers' main goal is to maximize hatchability by reducing the spread of the hatching period (Decuypere et al., 2001; Tona et al., 2003). If the hatching window is large, then this will delay water and feed intake for the already hatched chicks which can negatively impact their welfare (Bamelis et al., 2005). This may result in decreased growth rate and body weight gain which are important performance parameters in the broiler industry (Decuypere et al., 2001; Geyra et al., 2001; Gonzales et al., 2003; Noy & Sklan, 1999). The already established approaches to shrinking the time between the first and last hatched bird such as temperature control (Decuypere & Michels, 1992), relative humidity, and carbon dioxide production (De Smit et al., 2006; Sadler et al., 1954) have been used in commercial hatcheries, however, many face the challenge of controlling all of these factors at the same time (Bamelis et al., 2005). A new approach, acoustic methodology, has gained traction in accessing the incubation time in chicks, however, there has been limited studies.

There is a potential of utilizing sound as being a sole feature to optimize hatching success. In small-scale testing, researchers placed ten eggs in individualized incubators and recorded the noise being produced by the chicks. The frequency of the chick's vocalization was

significantly different in the internal pipping, external pipping, and after hatch stages of incubation (Exadaktylos et al., 2011). This finding can help researchers develop an algorithm that can effectively tell producers what stage the chicks are in in order to adjust any factors so that the hatching window is reduced. In a different study, Bamelis et al. (2005) were able to monitor the start of the hatching process and the mean hatching time by calculating the energy level from the vocalizations the chicks produced. The results of the preliminary small-scale study were sufficiently promising that the idea was tested in a commercial scale hatchery. Researchers used an industrialized-scale hatch setter and electret microphones to surveillence 19,200 eggs in five repeated trials. Their goal was to solve how long it took all the chicks in the incubator to pass the internal pipping stage. The findings suggest that hatching time was within 3 hours of manual inspection (visual observations using candling) (Silva et al., 2010). Using acoustic monitoring that automatically detects hatching is a promising tool as it eliminates subjective bias, requires no contact with the eggs, and can simultaneously gather the sound from multiple chicks at once. Producers can know exactly when the eggs are being hatched and remove the ones that are already hatched so that their welfare is not impaired due to water and food deprivation. However, further research needs to focus on improving the algorithms so that the acoustic measurements better reflect actual hatch times.

3. Pain and stress detection

One way that researchers can determine the affective state of an animal is by understanding the acoustic signals that the animal is providing about its needs and conditions (Weary & Fraser, 1995). Stress-related vocalizations can be an indicator that the well-being of the animal is being disrupted. The establishment of a tool that classifies stress vocalizations in

animals in real-time will eliminate subjective perspectives in measuring various stress levels in agricultural species (Marx et al., 2003; Schön et al., 2004). Stressors such as castration in piglets, feather pecking in chickens, and heat stress in various agricultural species can be assess using acoustic monitoring which allows producers to identify the problems and improve on operational outcomes. By knowing the animal's natural history, biological functioning, and emotional states, producers and researchers can gain insight on the animal's welfare (Duncan & Dawkins, 1983; Fraser, 1993; Weary & Fraser, 1995).

3.1 Castration

A common practice done on domestic pigs is castration to eliminate aggression and boar taint in male pigs (Fredriksen et al., 2011). Physiological responses and the effects of castrations in piglets have been well studied in terms of pain level experienced and the use of local anesthetics (Moya et al., 2008; Prunier et al., 2006; Von Borell et al., 2009). Measuring cortisol levels by collecting blood samples is a common method to evaluate pain and stress in castrated piglets (Byrd et al., 2020; Gottardo et al., 2016; Kluivers-Poodt et al., 2012; Sutherland et al., 2012); however, this approach can be stress-inducing to the piglets.

One way to access pain and stress in piglets during castration that is non-invasive is by studying their stress vocalizations (Leidig et al., 2009; Sutherland et al., 2010; Weary et al., 1998; White et al., 1995). An automatic monitoring system designed to detect stress vocalizations in pigs has been developed in Germany called STREMODO (stress monitoring and documentation) (Manteuffel & Schön, 2002; Schön et al., 2001). This system utilizes linear production coding and artificial neural network in order to only recognize and classify stress vocalizations from pigs (Schön et al., 2004). STREMODO has allowed researchers to access

different products and methods of analgesia (Leidig et al., 2009; Sutherland et al., 2010) and to quantify the stress in piglets without anesthesia (Manteuffel & Schön, 2004; Puppe et al., 2005) during castration. Studies that used STEMODO have had a reliability of more than 95% when detecting stressful calls in pigs even with a noisy environment (Manteuffel & Schön, 2004; Puppe et al., 2005; Schön et al., 2004). With the help of STREMODODO, producers could find the best plan for castration in a way that minimizing stress and pain calls. Future studies should explore using the STREMODODO system for other domesticated species like cattle, goats, and sheep that uses castration practices for production purposes.

3.3 Feather pecking in chickens

The different types of calls (Zimmerman et al., 2000) and specific acoustic parameters in domestic fowl's vocalizations (Jones et al., 1998; Marx et al., 2001) can be useful indices when determining the quality of their welfare. Feather pecking in poultry is an aversive experience that can lead to decreased productivity and increased feed consumption in both laying hens and broiler chickens (Nicol et al., 2013). If managerial actions don't take place right away then mortality can rise in the flocks due to feather pecking (Keeling & Willhelmson, 1997; Johnsen et al., 1998; Rodenburg et al., 2008).

Acoustic monitoring has the potential of detecting feather pecking early so that it doesn't become problematic issue in the chicken's welfare that can't be reverse. Bright (2008) compared the vocalization rates in non-feather pecking and feather pecking farms in a commercial setting. The results showed that there was a significant difference in that the total vocalizations in feather pecking farms were more than non-feather pecking farm. Rodenburg and Koene (2003) also confirmed high total vocalization rates based on the rate of feather pecking. There has been

anecdotal evidence that suggest noise coming from groups of feather pecking hens sound different than non-feather pecking hens at the beginning stage of feather pecking (Bestman & Wagenaar, 2003; Bright, 2007). This would be a perfect opportunity to utilize acoustical devices to supply factual evidence to support this claim. If producers can recognize that their flocks are experiencing feather pecking via acoustic monitoring, then it gives them the opportunity to find the cause that is compromising the chickens' welfare (i.e. high density, heat stress, injuries of other birds, and inadequate nutrition) and be able to fix the problem in a timely manner.

3.3 Thermal stress

Understanding optimal temperature ranges for various agricultural species is key for successful production. Thermal stress is detrimental to an animal's health and productivity (Ferrari et al., 2013). In the poultry industry alone, losses of \$128-165 million annually have been estimated due to the consequence of heat stress (St-Pierre et al., 2003). Physiological and behavioral adaptations are needed for animals to cope with the changes in ambient temperatures which may decrease efficiency in production livestock (Das et al., 2016; Lara & Rostagno, 2013). This means that management needs to observe thermal stress and make decisions promptly and effectively.

Analyzing vocalizations using sound technology has been a useful non-invasive tool to evaluate early warning levels of thermal stress. Studies have found that an increment in peak frequency in the vocalization of chickens and swine is correlated with a rise in environmental temperatures (De Moura et al., 2008; Ferrari et al., 2011, 2013). Also, animals, such as broilers and pigs, that are experiencing thermal stress increase their number of times vocalizing compared to non-stressed broilers and pigs (Curtin et al., 2014; S. Ferrari et al., 2011). This

finding was supported by Liu et al. (2018), who found that the turkeys who were heat stressed vocalized 43% more than the control group. In one study, researchers evaluated the thermal comfort of 100 laying hens using a noise-based monitoring system. After the sound of the hens were collect, the results revealed that hen's alarm and squawk calls were notably correlated with heat stress indices (Du et al., 2020). An acoustical analysis is a propitious method that is reliable compared to the conventional method of thoroughly inspecting all the animals visually, which can be time- consuming on a commercial farm and the person needs to have complete training in order to observe signs of heat stress in animals. In addition, having a continuous monitoring system allows producers to make quick decisions before the effects of heat stress are detrimental.

4. Health Management

Producers are constantly striving to have their animals healthy and growing efficiently. If an animal's health is compromised, then it is critically important for producers to make quick and proper decisions in order to alleviate the issues. From weighing poultry to accessing respiratory diseases in intensive farming, acoustic monitoring has allowed for continuous assessment on the animals' well-being. Early detection of health-related issues using acoustic methodology increases the survivability of the animal and promotes economic growth in the production animal industry (Matthews et al., 2016).

4.1 Growth in turkeys and broilers

Weighing is a routine husbandry practice that allows producers to determine if the animals are properly growing, sick or healthy, and if different feed diets need to be implemented

(Lawrence et al., 2017). However, human contact, isolation in individualized scales, and inadequate handling skills can cause fear responses and hinder the animal's welfare (Grandin, 1989; Grandin, 1997). In broiler commercial farms, automatic weighing systems called "step-on scales" are used to take an average weight of the flock without having human contact (Fontana et al., 2017). However, this system induces bias measurements. Birds that are heavy, lame, or sick are reluctant to walk (Bokkers & Koene, 2003; Nääs et al., 2009) and most likely unwilling to step on the scale. Chedad et al. (2003) collected images of broilers on the scale platform and of the near surroundings of the platform for 42 days to confirm their hypothesis that heavier birds do indeed approach the automatic scale less in comparison with lighter birds, especially towards the end of their growing period.

Using the animal's vocalizations can be an objective tool to measure body weight. The relationship between vocalization and body size was first introduced by Eugene Morton in 1977 (Bowling et al., 2017). Since then, recent studies have used this concept to develop an automatic tool to accurately predict the weight of turkeys (Abdel-Kafy et al., 2020) and broiler chickens (Fontana et al., 2017). Studies have shown that the noise that chickens produce decreases in frequency as the birds age (Fontana et al., 2014; 2015; 2017). An experiment done with 570 turkeys recorded the peak frequency of their vocalizations as well as random samples were selected to be manually weighed from 13 to 128 days of age in four different trials. By combining all of the data from all the trials, researchers were able to confirm that there was a significant negative correlation ($r^2 = 0.97$) between the weight and peak frequency of the turkey's vocalizations based on the age of the bird (Abdel-Kafy et al., 2020). Audio data that is collected from turkey vocalizations is a great tool that will allow producers to monitor the growth of turkeys without physically handling or causing stress to them. The next future step is to

implement acoustic monitoring to measure animal's weight into the PLF system in order to test the validity in farm use.

4.2 Respiratory Diseases

Early detection of abnormalities involving health is an important step in management in intensive production farms (Chung et al., 2013). If the illness is left untreated right away, the animal will reduce productivity, have an increased chance of mortality, and intervention from the veterinarians could get costly (Gerhard Manteuffel et al., 2004; Neethirajan et al., 2017; Rizwan et al., 2017). Coughing can be a symptom of potential respiratory complications (Chedad et al., 2003; Chung et al., 2013; Vandermeulen et al., 2016). Taking a Precision Livestock Farming approach, researchers have been creating continuous and automatic monitoring systems to recognize the early signs of respiratory diseases by investigating the different sounds produced by coughing.

In the swine industry, researchers have attempted to localize coughs to a particular pen. Researchers used electret microphones to triangulate the cough sounds and thus create a pinpoint location of where the coughing takes place (Exadaktylos et al., 2008b; Silva et al., 2008). Localizing the cough sounds can allow veterinarians to quantify and assess the conditions of the pigs (Silva et al., 2008). Research has also looked to see if acoustic tools can identify sick vs. non-sick pig coughs (Chung et al., 2013; Exadaktylos et al., 2008a; Ferrari et al., 2008). One group of pigs were infected with bacterial strains that induced pneumonia while another group were exposed to citric acid solution to promote chemically induced coughs (Exadaktylos et al., 2008a; Ferrari et al., 2008). Pigs exposed to pneumonia had a shorter mean duration, longer length of single cough, and lower peak frequency in comparison to non-

infectious coughs (Ferrari et al., 2008). Immediate actions can take place by the producers due to automatic data collection using acoustic technology.

There are many highly pathogenic respiratory diseases in poultry species (Swayne & King, 2003). These diseases are a huge problem in the poultry industry due to being extremely contagious, have high mortality rates, and possible complete eradication of an infected barn full of thousands of birds (Al-Dabhawe et al., 2013; Shankar, 2008). One small-scale study looked to create an artificial intelligence system based on chicken's vocalizations to diagnose diseases (Banakar et al., 2016). Researchers placed 4 separate groups of 60 chickens each (one was the control group and the other three were infected with Newcastle Disease, Bronchitis Virus, and Avian Influenza respectively) in a box to collect their sounds using a microphone. By creating an algorithm using the extracted sounds, researchers were able to create an automatic detection system that was 91% accurate in identifying Avian Influenza, Newcastle Disease, and Bronchitis Virus (Banakar et al., 2016). Another study looked solely on Bronchitis Virus using audio signals and saw 97% accuracy when investigating the rale sounds of chickens (Rizwan et al., 2017). Producers are able to get rapid and accurate diagnosis so that the animals receive proper treatment in order to improve their health, welfare, and production. Future studies should explore the validity of using acoustic technology to monitor respiratory health in large commercial setting

5. Conclusion

The push to create validated welfare measurements is largely due to the public concerns over animal's well-being (Sassi et al., 2016). Over the past decade, technology has increasingly been adopted by animal production, including its use for monitoring animal welfare. Acoustical

devices have provided researchers with the ability to examine animal health, behavior, and environmental parameters in real-time. Information gathered on sex determination on chicks and pigs (Pereira et al., 2015; Cordeiro et al., 2018), heat stress in poultry (Du et al., 2020; Liu et al., 2018), and respiratory diseases in pigs (Chung et al., 2013) have been accomplished using sound analysis.

Although some studies have only been done in a lab setting, researchers have been moving towards integrating acoustic monitoring in commercial conditions. Even though technology is becoming advanced, the movement to commercial applications may bring limitations. It is essential that both the acoustic set-up and the use of algorithms can be easily performed by producers and laborers. Fully adapting to a complex system may be time-consuming to learn. Also, lab settings can control many variables, so it may be challenging to apply acoustic monitoring when taking account of the different and unpredictable variables in a commercial setting. This means that acoustic devices need to be durable, long-lasting, and withstand environmental states. The progression in the use of wearable sensors have gained importance in animal welfare management. It is valuable to note that it is critical for wearable sensors to not put stress, change the behaviors, or draw attention to other animals which could lead to biting/pecking on the animal who is wearing the sensor. Previously mentioned in this paper, some applications using acoustics were only species-specific. Future studies should explore using the same methods in other livestock and poultry species. This will increase validity with the methods and algorithms and would benefit producers with multi-species farms economically.

Over the past many decades, global meat, dairy, and egg consumption have increased by rapid rates. By 2030, meat production is forecasted to reach over 450 million tonnes (Alexandratos & Bruinsma, 2012). The need for continuous real-time data is important as ever in order to gain efficiency and productivity in animals to feed the world's population. Many studies showed the capability of utilizing acoustic monitoring as a way to assess animal welfare; however, these studies are conducted in a research setting. In order to establish acoustic monitoring as an approach in Precision Livestock Farming, they need to be integrated and validated in farm use. Automated and continuous sound detection systems using acoustic monitoring has the potential in delivering a minimally invasive and explicit method to measure animal welfare in commercial farms.

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CHAPTER 2

A wearable sensor system to assess behavior of chickens infested with Northern Fowl Mites (*Ornithonyssus sylviarum*)

Abstract

In North America, northern fowl mite (NFM; *Ornithonyssus sylviarum*) is one of the most common mites in commercial poultry production. When infested with NFM, chickens can become anemic, reduce egg production, experience pain and stress, have signs of weight and feather loss, and if left untreated can lead to death. To understand the impacts of NFM on hen's behavior throughout the different stages of infestation, we fitted three-axis accelerometer sensors (a common wearable device to measure activity and movement) on Hy-Line Brown laying hens (N = 48) to record the duration of foraging, preening, dustbathing, and shaking during different levels of infestation of NFM. Movements recorded by sensors were identified to specific bird behaviors through a previously trained algorithm, with frequency of these behaviors recorded for individual birds. Data collection took place during four infestation phases: Phase 1= prior to infestation, Phase 2= low levels of mites (early infestation), Phase 3= high levels of mites (peak infestation), Phase 4= treated (no mites). Accelerometer measurements revealed that hens significantly spent increasing amount of time preening and dustbathing as mite infestation levels increased ($p < 0.0001$) and significantly reduced the amount of time performing these behaviors after treated with acaricide. Foraging significantly increased from Phase 1 to Phase 2 and stayed consistently high throughout the entire study, however there was no significant differences. There was significant decrease in shaking behavior when hens went from no mites to low levels of mites, and then it significantly increased after that. The treated birds perform

shaking behaviors approximately the same amount of time as the baseline. Overall, preening and dustbathing behaviors had a positive correlation and shaking had a negative correlation to NFM infestation levels. The three-axis accelerometer has the potential to be a useful tool for detecting behavioral changes throughout a mite outbreak in chickens.

1. Introduction

Over the last two decades, there has been growing interest in the use of wearable sensor technology to assess animal welfare (Harrop et al., 2017). Wearable sensors provide real-time and reliable monitoring that can detect health issues in a timely manner (Neethirajan, 2017) and assess behavioral parameters, such as activity budgets and locomotion of various animals (Neethirajan, 2017; Pons et al., 2017). These devices used as an implement to analyze animal welfare would bring potential advantages to producers as they can collect continuous data to make quick decisions to prevent/reduce the spread of diseases and adjust management protocols to increase welfare and productivity (Neethirajan, 2020).

One such commonly used wearable device for animal monitoring is the accelerometer, which measures activity and movement based on changes in the animal's velocity in a period of time (D. Brown et al., 2013; Halachmi et al., 2019). Most common accelerometers are single-axis, dual-axis, or triaxial depending on how many orthogonal directions one wants to measure simultaneously. The use of accelerometers supplies real-time information as it can detect changes in an individual's behavior over a period of time which is important when measuring animal's well-being (Li et al., 2019; Pastell et al., 2009), however, there have been limited studies that measure behaviors of chickens using accelerometers. Banerjee et al. (2012)

accurately detected six behaviors based on the output of a dual-axis accelerometer using computer algorithms. In another study, the researchers fitted a jump-detection wearable device on chickens in non-cage housing system. The accelerometer in the device was able to detect the height of jump, landing force, and time to land (Banerjee et al., 2014). Researchers compared physical activity levels in laying hens based on age using three-axis accelerometer mounted on the hens. The results showed as birds aged from young pullets to mature hens that the percentage of time spent executing high-intensity physical activity decreased (Kozak et al., 2016). These findings can provide important insight on how hens change their activity budget and utilize their space over a period of time. This allows producers to optimize chicken welfare by developing better housing systems and remotely monitoring the chickens' behaviors on an individual level.

Wearable accelerometer system has the potential of not only collecting continuous data but also may provide early detection of infestation. Researchers developed a wearable system to monitor activity levels of chickens who were exposed to the highly pathogenic Avian Influenza Virus. By counting the number of accelerations above the threshold (the activities during the latest 3 hours are lower than the minimum average of daytime activities when chickens were not infected), the researchers could detect infectious behaviors twice as early compared to using body temperature sensors (Okada et al., 2014) and diagnose the infection ten hours before death (Okada et al., 2010). A tool that can detect early infectious outbreaks lets producers to take immediate and proper actions in order to save the animals' lives.

In North America, the northern fowl mite (NFM; *Ornithonyssus sylviarum*) is one of the most common mites in commercial poultry (McCulloch et al., 2020; Owen et al., 2009). These mites feed off the chicken's blood and are economically damaging because it hard to eradicate them in a flock (Crystal, 1986; Mullens et al., 2010). The infestation can occur in both

conventional (Arther & Axtell, 1983; Mullens et al., 200) and caged-free housing systems (Mullens & Murillo, 2017). Chickens can become anemic, reduce egg production, experience pain and stress, have signs of weight and feather loss, and if left untreated can lead to death (Jacobs et al., 2019; Mullens & Murillo, 2017; Vezzoli et al., 2016). A recently published article found that feather damage on the head and neck and skin lesions were worsened over time in the treatment groups that were infested with northern fowl mites compared to the control group (Jarrett, 2020). To study the impact of northern fowl mites on egg production, researchers recorded daily egg production of broiler breeder layers that were infested with northern fowl mites and ones that were free from northern fowl mites. At the end of study, the layers that were free from northern fowl mites produced over three thousand dozen more eggs than the layers that were infested with northern fowl mites (Arends et al., 1984).

Since ectoparasites, such as the northern fowl mites, are in contact with the host skin and/or feather, there is no surprise that different grooming behaviors play a crucial role in the behavioral repertoire of birds. Preening and dustbathing are common grooming behaviors performed by avian species, including chickens, experiencing ectoparasite infestation (Bush & Clayton, 2018; Clayton et al., 2014). Based on a comparative analysis of 62 avian species, Cotegreave and Clayton (1994) noted that parasite infestation levels highly influence the amount of time birds spent grooming.

The few studies that have observed the effects of NFM on chicken behaviors had limitations. The way these studies were measured was by human observations and video recordings (Jacobs et al., 2019; Vezzoli et al., 2015, 2016) which are labor-intensive and restricts sampling intervals and duration. These limitations in turn restrict the number of chickens used, observational period per chicken, and how many studies are conducted. Using sensors to study

the effect of mite-infested chicken's behavior offers a good tool to accurately assess the welfare of the chickens in real-time. This experiment was part of a larger study from Murillo et al. (2020) which investigated the welfare and behavior of chicken with no, low, and high mite scores. The purpose of this study is to evaluate the activity budget of chicken behaviors before, during, and after NFM infestation using a wearable three-axis accelerometer system. We hypothesized that the time spent performing behaviors by hens will be different during different infestation levels due to alleviating the stress and discomfort of the mite infestation. We predicted that as infestation levels increase that the hens would spend more time foraging, preening, dustbathing, and shaking in order to combat the effects of northern fowl mites.

2. Methods

2.1 Ethical statement

This study was approved by and conducted in accordance with the University of California Riverside Institutional Animal Care and Use Committee.

2.2 Animals and housing

Forty-eight Hy-Line Brown laying hens were housed at the Poultry Research Facility at the University of California Riverside Agricultural Operations (Riverside, California, USA). Hens were 22 weeks old at the beginning of the study and were received from a local poultry facility. The birds were separated into four groups: Flock 1, Flock 2, Flock 3, Flock 4, (12 birds per group). Each group was housed in a 1.9 x 2.9 m floor pen bedded with straw. Hens had access to *ad libitum* feed and water as well as nest boxes. Lights were maintained on a 16:8

(L:D) cycle. Individual hens in each flock were distinctly marked using colored leg bands for bird identification.

2.3 Wearable sensors

Hens were fitted with three-axis accelerometers (AX3, Axivity Ltd, UK) to record the orientation and magnitude of acceleration as the chickens moved or changed body positions within their designated pens. Sensors were positioned in plastic “backpacks” (Hero 4 AHDBT-401 plastic case, Amazon.com, Seattle, WA, USA) secured to the back of each bird using elastic bands stretched around the base of each wing (**Figure 1**). The data from the sensors were collected at a rate of 100 readings/sec.



Figure 1. Chickens wearing the “backpacks” with the three-axis accelerometers inside (left). The three-axis accelerometer in the plastic case (top right). The backpack was made of a plastic case, two elastic bands, and color tape to mark the backpacks (bottom right).

*Photo credit: Amy Murillo

2.4 Behavior

In order to classify behaviors performed by birds, a “behavior dictionary” was developed (as described in Murillo et al., 2020). In brief, data was collected from ten different birds (recorded for ≥ 4 hours at a time) over the span of several months to create sensor data which was used to build and evaluate the behavior dictionary. In previous study, video recordings of the test birds were synced with sensor output data, and visually observable and distinct behaviors were annotated by a single observer using ELAN open-access software (Max Planck Institute for Psycholinguistics, The Language Archive, Nijmegen, The Netherlands, v. 5.2, <https://tla.mpi.nl/tools/tla-tools/elan/>) (Murillo et al., 2020). The details on how the algorithms classified hen behavior are described by Abdoli et al. (2019) and Abdoli et al. (2020). The algorithms were validated using video recordings with an average accuracy rate of 94%. The four behaviors of interest that were recorded were pecking, preening, dustbathing, and shaking behaviors (see **Table 1**) because they were distinguishable from one another and are associated with chicken welfare.

BEHAVIOR	DEFINITION
FORAGING	Beak tapping the ground in search of substrates

PREENING	Running beak in stroking motion through feathers to arrange, manipulate, or clean body feathers
DUSTBATHING	The bird is squatted on the ground, throws litter substrate over their body by ruffling feathers and rubbing ground
SHAKING*	Moving body side-to-side vigorously in standing or sitting position

Table 1. Behavioral definitions

*Shaking is a component to dustbathing

2.5 Northern Fowl Mite Infestation

This study This study was conducted over 12 weeks from November 2017 to February 2018. Phases of the study corresponded to infestation levels. Procedures on how the birds were inoculated with northern fowl mites are described in detail by Martin and Mullens (2012). To summarize, NFM were collected from source hens using capillary pipettes. Each hen in our study was infested with approximately 20-30 mites that was placed in the skin and feathers of the vent area. There were four phases of mite infestation, During Phase 1, hens' behaviors were recording during week 1. The hens were examined and found not to have any NFM (**Table 2**). This phase served as a baseline for behavioral data collection. Phase 2 began at week 2 and lasted through week 4. During phase 2 most of the chickens were naturally infested with low

levels of NFM by week 4 (45/48 hens infested) (**Table 2**). Throughout phase 3, all chickens were deliberately inoculated with NFM at the beginning of week 5 to ensure that all birds in each flock were exposed to a similar infestation level of mites from week 5 to end of week 8. At this phase, all the birds were infested with high levels of NFM (**Table 2**). During phase 4, all birds were treated with an acaricide, RaVap (Bayer, Shawnee Mission, Kansas, USA), following label instructions to eliminate mites at two different weeks. The hens were fitted with the backpack containing the sensor to record the bird’s behavior during weeks 1, 4, 7, and 12 which correlates with Phase 1, 2, 3, and 4 respectively (**see Table 2**). After each recording period, data were downloaded from each sensor and run through the developed algorithm (Abdoli et al., 2020; Appleby et al., 2004) to identify and tally each behavior event recorded for the defined behaviors of interest. Infestation levels were assessed by calculating the average mite score based on the mite density in the vent area by a single researcher for consistency in score (Murillo et al., 2020).

Phase	Week	Infestation level	Behaviors recorded	Average mite score*
1	1	none	Week 1	Week 1= 0.0 ± 0.0
2	2-4	low (naturally infested)	Week 4	Week 4= 2.10 ± 0.22
3	5-8	high	Week 7	Week 7= 4.83 ± 0.14
4	9 & 11	none (treated with acaricide)	Week 12	Week 12= 0.0 ± 0.0

*Mite score = number of mites: 1 (1-10 mites), 2 (11-50 mites), 3 (51-100 mites), 4 (101-500 mites), 5 (501-1000 mites), 6 (1001-10000 mites), and 7 (>10000 mites) (Arthur & Axtell, 1983)

2.6 Statistical analysis

Data were analyzed using SAS software (SAS Institute Inc., Cary, NC, 2012, v. 9.4.), with PROC MEANS used to generate means and standard errors for behavior duration in seconds (s). A general linear model (PROC GLM) and least squares means were used to detect whether average duration of all the hens' behaviors differed across the four different phases. The repeated variable was phases, independent was flock, and the dependent variables were the four behaviors observed in this study (foraging, preening, dustbathing, and shaking). A Tukey's post hoc test was used to compare between means. Statistical significance was analyzed at a threshold of $\alpha = 0.05$.

3. Results

3.1 Foraging

Hens foraged less in Phase 1 than all subsequent phases ($p < 0.0001$) (**Figure 1**). Time spent foraging during low levels (Phase 2), high levels (Phase 3), and treated (Phase 4) were consistently high (1729.8s, 1704.81s, and 1799.72s respectively), however, there was no significant difference between these phases.

3.2 Preening

The duration of preening significantly increased from the baseline of this study to when the chickens had low levels and from low levels to high levels of NFM (**Figure 2**). In fact, the chickens in Phase 3 (mean \pm SE: 3545.11s \pm 78.61) preened almost 57.5% more than they did when they had no mites at the beginning of the study (mean \pm SE: 1503.85s \pm 37.44, $p < 0.0001$). After the birds were treated with the acaricide, time spent preening significantly decreased compared to when the birds were exposed to high levels of NFM ($p < 0.0001$), and the average duration of preening was similar to the baseline of the study.

3.3 Dustbathing and shaking

As shown in **Figure 3**, there was a significant increase in the time spent dustbathing from no mites (Phase 1) through high levels (Phase 3) ($p < 0.0001$). The time spent dustbathing between the high levels and after the chickens were treated with the acaricide (Phase 4) significantly decreased ($p < 0.0001$) to a similar value to when the chickens were experiencing low levels of mite infestation. The chickens significantly decreased time spent shaking from no mites (Phase) 1 to low levels (Phase 2). Time spent performing shaking behaviors by the hens increased significantly from low levels (Phase 2) to when they got treated (Phase 4). The average time spent shaking during when the hens were treated was similar to the baseline of the study.

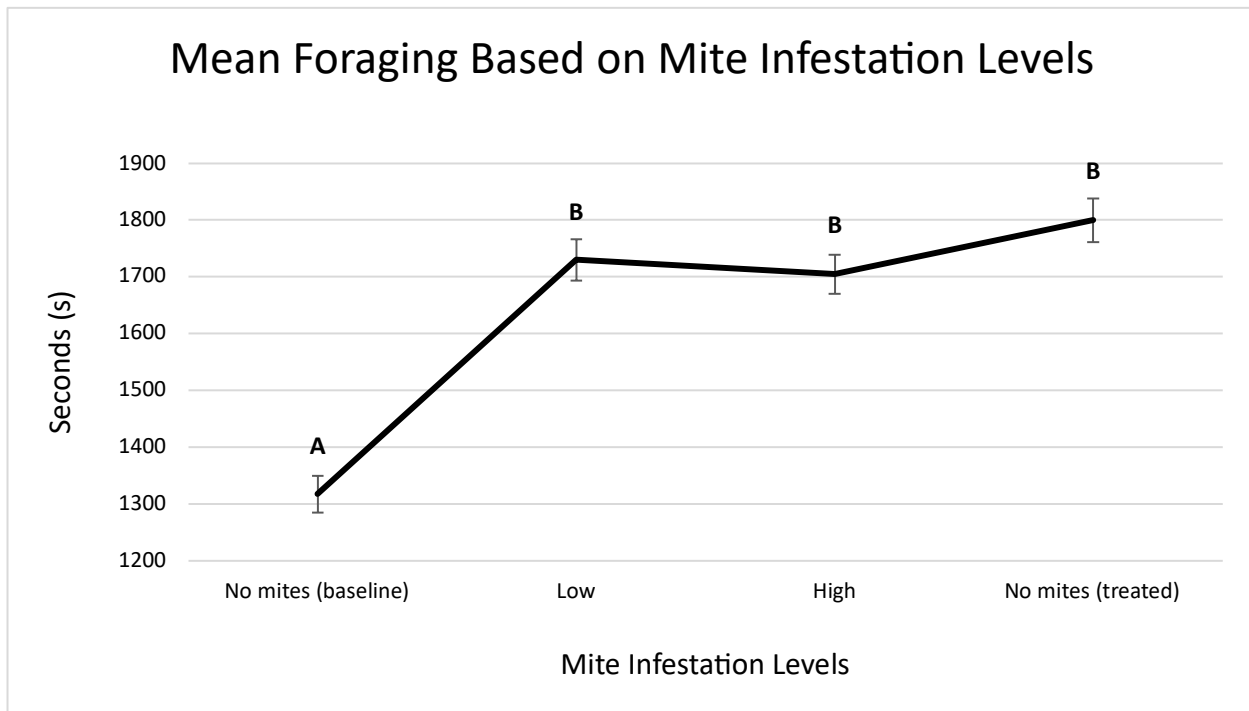


Figure 1. Mean time spent foraging per designated week of recording for all birds. The chickens were recorded during the weeks of 1, 4, 7, and 12 that represents phases 1, 2, 3, and 4 respectively. The mite infestation level at each week is as follows: no mites (baseline) = Phase 1, low levels of mites = Phase 2, high levels of mites = Phase 3, no mites (treated)= Phase 4. Different letters indicate significant differences between the phases.

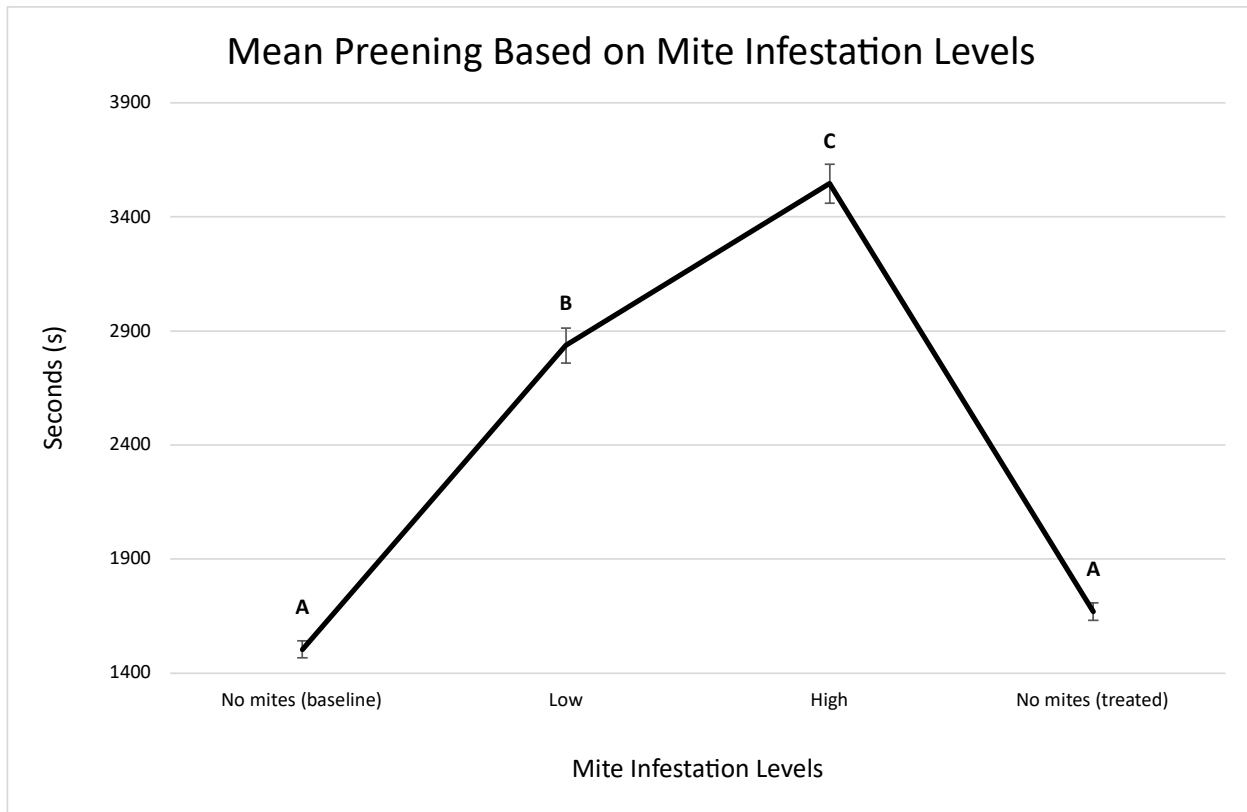


Figure 2. Mean time spent preening per designated week of recording for all birds. The chickens were recorded during the weeks of 1, 4, 7, and 12 that represents phases 1, 2, 3, and 4 respectively. The mite infestation level at each week is as follows: no mites (baseline) = Phase 1, low levels of mites = Phase 2, high levels of mites = Phase 3, no mites (treated)= Phase 4. Different letters indicate significant differences between the phases.

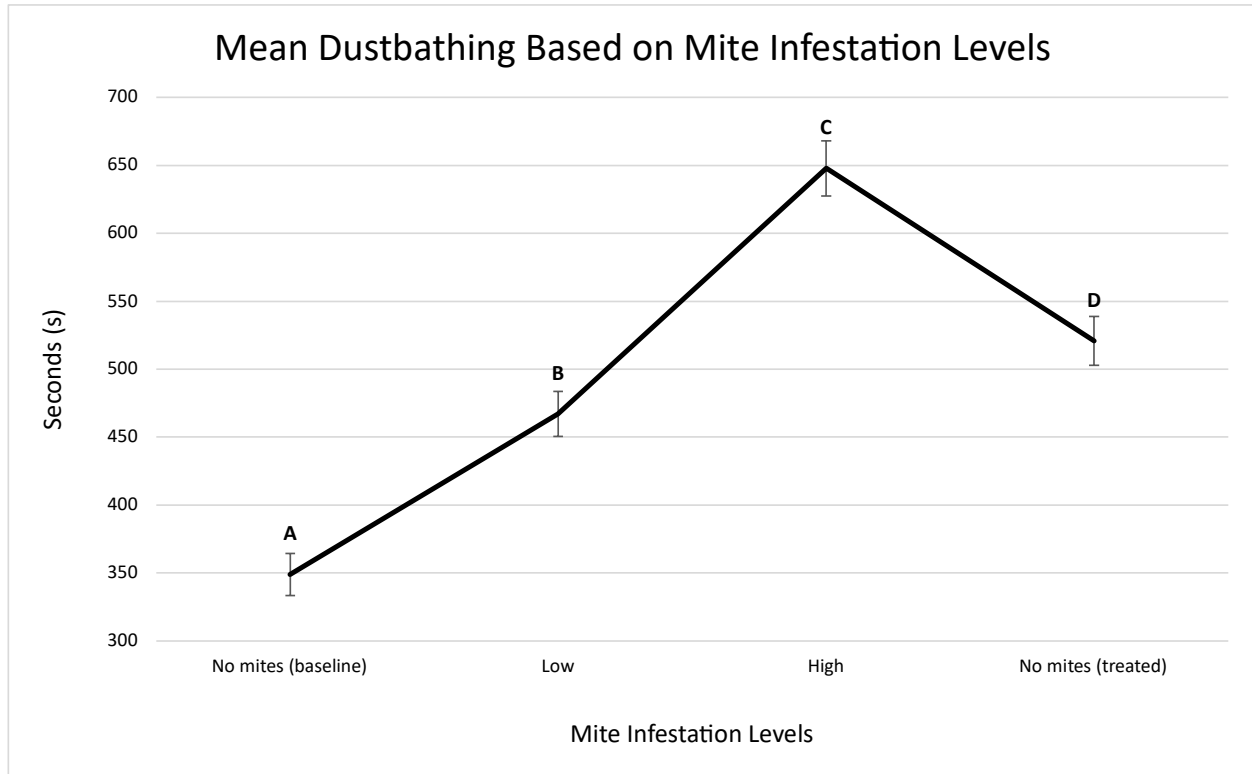


Figure 3. Mean time spent dustbathing per designated week of recording for all birds. The chickens were recorded during the weeks of 1, 4, 7, and 12 that represents phases 1, 2, 3, and 4 respectively. The mite infestation level at each week is as follows: no mites (baseline) = Phase 1, low levels of mites = Phase 2, high levels of mites = Phase 3, no mites (treated)= Phase 4. Different letters indicate significant differences between the phases.

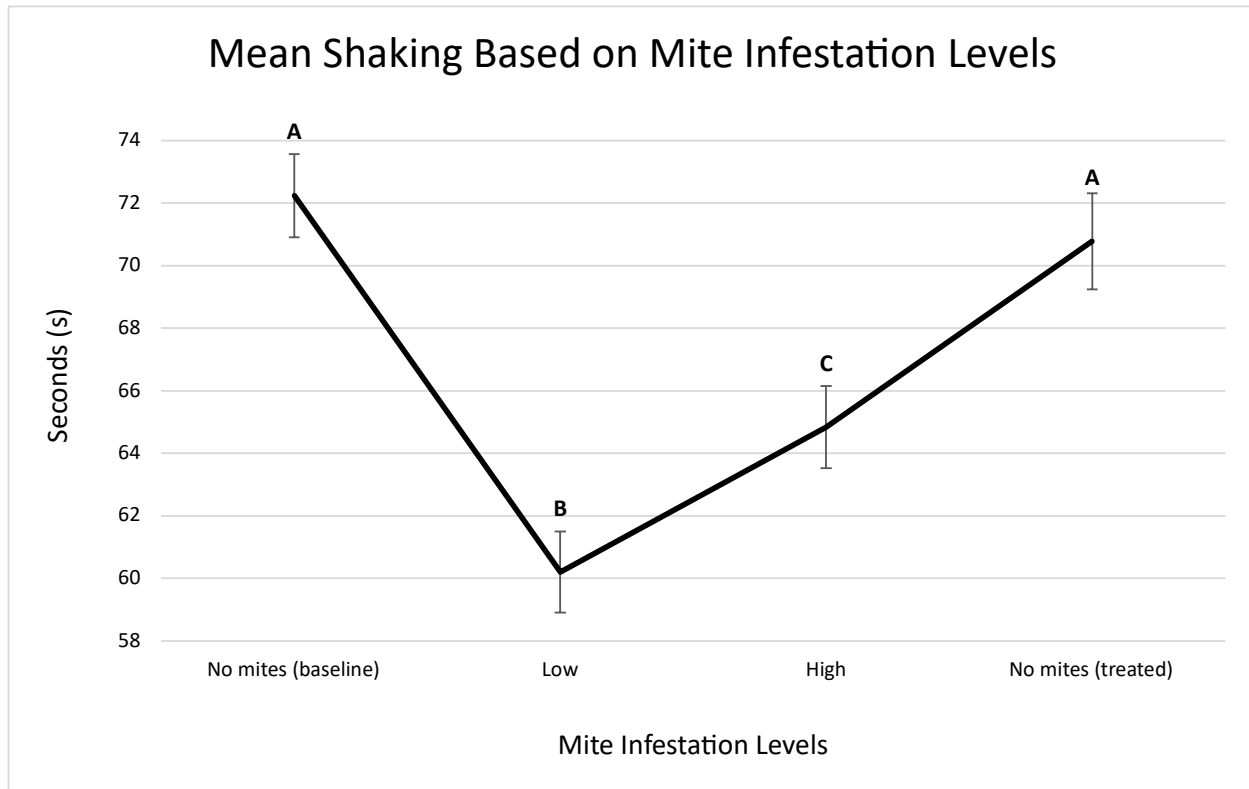


Figure 4. Mean time spent shaking per designated week of recording for all birds. The chickens were recorded during the weeks of 1, 4, 7, and 12 that represents phases 1, 2, 3, and 4 respectively. The mite infestation level at each week is as follows: no mites (baseline) = Phase 1, low levels of mites = Phase 2, high levels of mites = Phase 3, no mites (treated)= Phase 4. Different letters indicate significant differences between the phases.

4. Discussion

The three-axis accelerometers allowed for continuous recordings of four distinct chicken behaviors associated with ectoparasite infestation. We predicted that as mite infestation levels become greater that time spent preening, dustbathing, and shaking would increase and foraging would decrease. Our results revealed that only preening and dustbathing fit this pattern and that shaking and foraging behaviors were inconsistent with our prediction.

Preening behavior had a positive correlation with NFM levels, with the most time spent preening peaked during Phase 3 when mite levels were at the highest. Preening is an important control mechanism against ectoparasites in many avian species (Bush & Clayton, 2018; Clayton, 1991; Clayton et al., 2014). Studies have shown that avian species that have the ability to perform preening behaviors properly can remove ectoparasites effectively. For example, beak-trimmed hens had a higher ectoparasite (northern fowl mite and chicken body louse) counts compared to hens with intact beaks (Chen et al., 2011). Waite et al. (2012) conducted two experiments on the effectiveness of preening in captive pigeons infested with hippoboscid flies. The first experiment tested whether preening would be performed more by the pigeons if they were infested with flies by comparing the number of times preening of pigeons not infested versus infested by using instantaneous scan sampling. The results revealed that the pigeons that were infested with hippoboscid flies preened twice as pigeons free from flies. The second experiment examined the effectiveness of preening eliminating flies. Researchers counted and compared the number of dead flies by pigeons with impaired preening and pigeons with normal preening for one week. The pigeons that were able to preen normally killed twice as many flies as the pigeons with impaired preening (Waite et al., 2012) These studies show that proper preening behaviors can highly influence ectoparasite infestation levels. While the present study

examined the duration of preening instead of number of times preening, these authors recorded preening behaviors by visually inspecting the birds. This technique is labor intensive and limits sample size as well as number of times recording. In our study, on-animal sensors avoid human-animal contact and allows for continuous data collection for several days.

Time spent foraging increased the first four weeks of the study, but after that it did not change significantly. Daigle and Sigford (2014) examined time spent foraging in healthy Hy-Line Brown laying hens at 19, 28, 48, 66 weeks of age and found no significant difference in time spent foraging across the four different ages. Since there was a significant increase in foraging at the beginning of the study and the time spent foraging stayed consistently high, then more definitively we can attribute this to infestation rate changes because foraging at this time period doesn't seem to change. However, hens spent the most time foraging when they were treated for NFM. A possible reason may be the alleviation of NFM allows them to perform more exploratory behaviors like foraging. Future studies should investigate if foraging is influential to the energy expenditures of shaking, dustbathing, and preening, during the different levels of infestation.

When we examined the time spent dustbathing, results revealed that as mite levels increase, so did time spent on dustbathing behaviors. Chickens carry out dustbathing behaviors to remove excess lipids found on the feathers in order to keep plumage in good condition and may indirectly remove ectoparasites (Appleby M.C., 1993; van Liere, 1992). Martin and Mullens (2012) found that the birds used dustbox more as northern fowl mite scores increased. Their study scored the mites by visual observations and did not examine the total time spent dustbathing and shaking. Complimentary to their study, dustbathing behaviors can be a good predictor for infestation of mites. In our present study, as NFM infestation levels grew, so did

time spent dustbathing, and after the hens were treated with acaricide was when dustbathing duration decreased. However, external factors (i.e., substrate material, substrate deprivation, and social stimuli) can play an important role in dustbathing, changing the frequency and duration of the behavior (Borchelt et al., 1973; Olsson & Keeling, 2005; Weeks & Nicol, 2006). Future works can help determine if external factors are more influential than mite infestation levels. Interestingly though, shaking, a component of dustbathing, did not follow the same trend as dustbathing. Possible reason may be that shaking alone does not optimize the removal of mites compared to preening and dustbathing. Another reason may be since shaking is part of dustbathing that the accelerometer might have had issues distinguishing between the two behaviors. Even though dustbathing was highly influenced by the increased levels of NFM infestation throughout the study, the lack of uniformity between dustbathing and shaking indicates that these mite-driven behaviors need further investigation.

Based on the individual's behaviors, patterns appeared among the population of hens associated to mite infestation levels. As northern fowl mite infestation levels increased, so did time spent performing preening and dustbathing behaviors as well as increase in foraging at the beginning stage on infestation, which are import measures to assess chicken welfare. The use of on-animal sensors, in particular accelerometers, gives researchers and producers the ability to collect large datasets and monitor changes in behaviors continuously for several days. This approach alleviates the use of small sample sizes and does not restrict researchers with limited sampling intervals as compared to video recordings and in-person observations. Furthermore, the three-axis accelerometers have the potential to detect early levels of northern fowl mites based on the variances of foraging, preening, and dustbathing behaviors. Future studies should apply

wearable accelerometers in a commercial setting to verify the changes in time spent in behaviors between different levels of infestation of NFM.

5. Limitations to the current study

The behavior data analyzed in this study is based on accelerometer measures that were fed into an algorithm. The algorithm is reported to have an accuracy rate of 94% (Abdoli et al., 2020). However, the algorithm used in this study could not be independently validated due to technical difficulties with the type of video cameras used. One issue that arose in the data was an unexpected finding between dustbathing and shaking behaviors. The algorithm may have been unable to accurately distinguish between these two behavioral categories. The developed algorithm defined dustbathing as “sitting or rolling in dirt” (Abdoli et al., 2020). However, dustbathing consists of series of behaviors lumped together where some of these behaviors can be found outside of dustbathing. Olsson and Keeling (2005) point out the complexity of the ethological definition of dustbathing as it involves bill-raking, lying down, wing-shaking, head rubbing, and leg scratching. The accelerometer may not have known when to start or possibly had missing bouts since the behaviors that make up dustbathing are not in a set sequence. Shaking on the other hand is consistent behavior and generally occurs only in dustbathing. It may be worth using shaking component as the measure of dustbathing rather than the grander definition of dustbathing.

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