COMPARISON OF IMMOBILIZATION TECHNIQUES FOR WHITE-TAILED DEER

by

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(Under the Direction of Gino J. D'Angelo and Mark G. Ruder)

ABSTRACT

Safe and effective immobilization techniques for white-tailed deer (*Odocoileus virginianus*) are valuable tools for researchers and managers. The use of chemical immobilization agents is common to capture white-tailed deer, however, effects of immobilization are rarely monitored past the active immobilization period. Conducted electrical weapons (CEWs) are shown to immobilize other wildlife, but no research has been published on the use of CEWs on white-tailed deer. I tested immobilization efficacy of butorphanol-azaperone-medetomidine (BAM), nalbuphine-medetomidine-azaperone (NalMed-A), and CEWs by collecting physiological metrics, behavioral observations, feed measurements, and fecal samples pre-, post-, and during immobilizations.

INDEX WORDS: BAM, behavior, butorphanol-azaperone-medetomidine, CEW, conducted electrical weapon, immobilization, nalbuphine-medetomidine-azaperone, NalMed-A, *Odocoileus virginianus*, white-tailed deer

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Safe and effective capture of white-tailed deer (*Odocoileus virginianus*) is important for gathering information about the species (DelGiudice et al. 2005). The information gathered during capture is used to inform population management decisions (Miller et al. 2003, McDermott et al. 2020, Ortega et al. 2020). Temporary immobilization techniques are a valuable tool in research and management to ensure safety of deer and personnel involved in sampling (DelGiudice et al. 2005). Wildlife managers commonly affix tracking collars and collect biological data while animals are safely under the effects of chemical immobilization drugs (Arnemo et al. 2003, DelGiudice et al. 2005, Robert et al. 2012, Casady and Allen 2013). Physical capture methods (e.g., Clover trapping, rocket netting) of ungulates are useful, but chemical immobilization is a potential alternative. Chemical immobilization can be used as a stand-alone technique (e.g., free darting from a helicopter) or used in addition to physical capture methods. Chemical immobilization techniques can be used to resolve important public safety situations (e.g., wildlife entry into buildings) and wildlife welfare concerns (e.g., antlered deer locked together, animals caught in fences) in addition to research activities.

Additionally, increasing safety and effectiveness of new formulations of chemical immobilization drugs and existing immobilization procedures is a responsibility of researchers and managers (Casady and Allen 2013). Currently, the U.S. Drug Enforcement Administration (DEA) regulates many immobilization drugs used in wildlife research and management (DEA 2020). Each state in the U.S. may have additional permitting that is required along with the DEA

permitting immobilization drug use (Pleva and Davis 2017). Few personnel within an agency have required permits to use DEA regulated immobilization drugs, which makes it difficult or impossible to respond to all situations where wildlife need to be immobilized. More trained agency staff could store, transport, and use immobilizing drugs if they were not DEA regulated. Reduction of response times and need to euthanize animals could be accomplished with the greater flexibility and benefit of animal welfare with more readily available immobilization techniques (Lieske et al. 2018).

Butorphanol-Azaperone-Medetomidine

Butorphanol-azaperone-medetomidine (BAM; ZooPharm, Laramie, Wyoming, USA) is commonly used for sedation of wildlife because it is relatively safe, provides smooth induction and is effective on a wide range of wildlife species (Wolfe et al. 2008, Miller et al. 2009, Kreeger and Arnemo 2018). Commercially manufactured BAM is available in a concentration of 27.3 mg/mL butorphanol tartrate, 9.1 mg/mL azaperone tartrate and 10.9 mg/mL medetomidine HCL (ZooPharm 2020). Federal regulation of BAM occurs because butorphanol is a schedule IV controlled substance (Kreeger and Arnemo 2018, DEA 2020). Schedule IV controlled substances have less regulations than higher scheduled chemical immobilization agents (e.g., carfentanil, ketamine, sufentanil) that have been traditionally used in wildlife research and management (Kreeger and Arnemo 2018). All controlled substances, regardless of schedule level, are subject to federal and state permitting (Wolfe et al. 2016). Failure or delay in acquiring necessary permits can decrease the ability to complete rigorous field studies (Wolfe et al. 2016) which could encumber management efforts.

Butorphanol is a synthetic opioid that when combined with an alpha-2 adrenoreceptor, such as medetomidine, increases immobilization effectiveness (Miller et al. 2009, McDermott et

al. 2020). The addition of azaperone is intended to reduce stress on the animal during immobilization and has been shown to decrease excitability that can arise with an opioid induction (McDermott et al. 2020). The commercially available mixture of BAM provides strong sedation and has a high therapeutic index in wildlife (Wolfe et al. 2008, McDermott et al. 2020).

In recent years, BAM has been employed successfully to immobilize ungulates such as white-tailed deer, elk (*Cervus canadensis*), bighorn sheep (*Ovis canadensis*) (Miller et al. 2009, Kreeger et al. 2010, Garwood et al. 2020), and other wildlife (Semjonov et al. 2017, 2018). Studies in white-tailed deer showed BAM produces safe and reliable immobilization, but complete immobilization can take >10 minutes to achieve (Miller et al. 2009, McDermott et al. 2020). Experimental mixtures of BAM produced times to complete immobilization from 8.6 minutes (McDermott et al. 2020) to 17 minutes (Miller et al. 2009). When compared to drug combinations that include carfentanil and ketamine, with similar treatment methods, BAM had a noticeably slower time to complete immobilization (Storms et al. 2005, 2006, Miller et al. 2009).

General anesthesia of immobilized wildlife should be the desired effect of a chemical immobilization agent (Kreeger et al. 2010). General anesthesia is achieved when an animal loses consciousness and bodily function (Kreeger et al. 2010). However, opioids, such as carfentanil and butorphanol only produce a state of neuroleptanalgesia (i.e., central nervous system depression)(Kreeger et al. 2010). During neuroleptanalgesia animals may lose consciousness, but still have bodily functions when reacting to stimuli (Kreeger 2010). Kreeger et al. (2010) showed that BAM produces a deeper state of immobilization closer to general anesthesia than carfentanil-xylazine and can be used in elk with proper safety considerations.

Each drug contained in BAM can individually decrease respiratory function in white-tailed deer, and when used in combination, these effects have the opportunity to compound

(McDermott et al. 2020). Providing supplemental oxygen to sedated wildlife is encouraged, but where it is not feasible the lack of supplemental oxygen is not likely to increase capture myopathy (Wolfe et al. 2008, Miller et al. 2009). At high elevations (i.e., 1,585 m), Mich et al. (2008) observed significant hypoxemia in white-tailed deer immobilized with BAM, but supplemental oxygen reduced tissue hypoxia.

Federal Drug Administration guidelines for BAM residue (i.e., any component of an immobilization drug or reversal that remains in animal tissues after recovery of immobilization) in muscle tissue that is safe for human consumption is <0.01 ppm (Cook et al. 2016). No element of BAM was found in muscle and liver samples of white-tailed deer 11- and 21-days post treatment (Cook et al. 2016). Residual levels of BAM were found in tissue samples of American black bears ≥8 days post treatment (Wolfe et al. 2020). The use of BAM during periods when a person may harvest and consume wildlife can risk exposure of chemical agents to the public.

Nalbuphine-Medetomidine-Azaperone

Relative to many immobilization drugs, nalbuphine-medetomidine-azaperone (NalMed-A; ZooPharm, Laramie, Wyoming, USA) is more readily available for researchers and managers because it is not regulated under the DEA list of scheduled drugs (DEA 2020). Commercially manufactured NalMed-A is 40 mg/mL nalbuphine HCL, 10 mg/mL medetomidine HCL and 10 mg/mL azaperone tartrate (ZooPharm 2020). Medetomidine is an alpha-2 adrenoceptor agonist which provides potent sedative and analgesic effects (Kreeger and Arnemo 2018). Azaperone is a short-acting butyrophenone agonist that has a high therapeutic index and provides smooth anesthetic induction and recovery (Kreeger and Arnemo 2018). Medetomidine and azaperone have been used with other immobilizing drugs with positive results in American black bears (*Ursus americanus*) and white-tailed deer (Wolfe et al. 2008, Miller et al. 2009, McDermott et al.

2020). However, there has been limited testing with nalbuphine. Nalbuphine is a semi-synthetic opioid that can act as an agonist or antagonist (Kreeger and Arnemo 2018). Nalbuphine is 10 times more potent than butorphanol, a similar opioid, and the few wildlife studies using NalMed-A have shown its usage as a viable alternative to other more potent opioids (Wolfe et al. 2014, 2016; Kreeger and Arnemo 2018). The combination of NalMed-A is a more viable immobilization option than nalbuphine alone and was formulated as an alternative to BAM (Wolfe et al. 2016).

Original combinations of NalMed-A were tested with Rocky Mountain elk (*Cervus canadensis nelsoni*, Wolfe et al. 2014). Doses that contained 80 mg of nalbuphine produced the shortest induction times regardless if azaperone was used (Wolfe et al. 2014). In adult elk, a dose of 1.8–2.0 mL NalMed-A (40 mg/mL nalbuphine, 10 mg/mL medetomidine and 10 mg/mL azaperone) was recommended (Wolfe et al. 2014). White-tailed deer were successfully immobilized with the aforementioned NalMed-A combination by remote injection by dart and hand injection in multiple geographical areas (Wolfe et al. 2016b). The NalMed-A combination described above was used successfully in American black bears with a dose of less than 2.0 mL for most individuals (Wolfe et al. 2016a). American bison (*Bison bison*) were immobilized with the same concentration of NalMed-A in which almost all individuals reached complete immobilization (Wolfe et al. 2017). Safety, drug volume and efficacy of NalMed-A used in black bears was similar to BAM (Wolfe et al. 2016a). NalMed-A was safe for wildlife during immobilization, but physiological effects after immobilization were not well documented. Further research is needed to properly assess the overall effectiveness of NalMed-A on white-tailed deer.

Naltrexone, atipamezole and tolazoline have been used in conjunction with NalMed-A to reverse the immobilization effects (Wolfe et al. 2014). Tolazoline is an alpha-2 adrenoceptor antagonist that has been shown to increase rumen motility faster than similar drugs but should only be used to antagonize xylazine (Kreeger and Arnemo 2018). Atipamezole is an alpha-2 adrenoceptor antagonist specifically designed to reverse the effects of medetomidine (Kreeger and Arnemo 2018). Naltrexone is a synthetic opioid antagonist used to reverse the effects of nalbuphine (Kreeger and Arnemo 2018). Commonly used NalMed-A kits (ZooPharm, Laramie, Wyoming, USA) effectively use naltrexone and atipamezole without tolazoline to effectively reverse NalMed-A (ZooPharm 2020).

Federal Drug Administration guidelines for safe human consumption is <0.01 ppm NalMed-A drug residue in muscle tissue (Cook et al. 2016, KuKanich et al. 2005). NalMed-A residuals were measured at a minimum of 0.01 ppm in liver and muscle of Rocky Mountain elk and were only detected $\leq$24 hours post treatment (Wolfe et al. 2018). However, the reversal agents were detected at levels >0.01 ppm up to 21 days post treatment (Wolfe et al. 2018). Avoidance of residual drugs in tissue can be avoided by use of physical immobilization techniques.

Adequate chemical immobilization has been achieved by the use of NalMed-A in a variety of wildlife, but few studies have looked at physiological effects on animals. Wildlife may experience long-term physiological effects caused by the use of immobilization drugs such as NalMed-A (West et al. 2007). Continued research of NalMed-A and its physiological effects is needed to ensure long-term health of immobilized wildlife.

Conducted Electrical Weapon

The first handheld conducted electrical weapon (CEW) was developed in 1970 by a NASA scientist (Biria et al. 2010) to provide rapid and short-term immobilization of humans (Kuersten 2020). Approximately, 90 percent of law enforcement agencies currently issue CEWs to their officers (Kuersten 2020). In a majority of instances of CEW use on humans there was mild to no injuries reported (Bozeman et al. 2009). The use of CEWs has been associated with reduced injury rates for both suspected criminals and police officers because of the reduction in use of lethal force and physical restraint (Bozeman et al. 2009). Wildlife law enforcement officers carry CEWs and are commonly the first responders of wildlife emergencies (e.g., sick, injured, entrapped or aggressive wildlife; Eliason 2011). If CEWs are shown to safely immobilize wildlife, agencies can reduce use of controlled substances and provide prompt resolutions to wildlife emergencies. In addition, CEWs would eliminate concerns related to the use of immobilization drugs and their reversal agents immediately before and during harvest seasons when wildlife may be utilized for human consumption (Clapham et al. 2019).

Pulses of electricity that are produced by the CEW are captured by motor neurons in the skeletal muscle and provide temporary immobilization by uncontrolled contractions of the muscles (Biria et al. 2010). Motor neurons function similarly in vertebrate species wherein the neuron is the driving force behind muscle contraction and relaxation (Peters 1989). If CEWs provide safe and practical immobilization of human subjects, an assumption can be drawn that CEWs will provide safe and practical short-term immobilization of vertebrate wildlife species. However, few studies have evaluated CEW application in wildlife.

The American Veterinarian Medical Association does not endorse the use of electro-immobilization for the routine capture or restraint of wildlife (American Veterinary Medical

Association 2008, 2010). Although preferred techniques for immobilization should provide anesthesia and analgesia (American Veterinary Medical Association 2008), electro-immobilization has not proven to provide either. Temporary electro-immobilization may be appropriate when traditional capture or restraint methods (e.g., chemical agents, live traps, rocket nets) threaten public safety or local regulations restrict capture equipment (e.g., dart projectors, net guns; Lieske et al. 2018).

There are only two known published articles about the effects of short-term CEW use on wildlife (Lewis et al. 2012, Lieske et al. 2018) and no studies have reported CEW use in white-tailed deer. Previous studies with CEWs showed promising results, but physiological and long-term health effects were not measured beyond subjective observations. Captive reindeer (*Rangifer tarandus tarandus*) exposed to a 10-second CEW exposure did not develop long-term health consequences (Lieske et al. 2018). Reindeer were exposed to the CEW for the same time period while immobilized with carfentanil and xylazine (Lieske et al. 2018). Carfentanil and xylazine used alone and paired with the CEW showed no differences in development of capture myopathy (Lieske et al. 2018). Domestic pigs (*Sus scrofa*) anesthetized and mechanically ventilated with 100 percent oxygen survived 3 minutes of continuous exposure to a CEW (Jenkins et al. 2013, Ziriax et al. 2014, Jauchem 2015). Werner et al. (2012) exposed pigs to different CEWs in continuous 1-min increments. After 4-6-min of exposure, 3 of the 18 pigs died (Werner et al. 2012). Anesthetized pigs exposed to a TASER X26 (Axon Enterprise Scottsdale, AZ, USA) for >10 min showed rapid cardiac rhythm and significant decline in systolic function monitored by an electrocardiogram (Walter et al. 2008). Prolonged exposure to CEWs has been shown to be dangerous and fatal to domestic pigs (Walter et al. 2008, Werner et al. 2012, Jauchem 2015). However, short-term CEW exposure on domestic and wild animals has not

shown any lasting physiological effects and may be useful in rapid immobilization procedures (Jenkins et al. 2013, Jauchem 2015, Lieske et al. 2018). It is important to note that many studies of CEW usage on domestic pigs, including the studies listed above (e.g., Jauchem 2015), were conducted to evaluate potential long-term and repeated CEW use on human subjects rather than short-term immobilization of wildlife.

OBJECTIVES

Chemical Immobilization

We immobilized captive white-tailed deer with either BAM or NalMed-A for a total of 20 minutes. Our objectives were to: 1) determine if the manufacturer's suggested dose of 1.5 mL for both BAM and NalMed-A provided complete immobilization, 2) determine physiological effects of chemical immobilization, and 3) determine extended physiological effects after the immobilization drugs were no longer exhibiting effects.

CEW Immobilization

We exposed deer to a 5- or 15-second exposure to the CEW while deer were chemically immobilized and without any immobilization or restraint. Our objectives were to: 1) determine physiological effects caused by an exposure to a CEW, 2) determine extended physiological effects after a CEW exposure had occurred, and 3) determine if CEW usage is appropriate for wildlife researchers and managers in real-world scenarios.

THESIS FORMAT

Chapter 1 is an extensive literature review of the selected research topics and provides background information on the importance of the selected topics. Chapter 2 is presented in manuscript format to compare physiological effects of chemical immobilization with either BAM or NalMed-A in captive white-tailed deer. Chapter 3 is presented in manuscript format to

compare the physiological effects of temporary immobilization of captive white-tailed deer with a CEW. Chapter 4 provides overall conclusions and possible management implications based off of these study results.

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CHAPTER 2

IMMOBILIZATION EFFICACY OF BUTORPHANOL-AZPAERONE-MEDETOMIDINE AND NALBUPHINE-MEDETOMIDINE-AZAPERONE FOR WHITE-TAILED DEER[1]

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ABSTRACT

Previous studies involving white-tailed deer (*Odocoileus virginianus*) have used various chemical agents to effectively immobilize animals and increase safety for deer and researchers during biological sampling. Current combinations of butorphanol-azaperone-medetomidine (BAM) are known to produce complete and long-lasting immobilization in white-tailed deer, but previous research did not focus on post-immobilization physiological effects. Nalbuphine-medetomidine-azaperone (NalMed-A) is an alternative to BAM, that has less stringent regulatory requirements for transport, storage, and use. Currently, there are no published studies on the use of NalMed-A in white-tailed deer, but one study with Rocky Mountain elk (*Cervus canadensis*) showed promise for use in other cervids. To compare immobilization efficacy, quality, and residual effects of BAM and NalMed-A, our research focused on potential physiological and behavioral changes produced by the respective chemical immobilizations in captive white-tailed deer. We administered a dose of either 1.5 mL BAM, 1.5 mL NalMed-A, or 2.0 mL NalMed-A to deer in 3 treatment groups (n=10 for each treatment). Before, during, and after immobilization treatments, we collected biological samples to measure glucocorticoid stress hormones and blood gas values. We conducted behavioral observations to determine treatment-related variations in stress levels and behavioral patterns. We measured respiration rate, pulse, rectal temperature, and hemoglobin oxygen saturation during immobilization to determine immediate physiological effects. Times to complete immobilization were similar among all treatments at each immobilization stage. Serum cortisol concentrations were similar among each treatment and decreased throughout the immobilization period. All physiological effects were similar among all treatments of BAM and NalMed-A indicating both are satisfactory immobilization drugs.

KEY WORDS BAM, chemical immobilization, NalMed-A, *Odocoileus virginianus*, stress, white-tailed deer

INTRODUCTION

Temporary immobilization of white-tailed deer (*Odocoileus virginianus*) is a valuable tool in research and management of the species (DelGiudice et al. 2005). Safe and effective capture of wildlife aids in gathering important information about individuals which can inform population management decisions (Miller et al. 2003; McDermott et al. 2020; Ortega et al. 2020). Wildlife managers commonly use immobilization drugs to safely restrain animals to affix tracking collars and collect biological data (Arnemo et al. 2003; DelGiudice et al. 2005; Robert et al. 2012; Casady and Allen 2013). Chemical immobilization may also allow personnel to more rapidly resolve emergency situations involving wildlife including public safety situations (e.g., wildlife entry into buildings) and animal welfare concerns (e.g., antlered deer locked together). Chemical immobilization of ungulates is used in combination with physical capture methods (e.g., rocket and drop nets) or can be used as a stand-alone technique (e.g., free darting).

Continually, researchers and managers are looking to increase safety and effectiveness of immobilizing procedures for wildlife (Casady and Allen 2013). Immobilization of white-tailed deer with butorphanol-azaperone-medetomidine (BAM) is common, however BAM is federally regulated as a controlled substance by the Drug Enforcement Administration (DEA 2020). Along with federal permits to use controlled substances, each state may have additional permitting requirements (Pleva and Davis 2017). Because situations where wildlife need to be immobilized can occur anywhere and at any time, it is not feasible for a few people with required permits (e.g., veterinarians) to always be available to respond. In an effort to develop a drug combination with minimal regulations, a combination of nalbuphine-medetomidine-azaperone (NalMed-A)

was developed (Wolfe et al. 2014). With reduced permitting requirements, agencies could allow more trained personnel to store, transport, and use NalMed-A compared with BAM. Improved availability of immobilization techniques could provide wildlife managers greater flexibility and benefit animal welfare by reducing response times and lessening the need to euthanize animals (Lieske et al. 2018).

To compare safety and physiological effects of BAM and NalMed-A a variety of measurements should be analyzed. Vital signs and blood samples, to analyze cortisol levels, are important measurements to be monitored during the immobilization period (Sente et al. 2014). To monitor long-term effects of immobilization, behavioral observations and fecal samples can be collected to monitor changes across time. Changes in frequency of behaviors and concentrations of stress related hormones can indicate a response to immobilization (Durnin et al. 2010). Typically, indications of stress in fecal samples are determined by cortisol metabolite concentrations (Millspaugh et al. 2001). However, fecal progesterone metabolite concentrations can be analyzed to determine potential stress events along with pregnancy status (Plotka et al. 1983). Our objective was to compare BAM and NalMed-A's sedation efficacy and physiological effects using the samples described above.

MATERIALS AND METHODS

The study was conducted at the Whitehall Deer Research Facility at the Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia, USA. The facility is 2.6 ha and contains 5 outdoor paddocks (0.4 – 0.8 ha), 3 sorting pens (15 x 20 m) and a 20-stall (3 x 6 m) barn. The entire property is enclosed by a 3.0-m tall woven-wire fence and a 2-strand electric fence at ground level to deter entry of domestic dogs (*Canis lupus familiaris*) and predators.

Trees in the paddocks primarily consisted of large pines (*Pinus spp.)* and oaks (*Quercus spp.*). Deer were provided a pelletized ration (AntlerMax® Breeder 17-6, Purina Animal Nutrition, Arden Hill, MN, USA), perennial peanut (*Arachis glabrata*) hay, and water *ad libitum*. Monthly average temperatures in Athens, GA during the research period ranged from a high of 23.3° C to a low of 2.2° C. The average monthly rainfall was 2.5 cm (U.S. Climate Data 2022). Animals in this study were captive raised and in good general health.

February through April of 2021, we randomly assigned 30 captive, female, adult white-tailed deer to 1 of 3 treatment groups (n = 10 for each treatment). The treatment groups included immobilization with: 1) BAM (1.5 mL), 2) NalMed-A (1.5 mL), and 3) NalMed-A (2.0 mL). We housed all deer in a treatment group in the same paddock separate from other treatment groups. All aspects of this study were approved by the University of Georgia Institutional Animal Care and Use Committee under Animal Use Proposal A2021 01-028-Y1-A2.

Behavioral Observations

We monitored deer behavior for 30 days to evaluate potential effects of treatments, including 15 days before and 15 days after treatments, with the treatment occurring on day 15 of observations. We monitored group-housed deer in paddocks during the first 10 days and the final 10 days of the 30-day observation period. During days 11-20, we monitored deer in isolated barn stalls. Observations occurred throughout daylight hours, but typically sessions began at sunrise. Deer were identified by unique ear tag numbers. We recorded behavioral observations pre- and post-treatment that included frequency and amount of time standing, lying, moving, foraging or being vigilant (Table 2.1).

Group-housed Observations – Observers waited 15 minutes after situating all equipment (e.g., binoculars, datasheet) and themselves before beginning observations to allow deer to

acclimate to the presence of the observer. Observers monitored each deer for 10 minutes from an observation tower within the outdoor paddock. Observers monitored deer opportunistically each day until all deer in a treatment group were observed.

Isolated Barn Stall Observations – We conducted remote observations of deer in barn stalls by reviewing videos obtained by a video recorder (Panasonic 25x i.zoom, Panasonic Corporation, Kadoma, Osaka, Japan). The observers placed a camera mounted to a tripod in the hallway between stalls directed into the focal animal's stall. The observer then left the hallway for 15 minutes. The first 5 minutes allowed the deer to resume normal behaviors (i.e., not influenced by the presence of the researcher) and the following 10 minutes were used to classify behaviors. After all deer in a treatment had been monitored, the researchers watched the video recordings and classified each deer's behavior.

Immobilization Treatment Observations – We measured specific body orientations and alertness during immobilization treatments that included: first noticeable sign of immobilization, drooping head, sternal recumbency, lateral recumbency, head down on the ground, and complete immobilization. We measured body orientation and alertness, in minutes, from when a treatment was applied to when the individual deer did not react to stimuli. We monitored body orientation and alertness after the treatment was reversed until the individual appeared to have no visual remnant effects of the treatment (e.g., did not stumble when walking, head held upright). Measurements taken after the treatment was reversed included: first sign of reversal, time to sternal recumbency, head up, standing, and no sign of immobilization. We used 2 independent observers to score quality of induction using the same criteria as Storms et al. (2005). Induction scores were combined from each observer for a possible range from 0-18 with a score $\geq$12 considered desirable (Storms et al. 2005). The same 2 independent observers scored quality of

reversal based on criteria derived from Storms et al. (2005; Table 2.2). Reversal scores were combined from each observer for a possible range from 0-18 with a score of ≥12 considered desirable.

Immobilization Treatment

We transferred deer from paddocks into individual barn stalls 5 days before application of treatments. To prevent aspiration of rumen contents during immobilization, we fasted deer for ≥16 hours before treatment (Lin and Walz 2014). We moved individual deer into a restraint chute, dropped the floor of the chute to suspend the deer's feet above the ground, and a handler physically restrained the deer's head to allow for hand-injection of immobilization drugs. We released deer into a holding pen (15 x 20 m) after the application of BAM or NalMed-A. Once each deer was deemed completely immobilized, a blindfold was placed over the deer's eyes and the deer was kept in sternal recumbency throughout sedation. We administered reversals 20 minutes after complete immobilization was determined. After immobilization effects had diminished, we returned deer to individual barn stalls for five days of observation before being released into paddocks.

Butorphanol-azaperone-medetomidine – We immobilized 10 deer with the manufacturer's suggested dose for BAM of 1.5 mL (27.3 mg/mL butorphanol, 9.1 mg/mL azaperone, 10.9 mg/mL medetomidine; ZooPharm, Laramie, Wyoming) with a single IM injection per deer to the left hindquarter. We administered 0.5 mL naltrexone (50 mg/mL) and 3.0 mL atipamezole (25 mg/mL) to antagonize the butorphanol and medetomidine, respectively. We administered each antagonist separately via a single IM injection in the left hindquarter at 20 minutes after complete immobilization.

Nalbuphine-medetomidine-azaperone – We immobilized 9 deer with the manufacturer's suggested dose of 1.5 mL of NalMed-A (40 mg/mL nalbuphine, 10 mg/ml medetomidine, 10 mg/ml azaperone; ZooPharm, Laramie, Wyoming) and we immobilized a separate 9 deer at a dose of 2.0 mL per deer by a single intramuscular (IM) injection in the left hindquarter. A tenth deer intended for the 1.5 mL NalMed-A treatment was mistakenly given an antagonist and did not receive NalMed-A. A tenth deer intended for the 2.0 mL NalMed-A treatment was injured during handling and did not receive NalMed-A. Both deer were censored from analyses. We administered 0.5 mL naltrexone (50 mg/mL) and 3.0 mL atipamezole (25 mg/mL) to antagonize the nalbuphine and medetomidine, respectively. We administered each antagonist separately via a single IM injection in the left hindquarter at 20 minutes after complete immobilization.

Physiological Measurements

We collected 10 mL of blood from the jugular vein in additive free tubes at: 1) the time of complete immobilization (T0), 2) 10 minutes from complete immobilization (T1), and 3) 20 minutes from complete immobilization (T2). We collected 0.1 to 0.6 mL of blood in heparinized syringes from the auricular artery at T0, T1, and T2.

We centrifuged (1775 xg) venous blood samples for 10 minutes and collected serum to analyze cortisol levels. We froze serum samples at -20° C until analysis occurred. We used DetectX® Cortisol Immunoassay Kits (Arbor Assays, Ann Arbor, Michigan, USA) according to manufacturer instructions to analyze serum samples at the University of Tennessee Institute of Agriculture (Knoxville, Tennessee, USA). We immediately sampled arterial blood samples with an i-STAT point of care device (Abbott, Princeton, New Jersey, USA). We used the CG4+ sample cartridge to analyze pH, partial pressure carbon dioxide (pCO_2), partial pressure oxygen

(pO_2), base excess (BEecf), bicarbonate (HCO_3), total carbon dioxide (TCO_2), percent blood oxygen ($s0_2$) and lactate.

We monitored rectal temperature, respiration rate, pulse, and percent blood oxygen every 2 minutes during immobilization. We monitored pulse and percent blood oxygen with a pulse oximeter (Masimo Corporation, Irvine, California, USA) attached to the tongue. We immediately gave a cool water enema to individual deer with a temperature above 41.3° C. Deer with a temperature above 40° C that was continuing to increase received a cool water enema. We provided supplemental oxygen if percent blood oxygen fell ≤70 percent.

Fecal Collection

To monitor individual and group fecal cortisol and progesterone concentrations, we collected fresh fecal samples daily throughout the 30-day observation period. We randomly collected a total of 10 fecal samples (5-10 pellets) from separate fecal deposits each day from each treatment group in paddocks for the first 10 days and final 10 days of the 30-day observation period. To determine individual fecal cortisol and progesterone levels, we collected fecal samples daily from individual deer in barn stalls 4 days before treatment, during the treatment, and 5 days post-treatment. Individual samples were taken from assumed pregnant and non-pregnant deer. Assumed pregnant deer were housed with male deer for the entirety of the breeding season and non-pregnant deer were housed separately from male deer. We froze fecal samples at -20° C until further processing and analysis occurred.

To process feces, we dried samples for ≥24 hours in a drying oven at 50° C, ground pellets using a mortar and pestle, and returned samples to -20° C until analysis. We used DetectX® Cortisol Immunoassay kits (Arbor Assays, Ann Arbor, Michigan, USA) according to manufacturer's guidelines for fecal extraction of cortisol at the University of Tennessee Institute

of Agriculture (Knoxville, Tennessee, USA). We weighed 0.2-0.4 g of ground fecal material and added 1 ml of 200% absolute ethanol (Fisher Scientific, Hampton, New Hampshire, USA) for every 0.1 g fecal material for extraction of steroids. The ethanol mixture was shaken for 30 minutes at room temperature on a rocking shaker. We pipetted the supernatant into 2 mL microcentrifuge tubes. The supernatant was either assayed directly or stored at -20 C for up to 30 days.

We used the Arbor Assays Progesterone EIA kit (K025; Arbor Assays, Ann Arbor, Michigan, USA) and followed assay protocols for measuring fecal progesterone metabolites. We reran any sample with coefficient of variation (CV) >20% or when the concentration was not within the linear portion of the standard curve (20-80% binding). We tested 5 dilutions of assumed pregnant (1:150 – 1:250) and non-pregnant (1:20-1:100) samples for parallelism. We calculated extraction efficiency by adding 10 uL of 1,000 ng/mL progesterone standard (Arbor Assays) to two dried and weighed fecal samples before extraction and compared to a control sample without added progesterone. We calculated extraction efficiency as ((spiked sample – control)/spiked sample)*100. We calculated intra-assay variability by averaging the CV of all duplicate samples assayed. We calculated the inter-assay variability by using a pooled mix of samples run with every plate. Due to the sample stability of only 30 days, we used 4 different extraction mixes across all plates. We averaged the variability for each mix and provided a final average across all four samples.

Feed Measurements

We measured feed consumption of each treatment group using the same schedule as fecal collection. We weighed new feed (kg) before placing it in the paddocks or barn stalls. We

weighed residual and additional feed daily. We determined daily feed consumption by subtracting residual feed mass from total mass of feed provided the previous day.

Data Analysis

We analyzed data using program R 4.1.0 (R Core Team 2021). We used a one-way ANOVA with a post-hoc Tukey's test to determine differences among treatments when measuring mean time to immobilization stages, quality of induction, time to reversal stages, quality of reversal, serum cortisol, blood gas values, and daily feed consumption. For behavioral observation analysis, we grouped calm movement, bedded relaxed, standing relaxed, foraging, grooming, and groomed behaviors into the calm classification and rapid movement, bedded alert and standing alert behaviors into the alert classification. We analyzed frequency of calm and alert behaviors using a hypothesis based linear mixed effects model with treatment and day of observation as fixed effects and the individual deer as a random effect. We analyzed changes in progesterone levels using a linear mixed effects model with pregnancy status, stress event periods, and treatment as fixed effects and the individual deer as a random effect. Stress effect periods were arranged into 3 groups including: 1) before (i.e., time in isolated barn stalls before treatment), 2) treatment (i.e., the treatment day and the 2 following days), and 3) after (i.e., remaining 3 days in isolated barn stalls) during the 10 days deer were in isolated barn stalls. We used Akaike's Information Criterion for small sample sizes to determine the most influential fixed effects on progesterone. We used alpha of <0.05 to determine statistical significance for all analyses.

RESULTS

All deer reached complete immobilization when immobilized with 1.5 mL BAM and 2.0 mL NalMed-A. Eight of 9 deer (88.9%) injected with 1.5 mL of NalMed-A reached complete

immobilization. After 60 minutes, 1 deer in the 1.5 mL NalMed-A treatment was still reactive to physical stimulus and was given antagonists. A control treatment was originally planned, but failure for all individuals in the 1.5 mL NalMed-A treatment to reach complete immobilization prompted an additional treatment with an increased dose of NalMed-A to 2.0 mL. The number of animals at the research facility did not allow for fourth treatment to act as a control because all adult, female deer were already part of a prior treatment.

Time to first sign of induction, head droop, sternal recumbency, lateral recumbency, head down, and complete immobilization during induction (Table 2.3) and time to first sign, head up, sternal recumbency, standing, and no immobilization during reversal (Table 2.4) were similar in deer among all treatments excluding the individual that did not reach complete immobilization. Quality of induction scores were similar among all treatments ($F_{2,24}$=0.21, P=0.81). Mean quality of induction scores were 13.4 (Range 8-18) for deer in the 1.5 mL BAM treatment, 13.8 (Range 9-18) for deer in the 1.5 mL NalMed-A treatment, and 12.8 (Range 8-18) for deer in the 2.0 mL NalMed-A treatment. Quality of reversal scores were similar among all treatments ($F_{2,24}$=1.09, P=0.35). Mean quality of reversal scores were 16.8 (Range 11-18) for deer in the 1.5 mL BAM treatment, 15.9 (Range 12-18) for deer in the 1.5 mL NalMed-A treatment, and 17.2 (Range 15-18) for deer in the 2.0 mL NalMed-A treatment.

Blood gas values were similar among deer in all treatments at T0, T1 and T2 (Table 2.5). All blood gas values were similar across T0, T1, and T2 within treatments except for lactate in deer in the 1.5 mL NalMed-A treatment, which was significantly lower at T2 than at T0 ($F_{2,17}$=8.22, P=0.003). Hypoxemia (pO_2<80 mmHg; Read 2003) was observed in deer in each treatment at all three time points. We provided supplemental oxygen to 3 deer (30%) in the 1.5 mL BAM treatment before T2, 2 deer (25%) in the 1.5 mL NalMed-A treatment before T1, and 1

deer (11%) in the 2.0 mL NalMed-A treatment before T2. All deer that received supplemental oxygen were censored from the blood gas analyses starting at the point oxygen supplementation started.

Overall feed consumption did not change from before to after treatment for all treatments (Table 2.6). Feed consumption decreased when treatment groups were moved to individual barn stalls ($F_{1,323}$=155.30, P=<0.0001), but consumption was consistent and did not change over time in relation to the immobilization treatment. The only exception was an increase in feed consumption from before to after treatment ($F_{1,98}$=13.44, P=0.0004) for deer in the 1.5 mL NalMed-A treatment while in isolated barn stalls.

Mean rectal temperature (1.5 mL BAM, $F_{10,77}$=0.46, P=0.91; 1.5 mL NalMed-A, $F_{10,70}$=0.69, P=0.73; 2.0 mL NalMed-A, $F_{10,79}$=0.76, P=0.67) and respiration rates (1.5mL BAM, $F_{10,96}$=0.10, P=1.00; 1.5 mL NalMed-A, $F_{10,76}$=0.39, P=0.95; 2.0 mL NalMed-A, $F_{10,87}$=0.14, P=0.99) were similar across time within each treatment. Mean rectal temperature for deer in the 1.5 mL NalMed-A treatment was significantly higher than deer in 1.5 mL BAM treatment and 2.0 mL NalMed-A treatment ($F_{2,256}$=6.57, P=0.002). Mean respiration rates for deer in the 1.5 mL BAM treatment were significantly higher than each NalMed-A treatment ($F_{2,289}$=12.27, P=<0.0001) Ranges of mean rectal temperatures in deer were 39.7° C (SE = 0.5) to 40.4° C (SE = 0.3) in the 1.5 mL BAM treatment, 40.2° C (SE = 0.1) to 40.7° C (SE = 0.2) in the 1.5 mL NalMed-A treatment, and 39.8° C (SE = 0.2) to 40.4° C (SE = 0.2) in the 2.0 mL NalMed-A treatment. Subjectively, researchers noticed that deer in direct sunlight would have increasing rectal temperatures throughout the immobilization until either a cool water enema was given, or shade was provided. Ranges of mean respiration rates in deer were 20.4 (SE = 2.5) breaths/min to 23.6 (SE = 2.8) breaths/min in the 1.5 mL BAM treatment, 15.5 (SE = 1.4) breaths/min to 19.4

(SE = 1.8) breaths/min in the 1.5 mL NalMed-A treatment, and 16.0 (SE = 2.5) breaths/min to 19.6 (SE = 2.1) breaths/min in the 2.0 mL NalMed-A treatment.

Serum cortisol values of deer were similar among all treatments at T0 ($F_{2,24}$=2.34, P=0.12), T1 ($F_{2,24}$=2.34, P=0.12), and T2 ($F_{2,24}$=2.34, P=0.12). Within each treatment, serum cortisol levels of deer at T2 were significantly lower than T0 (1.5 mL BAM, $F_{2,27}$=6.46, P=0.005; 1.5 mL NalMed-A, $F_{2,21}$=14.38, P=0.0001; 2.0 mL NalMed-A, $F_{2,24}$=3.59, P=0.04). Deer in the BAM 1.5 mL treatment had mean serum cortisol levels of 3.77 ug/dL (SE = 0.29) at T0, 3.26 ug/dL (SE = 0.29) at T1, and 2.44 ug/dL (SE = 0.21) at T2. Deer in the NalMed-A 1.5 mL treatment had mean serum cortisol levels of 2.73 ug/dL (SE = 0.19) at T0, 2.15 ug/dL (SE = 0.17) at T1, and 1.46 ug/dL (SE = 0.14) at T2. Deer in the NalMed-A 2.0 mL treatment had mean serum cortisol levels of 3.26 ug/dL (SE = 0.46) at T0, 2.56 ug/dL (SE = 0.46) at T1, and 1.75 ug/dL (SE = 0.25) at T2. The fecal cortisol assay had an accurate threshold of detecting cortisol down to 30 ng/g. All fecal samples analyzed were below the 30 ng/g threshold and could not accurately be compared from before to after treatment and among treatments.

Changes in progesterone concentrations were most accurately described by the linear mixed effects model that included pregnancy status and stress effect periods as fixed effects. Progesterone concentrations increased during the treatment stress effect period in comparison to the before stress effect period (Table 2.7). Pregnant deer had significantly increased progesterone levels compared to non-pregnant deer (Table 2.7). Mean peak progesterone levels were 1.3 days after treatment for deer in the BAM 1.5 mL treatment, 1.7 days after treatment for deer in the NalMed-A 1.5 mL treatment, and 1.4 days after treatment for deer in the NalMed-A 2.0 mL treatment. We found similar slopes for all diluted samples in both nonpregnant and pregnant progesterone metabolite concentrations. Extraction efficiency using 2 different samples was

89%. The Intra-assay variability was 4.8% and the Inter-assay variability across 16 plates (4 mixes) was 13.4%.

There was no relationship between the frequency at which behaviors were observed and the treatment a deer received, or the timing observations occurred in relation to the immobilization event according to the hypothesis-based model (Table 2.8). Frequency of calm behaviors ranged from 86% to 90% of observations/session for deer in the 1.5 mL BAM treatment, 88% to 93% of observations/session for deer in the 1.5 mL NalMed-A treatment, and 86% to 91% of observations/session for deer in the 2.0 mL NalMed-A treatment (Figure 2.1). Frequency of alert behaviors ranged from 10% to 14% of observations/session for deer in the 1.5 mL BAM treatment, 7% to 12% of observations/session for deer in the 1.5 mL NalMed-A treatment, and 9% to 13% of observations/session for deer in the 2.0 mL NalMed-A treatment (Figure 2.1).

DISCUSSION

Our data demonstrate that NalMed-A can be used at a dose of either 1.5 mL or 2.0 mL and BAM can be used at a dose of 1.5 mL to safely immobilize white-tailed deer without extended physiological effects. Also, in scenarios where BAM is typically used to immobilize deer, our data indicates NalMed-A can be used because of the similar physiological and behavioral results each treatment produced in our study. Even though one deer was not completely immobilized with 1.5 mL of NalMed-A, it was able to be approached to give either a supplemental NalMed-A dose or the antagonists, which is why we believe 1.5 mL NalMed-A can be considered an effective dose. Giving a booster of immobilization drugs to have an individual animal reach complete immobilization is not ideal, but commonly occurs because exact weights and stress levels of each individual are not typically known in field scenarios

which can lead to incomplete immobilization (DelGiudice et al. 2005). Therefore, some situations may call for using an initial increased dose (e.g., 2.0 mL) of NalMed-A to ensure immobilization after one injection of an animal.

In a field setting, a standard dose of immobilization drug is typically used because exact weights of individual animals are rarely known (Hawkins et al. 2019). For this reason, we conducted our study using standard doses of 1.5 mL and 2.0 mL for NalMed-A, and 1.5 mL for BAM. Standard doses allow for shorter duration from when a target animal is defined to the administration of immobilization drugs (McDermott et al. 2020). Using weight specific doses can increase chase times and the amount of time an individual is in a trap (McDermott et al. 2020). An increased standard dose may require changes in other immobilization equipment (e.g., darts), but still allows for a low volume of drug to be administered when using BAM and NalMed-A. An increased dose allows a wider range of target animals to be immobilized with the same equipment. In addition, an increased dose enhances the likelihood of larger individuals reaching complete immobilization without additional immobilization drugs being administered (Hampton et al. 2019; Latham et al. 2019).

With an increased dose of 1.5 mL to 2.0 mL of NalMed-A, there was no increase in negative physiological effects. Use of chemical immobilization drugs that do not increase stress levels outside of the immobilization period help reduce the overall stress of capture (Breed et al. 2019). Researchers and managers are responsible for reducing stress on immobilized animals and considering BAM and NalMed-A have similar immobilization and physiological effects, focus can be turned to specific capture techniques (e.g., Clover traps, drop nets, tranquilizer darts, etc.; DelGiudice et al. 2005).

Deer in our study reached complete immobilization 2.3 minutes faster to 6.1 minutes slower with BAM and 3.3 to 11.8 minutes slower with NalMed-A compared to previous studies which immobilized white-tailed deer with BAM (Mich et al. 2008; Miller et al. 2009; Kirschner and Rodenkirch 2017; McDermott et al. 2020). Inconsistency in defining induction stages could have potentially led to a larger range of complete immobilization times in the literature. For example, Mich et al. (2008) listed "lateral recumbency/safe approach" as the final induction stage, whereas our study measured lateral recumbency and time to complete immobilization separately. Even though deer received different doses of NalMed-A, times to immobilization stages did not differ among all treatment groups. Deer in the BAM treatment had a mean time to complete immobilization 5.6 and 5.7 minutes faster than deer in the 1.5 mL and 2.0 mL NalMed-A treatments, respectively. Regardless, if the time to complete immobilization was significantly different, time to complete immobilization was potentially biologically significant. However, time to complete immobilization should not be considered solely. All three treatments had statistically and biologically similar times to first sign of drug effect and sternal recumbency. In free-darting scenarios, time of first sign and sternal recumbency are initially more important than reaching complete immobilization as long as stimuli are minimized (Barros et al. 2018). If a drug combination or a lower dose allows for an animal to retain normal body function for longer, that individual has more potential to elude or extend recovery (Hewlett et al. 2020). Delayed immobilization can lead to animals becoming sedated in dangerous situations, allow individuals to cross property boundaries, and extend the overall sedation period (Grace et al. 2021).

Quality of induction scores reported in our study indicate a desirable induction period. Other drug combinations have produced faster times to immobilization, but quality of induction has been reported as less than desirable using the same scoring system (Storms et al. 2005,

Storms et al. 2006). Drug trial studies conducted at the Whitehall Deer Research Facility have adopted a reproducible scoring system that can compare induction quality among different drug combinations (Storms et al. 2005, Storms et al. 2006, Miller et al. 2009). We encourage review of literature before conducting a drug trial and develop a scoring system that can be compared to similar studies. Quality of reversal has not been recorded as consistently in past research with scores being related to length of sedation and less with overall quality (Miller et al. 2004). Similar to quality of induction, we believe a literature review should be conducted to develop a scoring system to compare reversal quality more readily among drug combinations.

Vital signs during immobilization in our study showed that 1.5 mL BAM, 1.5 mL NalMed-A, and 2.0 mL NalMed-A had similar physiological effects on treatment animals. Deer in all treatments had pO_2 rates lower than defined limits for hypoxemia (<80 mmHg; Read 2003). Hypoxemia occurred in deer of each treatment and supplemental oxygen was given based on oxygen saturation rates ≤70% which allowed for outlining of the base effects of BAM and NalMed-A at each dose while considering safety of research animals. Supplemental oxygen is recommended for all chemical immobilizations but may not be feasible in all situations (Miller et al. 2009). If immobilizations are designed to be short in duration, the provision of supplemental oxygen may increase handling time and is potentially unnecessary. However, routine administration of supplemental oxygen is still highly recommended (Mich et al. 2008; Grondahl et al. 2018), and our results support use of supplemental oxygen when administering 1.5 mL BAM, 1.5 mL, and 2.0 mL NalMed-A.

Serum cortisol levels in all treatments indicated that the immobilization period allowed for stress to decrease. It is likely that the handling procedure before injection of the immobilization drugs increased stress (i.e., cortisol) and the sedation period allowed cortisol to

decrease. We did not have a control group to compare cortisol levels, but research has shown cervids can have decreased levels of serum cortisol during immobilization (Cattet et al. 2004). Serum cortisol levels in our study were comparable to serum cortisol levels in white-tailed deer capture using Clover traps and rocket netting (DelGiudice et al. 1990). Although fecal cortisol levels were not able to be accurately measured below 30 ng/g, we can be confident that cortisol levels were not above 30 ng/g. Other fecal cortisol studies in white-tailed deer showed fecal cortisol levels ranged from 7.63 ng/g to 225 ng/g (Millspaugh et al. 2002; Millspaugh and Washburn 2003; Taillon and Côté 2008; Moll et al. 2009; Vega et al. 2020). Our fecal and serum cortisol levels show that both BAM and NalMed-A along with our handling procedure were comparable to other studies and do not create excessive stress on individual deer.

Progesterone and associated metabolites are linked to reproductive status of female mammals (Plotka et al. 1983). Our data indicates pregnant white-tailed deer had higher concentrations of progesterone metabolites. Additional research has indicated that progesterone may also be produced in stressful situations (e.g., physical capture) by the adrenal gland, metabolized, and excreted as feces within 12-24 hours (Plotka et al. 1983, Kapke et al. 1999). Sampling procedures during our study only allowed for 1 sample per day which led to mean peak progesterone levels past the 24-hour time frame indicated in previous studies. Multiple samples per day would have provided the opportunity to identify peak progesterone more precisely. Both pregnant and non-pregnant deer in all 3 treatments showed elevated concentrations of progesterone metabolites in fecal samples following the immobilization treatment indicating a possible stress response and adrenal production of progesterone. Chemical immobilization during gestation has the capability to cause mortality of the female or fetus, however no deer in this study presented pregnancy concerns (Casady and Allen 2013). Response from the adrenal

gland by producing progesterone has the ability to counteract negative effects associated with physical capture (DeNicola et al. 1997) which is shown by the increased progesterone of deer in our study after chemical immobilization without any changes in behavioral observations.

Non-invasive collection of fecal samples with assay of progesterone metabolites has been used to estimate pregnancy rates in cervids (Cervera-Hernández et al. 2015, Peter et al. 2018, Watson et al. 2022). Variation of progesterone metabolite concentrations in our study overlapped between non-pregnant deer and pregnant deer. Adrenal progesterone was likely released during handling, immobilization, and movement of deer in the facility leading to high variation and overlap of fecal progesterone metabolites. Researchers relying on non-invasive fecal sampling to estimate population pregnancy status should take into consideration the effect of non-capture stress events leading to increased fecal progesterone metabolite concentrations.

With the lack of a control treatment, it is unknown if chemical immobilization was a factor in results of behavioral observations, feed consumption, cortisol, or progesterone. Moving deer from paddocks into barn stalls and daily encounters with researchers collecting data could have been the sole driver of observed data. We justified that an increased dose of NalMed-A to 2.0 mL was appropriate because it is common for field research studies to increase the dose of BAM to 2.0 mL, and NalMed-A is designed to be a similar drug to BAM (Wolfe et al. 2014; Cook et al. 2016; Kirschner and Rodenkirch 2017; Walker et al. 2021). We believe that the similar physiological effects we observed between deer immobilized with 1.5 mL NalMed-A and 2.0 mL NalMed-A confirms that NalMed-A is safe to use at a dose of 2.0 mL on adult female white-tailed deer.

Similar to BAM, game animals chemically immobilized with NalMed-A should undergo a withdrawal time that allows the immobilization drugs and reversals to pass through the

animal's system before tissue is fit for human consumption (Wolfe et al. 2020). All components of BAM and reversals, naltrexone and atipamezole, were reported to be at undetectable levels (i.e., <0.01 ppm) in muscle and liver tissue at 11-days post immobilization in white-tailed deer (Cook et al. 2016). There have been no published withdrawal studies of NalMed-A in white-tailed deer. However, a study on withdrawal times of NalMed-A and reversals, naltrexone and atipamezole, in Rocky Mountain elk (*Cervus canadensis*) reported no tissue residue (<0.01 ppm) of any substance after 3 days in muscle or liver tissue (Wolfe et al. 2018). White-tailed deer sedated with BAM and NalMed-A in our study show no extended behavioral signs of being chemically immobilized. For the safety of the public all chemically immobilized deer should be marked to ensure humans do not consume any immobilization drugs in an animal's tissues (Cook et al. 2016; Wolfe et al. 2020).

The similarities of the physiological and immobilization effects of BAM and NalMed-A described in this study allow for use to be based on logistical factors rather than efficacy of each drug. Because NalMed-A is not DEA regulated, NalMed-A can be stored in an area where more personnel have access to chemical immobilization equipment (DEA 2020). In addition to storage more personnel would be able to transport and use NalMed-A without direct supervision of a veterinarian. However, a veterinarian is still required to prescribe NalMed-A and ensure safety procedures are in place. Having an option of a non-scheduled drug could allow for broader research efforts and faster response times to wildlife emergencies.

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Table 2.1. Ethogram for classifying focal animal behaviors exhibited by captive white-tailed deer (*Odocoileus virginianus*) for evaluation of immobilization with butorphanol-azaperone-medetomidine, or nalbuphine-medetomidine-azaperone at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA, during spring 2021.

Behavior	Definition
Calm movement	Deer's entire body is moving in a calm manner (e.g., walking)
Rapid movement	Deer's entire body is moving in a rapid manner (e.g., running)
Bedded, relaxed	Deer is bedded and appears to be calm
Bedded, alert	Deer is bedded with head up and appears attentive
Standing, relaxed	Deer is standing and appears to be calm
Standing, alert	Deer is standing and appears attentive
Foraging	Deer is actively drinking, eating, or searching for food
Grooming	Deer is grooming itself or another deer
Groomed	Deer is being groomed by another deer
Undefined	Deer is in the act of a behavior not defined above
Out of view	Deer is out of view of observer

Table 2.2. Scoring chart to determine reversal quality of captive white-tailed deer (*Odocoileus virginianus*) chemically immobilized with either 1.5 mL BAM, 1.5 mL NalMed-A, or 2.0 mL NalMed-A at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA, during spring 2021.

Quality Score	Scoring Definition
Excitability	
3	Very calm; no excitation during reversal
2	Mild excitement; procedure-related stress evident
1	Marked excitement; frantic behavior
0	Extreme excitement; frantic, reckless, or violent behavior
Muscle rigidity	
3	Complete muscle relaxation
2	Minimal and sporadic muscle rigidity and fasciculations
1	Marked intermittent muscle rigidity or fasciculations when recumbent
0	Extreme or continuous muscle rigidity or fasciculations when recumbent
Overall quality	
3	Rapid, smooth, optimal reversal
2	Relatively rapid and smooth but could be improved
1	Rough or extended unacceptable reversal
0	Extremely rough, lengthy, potentially dangerous reversal

Table 2.3. Mean time (SE) in minutes captive white-tailed deer (*Odocoileus virginianus*) showed first sign of immobilization, head droop, sternal recumbency, lateral positioning, head down and complete immobilization when sedated with 1.5 mL butorphanol-azaperone-medetomidine, 1.5 mL nalbuphine-medetomidine-azaperone or 2.0 mL nalbuphine-medetomidine-azaperone at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA, during spring 2021.

	Mean immobilization time in minutes (SE)				
Measurement	**BAM 1.5 mL**	**NalMed-A 1.5 mL**	**NalMed-A 2.0 mL**	**F Score**	**P Value**
First sign	2.4 (0.2)	2.6 (0.2)	2.7 (0.4)	$F_{2,25} = 0.19$	0.83
Head droop	4.1 (0.6)	4.0 (0.3)	3.9 (0.5)	$F_{2,24} = 2.62$	0.09
Sternal	6.3 (1.2)	6.5 (1.5)	5.0 (0.6)	$F_{2,25} = 1.90$	0.17
Lateral	6.3 (1.2)	7.0 (1.4)	5.5 (0.6)	$F_{2,25} = 2.57$	0.10
Head down	8.5 (2.1)	7.8 (1.4)	5.8 (0.6)	$F_{2,25} = 0.07$	0.93
Complete immobilization	14.7 (2.6)	20.3 (1.6)	20.4 (2.6)	$F_{2,18} = 0.32$	0.73

Table 2.4. Mean time in minutes captive white-tailed deer (*Odocoileus virginianus*) showed first sign of reversal, head up, sternal positioning, standing and no sign of immobilization after 3.0 mL atipamezole and 0.5 mL naltrexone were administered to reverse either 1.5 mL butorphanol-azaperone-medetomidine, 1.5 mL nalbuphine-medetomidine-azaperone, or 2.0 mL nalbuphine-medetomidine-azaperone in captive white-tailed deer at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA during spring 2021.

	Mean recovery time in minutes (SE)				
Measurement	**BAM 1.5 mL**	**NalMed-A 1.5 mL**	**NalMed-A 2.0 mL**	**F Score**	**P Value**
First sign reversal	3.6 (0.5)	5.9 (1.4)	4.9 (0.5)	$F_{2,24} = 0.08$	0.92
Head up	5.1 (0.6)	7.8 (1.8)	7.5 (1.0)	$F_{2,25} = 0.44$	0.65
Sternal	5.8 (0.6)	8.6 (1.9)	8.1 (0.9)	$F_{2,24} = 0.26$	0.77
Standing	6.9 (0.5)	9.7 (1.8)	9.1 (0.8)	$F_{2,25} = 1.02$	0.38
No immobilization	7.3 (0.5)	11.0 (1.9)	9.8 (0.8)	$F_{2,25} = 1.04$	0.34

Table 2.5. Mean values of pH, partial carbon dioxide (pCO_2), partial oxygen (pO_2), base excess (BEecf), bicarbonate (HCO_3), total carbon dioxide (TCO_2), percent blood oxygen ($s0_2$) and lactate at time of complete immobilization (T0), 10 minutes post complete immobilization (T1), and 20 minutes post complete immobilization (T2) in captive white-tailed deer (*Odocoileus virginianus*) at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA during spring 2021.

	BAM 1.5 mL	**NalMed-A 1.5 mL**	**NalMed-A 2.0 mL**		
Measurement (unit)	**Mean (SE)**	**Mean (SE)**	**Mean (SE)**	**F Score**	**P Value**
pH T0	7.24 (0.03)	7.30 (0.01)	7.27 (0.03)	$F_{2,24}$=1.66	0.21
pH T1	7.28 (0.02)	7.32 (0.03)	7.30 (0.02)	$F_{2,23}$=0.78	0.47
pH T2	7.29 (0.02)	7.31 (0.02)	7.32 (0.02)	$F_{2,23}$=0.60	0.56
pCO_2 T0 (mmHg)	47.3 (1.7)	46.1 (1.8)	44.4 (1.8)	$F_{2,24}$=0.71	0.50
pCO_2 T1 (mmHg)	45.8 (1.5)	49.1 (4.3)	45.5 (2.1)	$F_{2,23}$=0.51	0.61
pCO_2 T2 (mmHg)	48.8 (2.1)	51.1 (4.2)	48.2 (2.7)	$F_{2,23}$=0.24	0.79
pO_2 T0 (mmHg)	56 (3.9)	64 (10.9)	62 (5.8)	$F_{2,24}$=0.36	0.70
pO_2 T1 (mmHg)	56 (4.7)	84 (17.5)	51 (3.2)	$F_{2,23}$=3.03	0.07
pO_2 T2 (mmHg)	74 (18.3)	74 (17.5)	58 (8.9)	$F_{2,23}$=0.47	0.63
BEecf T0 (mmol/L)	-7 (1.8)	-4 (1.2)	-7 (1.9)	$F_{2,24}$=0.95	0.40
BEecf T1 (mmol/L)	-5 (1.5)	-2 (0.8)	-4 (1.7)	$F_{2,23}$=1.62	0.22
BEecf T2 (mmol/L)	-3 (1.7)	-1 (1.1)	-1 (1.6)	$F_{2,23}$=0.52	0.60
HCO_3 T0 (mmol/L)	20.5 (1.5)	22.6 (1.1)	20.4 (1.4)	$F_{2,24}$=0.78	0.47
HCO_3 T1 (mmol/L)	21.5 (1.1)	24.5 (0.9)	22.7 (1.4)	$F_{2,23}$=1.58	0.23

HCO_3 T2 (mmol/L)	23.8 (1.5)	25.6 (1.2)	24.9 (1.4)	$F_{2,23}$=0.43	0.66
TCO_2 T0 (mmol/L)	22 (1.5)	24 (1.2)	22 (1.5)	$F_{2,24}$=0.69	0.51
TCO_2 T1 (mmol/L)	23 (1.2)	26 (1.0)	24 (1.4)	$F_{2,23}$=1.63	0.22
TCO_2 T2 (mmol/L)	25 (1.6)	27 (1.3)	26 (1.4)	$F_{2,23}$=0.44	0.65
sO_2 T0 (%)	81 (2.8)	85 (2.7)	85 (2.1)	$F_{2,24}$=1.04	0.37
sO_2 T1 (%)	82 (2.9)	88 (3.4)	80 (2.4)	$F_{2,23}$=1.91	0.17
sO_2 T2 (%)	85 (3.3)	86 (3.7)	82 (2.9)	$F_{2,23}$=0.52	0.60
Lactate T0 (mmol/L)	8.88 (1.4)	7.03 (0.6)	8.75 (1.4)	$F_{2,24}$=0.63	0.54
Lactate T1 (mmol/L)	7.58 (1.4)	4.83 (0.5)	6.87 (1.2)	$F_{2,23}$=1.54	0.24
Lactate T2 (mmol/L)	5.87 (1.1)	3.60 (0.4)	5.01 (0.9)	$F_{2,23}$=1.62	0.22

Table 2.6. Mean (SE) feed consumption (kg/day/deer) for deer immobilized with 1.5 mL BAM, 1.5 mL NalMed-A, or 2.0 mL NalMed-A while group-housed in outdoor paddocks or housed in individual barn stalls before and after immobilization in captive white-tailed deer (*Odocoileus virginianus*) at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA during spring 2021.

Treatment	Location	Before Treatment (SE)	After Treatment (SE)	F Score	P Value
BAM 1.5 mL	Paddock	0.98 (0.18)	0.84 (0.10)	$F_{1,15}=0.57$	0.46
BAM 1.5 mL	Stall	0.31 (0.06)	0.36 (0.04)	$F_{1,88}=0.71$	0.40
NalMed-A 1.5 mL	Paddock	0.69 (0.16)	0.76 (0.07)	$F_{1,15}=0.18$	0.68
NalMed-A 1.5 mL	Stall	0.19 (0.02)	0.31 (0.03)	$F_{1,98}=13.44$	0.0004
NalMed-A 2.0 mL	Paddock	0.85 (0.05)	0.64 (0.11)	$F_{1,18}=3.01$	0.10
NalMed-A 2.0 mL	Stall	0.31 (0.03)	0.32 (0.04)	$F_{1,79}=0.11$	0.75

Table 2.7. Progesterone metabolite concentration differences of deer immobilized with 1.5 mL BAM, 1.5 mL NalMed-A, or 2.0 mL NalMed-A during stress event periods including 1) before (i.e., the first 4 days in isolated stalls), 2) treatment (i.e., the day of treatment and following 2 days), and 3) after (i.e., the final 3 days in isolated barn stalls) during the 10-day observation period in isolated barn stalls, based on mean estimates from the best fit generalized linear mixed model conducted at Whitehall Deer Research Facility in Athens, GA, USA, during 2021. Shown are regression coefficients (β), standard error (SE), *t*-scores, and *P*-values.

Predictor	β	SE	*t*	*P*
Before	885.41	309.00	2.87	0.0045
After	-49.42	261.64	-0.19	0.8503
Treatment	1008.23	260.82	3.87	0.0001
Pregnancy status	3077.51	481.46	6.39	0.0000

Table 2.8. Behavioral differences between treatments immobilized with 1.5 mL BAM, 1.5 mL NalMed-A, or 2.0 mL NalMed-A during the 30-day observation period, based on mean estimates from the hypothesis driven generalized linear mixed model conducted at Whitehall Deer Research Facility in Athens, GA, USA, during 2021. Shown are regression coefficients (β), standard error (SE), *t*-scores, and *P*-values.

Behavior	Predictor	β	SE	*t*	*P*
Calm	BAM 1.5 mL	0.86	0.02	35.87	$<0.002^{-13}$
	NalMed-A 1.5 mL	0.02	0.03	0.82	0.417
	NalMed-A 2.0 mL	0.01	0.03	0.19	0.855
	Day	0.00	0.00	1.78	0.076
Alert	BAM 1.5 mL	0.14	0.02	5.84	$<0.004^{-4}$
	NalMed-A 1.5 mL	-0.02	0.03	-0.80	0.434
	NalMed-A 2.0 mL	-0.01	0.03	-0.27	0.789
	Day	-0.00	0.00	-1.72	0.085

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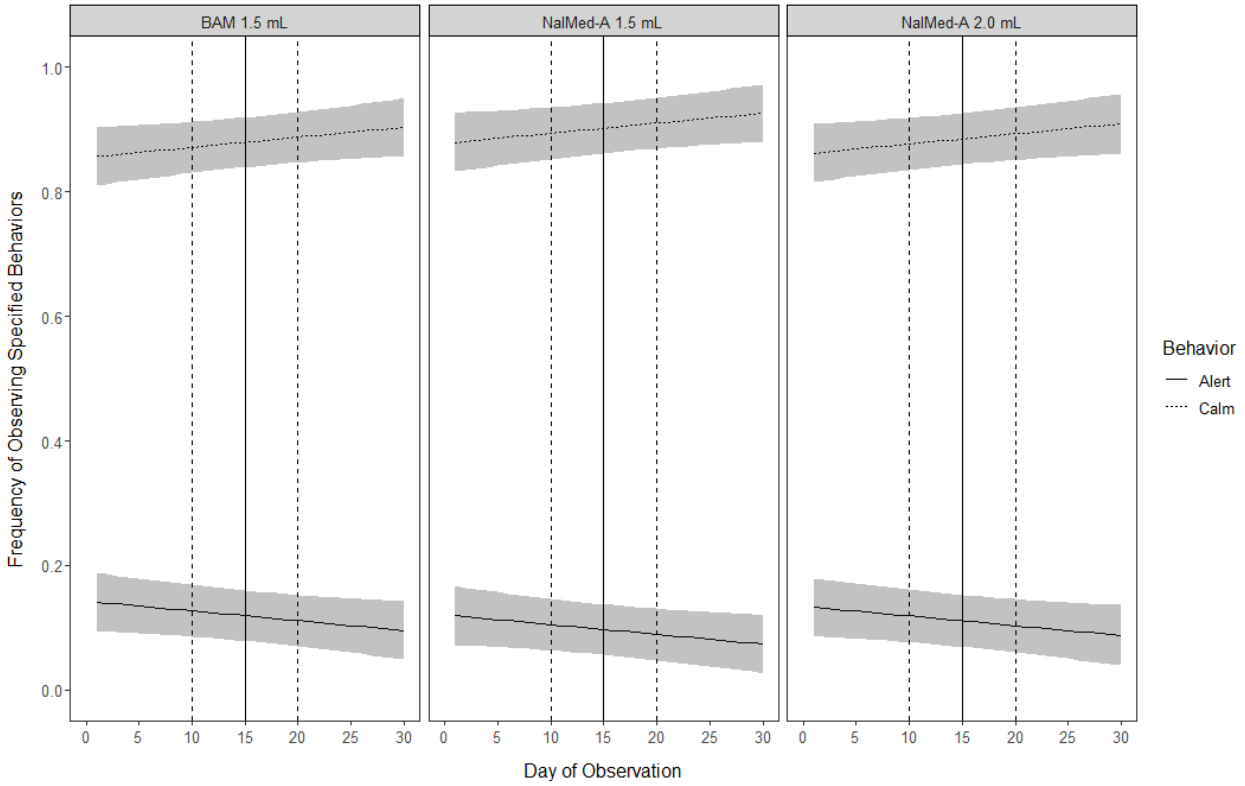

Figure 2.1. Mean frequency of observing calm and alert behaviors in captive white-tailed deer (*Odocoileus virginianus*) across the 30-day observation period for treatment deer immobilized with 1.5 mL butorphanol-azaperone-medetomidine, 1.5 mL nalbuphine-medetomidine-azaperone or 2.0 mL nalbuphine-medetomidine-azaperone at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA during spring 2021. Dashed lines indicate when deer were moved between outdoor paddocks and isolated barn stalls. The solid line indicates day of treatment.

CHAPTER 3

IMMOBILIZATION EFFICACY OF CONDUCTED ELECTRICAL WEAPONS ON CAPTIVE WHITE-TAILED DEER[1]

[1]Grunwald, P. J., M. G. Ruder, D. A. Osborn, L. I. Muller, K. O. Goode, and G. J. D'Angelo. To be submitted to *Journal of Wildlife Diseases.*

ABSTRACT

Wildlife emergencies (e.g., injured animals, etc.) often require capture or euthanasia of animals to resolve the situation. Conducted electrical weapons (CEWs) have the potential to immobilize white-tailed deer (*Odocoileus virginianus*) for a short duration avoiding potential risks of extended immobilization (e.g., chemical immobilization) and increasing safety during euthanasia methods (e.g., gunshot). To test the efficacy of CEW immobilization of white-tailed deer, we arranged 5 treatment groups of captive deer including: 1) chemical immobilization with 5-second CEW exposure (n=5), 2) chemical immobilization with 15-second CEW exposure (n=5), 3) 5-second CEW exposure with no chemical immobilization (n=10), 4) 15-second CEW exposure with no chemical immobilization (n=10), and 5) a control group with no chemical immobilization or CEW exposure (n=10). We collected blood from deer in treatments 1 and 2 immediately before CEW exposure, and 2-days and 5-days post exposure with chemical immobilization for serum biochemical analysis (to measure physiological markers associated with organ and tissue damage). We observed deer before, during, and after treatments to evaluate potential behavioral changes. All deer showed signs of muscle paralysis immediately after exposure to CEW and regained muscle control immediately after the exposure ended. Serum biochemistry results were unremarkable except for significant increases in creatine kinase (CK) and aspartate aminotransferase (AST) 2-days post treatment, suggesting temporary muscle damage. However, CK and AST returned to pre-exposure levels by day 5 post-exposure within both treatments. After 15-days post-exposure, deer that were only exposed to the CEW had 27 of 39 (69%) probes still attached. We detected localized scabbing at all probe sites, however there were no signs of infection or muscle tissue damage. Our findings suggest that short-term exposure of a CEW to immobilize white-tailed deer is a potential alternative to typical capture

techniques and would provide sufficient immobilization to approach and euthanize a deer if necessary. As with all capture techniques, trainings and protocols should be developed to ensure safety of personnel and animals during CEW exposures.

KEY WORDS behavior, controlled electrical weapon, CEW, *Odocoileus virginianus*, physiology, stress, TASER®, white-tailed deer

INTRODUCTION

As urban and suburban areas are continuing to expand, white-tailed deer (*Odocoileus virginianus*) populations are also continuing to grow within urban and suburban areas (Kilpatrick et al. 2004). White-tailed deer are highly adaptable and thrive in urban areas leading to a variety of deer-human interactions (Soulsbury and White 2015). Many deer-human interactions may be considered positive (e.g., viewing deer in a natural area), however some potentially negative interactions must be addressed for the safety of humans and deer (e.g., habituation, deer-vehicle collisions; Conover 2011; Stinchcomb et al. 2022). When potential injury of humans, deer, or both occur, wildlife managers must be able to respond in a safe, humane, and prompt manner (Curtis 2020). Each human-deer conflict presents a unique set of challenges that need to be overcome to provide human safety and animal welfare. A variety of safe capture and handling techniques is required to provide human safety and animal welfare in a range of conflicts.

Conducted electrical weapons (CEWs) are potential tools to temporarily immobilize wildlife to gain control of animals for handling (e.g., remove entanglements) or euthanasia, deter unwanted behaviors through aversive conditioning, and increase safety of personnel when working with wildlife (Lewis et al. 2012). Use of CEWs on wildlife currently occurs under specific policy guidelines throughout the United States, however only one study has measured physiological effects of CEW usage on wildlife (Lieske et al. 2018). Lieske et al. (2018)

determined that a 10-second exposure to a CEW in reindeer (*Rangifer tarandus tarandus*) produced similar physiological effects to chemical immobilization with carfentanil-xylazine.

The first CEW was developed in 1970 to provide rapid, short-term immobilization of humans by stimulating the neuromuscular system with pulses of electricity (Biria et al. 2010; Kuersten 2020). Modern CEWs have a similar outer appearance to handguns and are activated by a trigger pull; however, the intended use of CEWs is to avoid lethal situations (Sloane et al. 2008). The most common CEWs are activated by a compressed nitrogen cartridge that deploys two probes from a replaceable cartridge attached to the front of the weapon; the probes attach to the target with thin barbed metal shafts and electricity is transferred from a battery in the handheld portion to the probes through copper wire leads (Sloane et al. 2008). Electricity is automatically transferred between probes through the target for 5 seconds and duration of electrical pulses can be extended with specially programmed equipment (Sloane et al. 2008; Jenkins et al. 2013).

To determine safety and physiological effects of CEW exposure a variety of measurements should be analyzed. During the immobilization period vital signs and blood samples, to analyze cortisol levels, are important measurements to be monitored (Sente et al. 2014). Behavioral observations can be collected to monitor long-term effects. Responses to immobilization can be indicated by changes in frequency of behaviors (Durnin et al. 2010). Our objectives were to: 1) determine if immobilization with a conducted electrical weapon produces detrimental physiological effects based on measurements listed above, 2) determine if a CEW can produce adequate immobilization in deer-related emergencies, and 3) based on objectives 1 and 2, determine potential uses of CEW immobilization for wildlife managers. We hypothesized that exposing deer to a CEW for <15 seconds would provide safe and adequate immobilization

providing opportunity for use by wildlife managers. In addition, we hypothesized there would be no long-term physiological effects of CEW exposure to deer.

MATERIALS AND METHODS

This research study was conducted at the Whitehall Deer Research Facility at the Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, Georgia, USA. Approval of this study was issued by the University of Georgia's Institutional Animal Care and Use Committee under Animal Use Proposal A2021 05-002-Y1-A3. A 3.0-m tall woven-wire fence encompasses the entire property along with a 2-strand electric fence at ground level that deters entry of domestic dogs (*Canis lupus familiaris*) and predators. Total area of the facility is 2.6 ha and contains a 20-stall (3 m x 6 m) barn, 3 sorting pens (15 m x 20 m), and 5 outdoor paddocks (0.4 – 0.8 ha).

We provided deer with a pelletized ration (Antler Advantage Deer 20, Purina Animal Nutrition, Arden Hill, MN, USA), perennial peanut (*Arachis glabrata*) hay, and water *ad libitum*. Large oaks (*Quercus* spp.) and pines (*Pinus* spp.) were the primary trees in the paddocks. Monthly average rainfall during the study was 9.3 cm in Athens, GA and monthly average temperatures ranged from high of 32.2° C to a low of 10.6° C (U.S. Climate Data 2022).

August through October of 2021, we randomly assigned adult (>1 year old), captive, white-tailed deer to one of five treatment groups. Treatment groups included: 1) chemical immobilization and 5-second CEW exposure (Chem5, n = 5), 2) chemical immobilization and 15-second CEW exposure (Chem15, n = 5), 3) a 5-second CEW exposure with no chemical immobilization (CEW5, n = 10), 4) a 15-second CEW exposure with no chemical immobilization (CEW15, n = 10), and 5) a control with no chemical immobilization or CEW

exposure (n = 10). Deer in each treatment were group-housed in a paddock separate from other treatment groups.

Behavioral Observations

We created an ethogram of expected normal behaviors for deer and grouped them into calm or alert categories (Table 3.1). We observed each deer for a total of 30 days to determine changes in frequency of calm and alert behaviors, including 15 days before the treatment and 15 days after the treatment, with the treatment occurring on the 15th day. We observed deer in group-housed paddocks days 1-10 and 21-30. We observed deer in isolated barn stalls days 11-20. Individual deer were identified by uniquely numbered ear tags.

Group-housed Observations – To collect observations in group-housed paddocks we placed an elevated stand in each paddock that was easily accessible from the entrance and offered the least visual obstruction for the observer. Once observers entered the paddock and were situated in the stand, they waited 15 minutes for the deer to acclimate to their presence. Once the 15-minute period was over, observers opportunistically observed each deer individually for 10 minutes and every 30 seconds recorded the deer's most common behavior during that 30-second period for a total of 20 observations per deer per day.

Isolated Barn Stall Observations – We collected observations in barn stalls by reviewing videos captured with a video recorder (Panasonic 25x i.zoom, Panasonic Corporation, Kadoma, Osaka, Japan). Observers placed the camera mounted on a tripod in the hallway of the barn facing into the focal animal's stall and recorded separate 15-minute videos for each deer. The first 5 minutes allowed for the deer to acclimate to the presence of the camera after the observer had placed it and left the hallway. The remaining 10 minutes was used to classify behaviors in the same manner as the group-housed observations.

Immobilization Treatment

We transferred deer from paddocks into individual barn stalls 5 days before exposure to the CEW. We fasted deer for ≥16 hours before treatment to prevent aspiration of rumen contents during immobilization (Lin and Walz 2014). We returned deer to group-housed paddocks 5 days after exposure to the CEW occurred. All CEW exposures were carried out by a Georgia Department of Natural Resources Wildlife Law Enforcement Officer trained in the application of CEWs using a TASER X2 (Axon Enterprise, Scottsdale, Arizona, USA).

Welfare assessments – We assigned treatments Chem5 and Chem15 to determine short-term physiological effects on deer, related to overall welfare, after being exposed to a CEW. Treatments Chem5 and Chem15 were injected with a standard dose of 2.0 mL butorphanol-azaperone-medetomidine (BAM; 27.3 mg/mL butorphanol, 9.1 mg/mL azaperone, 10.9 mg/mL medetomidine; ZooPharm, Laramie, Wyoming, USA) via a dart projector (Telinject VARIO 4V, Telinject USA, Inc., Arleta, California, USA) in individual barn stalls at a distance of 5 m. Once completely immobilized, we placed deer in sternal recumbency against a padded wall and collected 10 mL of blood from the jugular vein in additive free vacutainer tubes. We deployed the CEW from an elevated (2.1 m) position roughly 5 m from the chemically immobilized deer to ensure appropriate spread of CEW probes. Appropriate spread is achieved when the probes contact the front and rear hemisphere of the deer. The front shoulder and hindquarter muscle mass were targeted on each deer to immobilize each respective hemisphere causing complete immobilization. Failure to expose multiple hemispheres to the CEW could lead to partial or no immobilization (Biria et al. 2010). We administered 0.5 mL naltrexone (50 mg/mL) and 3.0 mL atipamezole (25 mg/mL) to antagonize the butorphanol and medetomidine, respectively, via separate intramuscular injections to the hindquarter 20 minutes after CEW exposure. We chemically immobilized deer 2- and 5-days post CEW exposure using the same procedure noted

above to collect additional blood samples. On both days, we collected a 10-mL blood sample as described above. All blood samples were centrifuged (1775 xg) for 10 minutes and serum was collected to analyze for alanine transaminase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), bilirubin, creatine kinase (CK), creatinine, and lactate dehydrogenase (LDH). Serum samples were frozen at -20° C until analysis occurred. Serum samples were sent to the University of Georgia College of Veterinary Medicine Clinical Pathology Lab (Athens, Georgia, USA) for analysis. We removed all probes that were deployed by the CEW and remained attached to each deer on day 5 post exposure. We did not remove probes immediately to simulate real-world scenarios and to accurately determine potential infections and muscle damage at probe locations.

Practical use assessments – We assigned treatments CEW5 and CEW15 to determine how wildlife managers could use CEWs in real-world scenarios. We moved deer from individual stalls to a circular containment area (4.3 m diameter) with solid wooden walls and divided by 2 movable wooden walls (Figure 3.1). Conducting treatments in the larger containment area allowed for more realistic CEW exposures to mimic practical uses and allowed for more direct observation of the exposure. We observed and deployed the CEW from small windows in walls of the containment area while only the deer was in the exposure area. To test complete immobilization of deer, we deployed the CEW from ~ 2.5-3.7 m to target front and rear hemispheres of the deer's body (Figure 3.2). In addition, to evaluate poor shot placement (i.e., only one body hemisphere targeted) we tested immobilization in only one hemisphere on 3 deer where we deployed the CEW for 5 seconds into the hindquarter from ~1.5 m (Figure 3.3). The 3 additional deer were not part of either CEW5 or CEW15 and only observational data during the exposure was analyzed.

We used 2 independent observers to score general behaviors of deer during CEW exposures to determine overall effectiveness (Table 3.2). In addition, we scored the recovery period immediately after the exposure had ceased (Table 3.3). An observer's total score was determined by adding the scores from each category. The scores from each observer were averaged to create the final immobilization score. The CEW exposure score had a possible range between 0 and 16. A score of ≤4 was considered an effective immobilization. Desirable recovery score had a range between -3 and 3. A score of -1≤1 was considered a desirable recovery.

We returned deer to individual barn stalls immediately after the exposure. We returned deer to outdoor paddocks 5 days after the treatment. After the 15-day post exposure observation period ended, we returned deer to individual barn stalls. Deer were returned to the barn to be chemically immobilized, as described for Chem5 and Chem15 treatments, to remove probes and identify any potential muscle damage or infections.

Feed Measurements

We collected feed measurements daily for each treatment on the same schedule as behavioral observations. All feed was removed prior to the data collection period and new feed was weighed on day 1 of trials. Total daily feed consumed (kg) was measured by weighing the amount of residual feed and subtracting from the total amount of feed given the day prior.

Data Analysis

We analyzed data using program R 4.1.0 (R Core Team 2021). We used a one-way ANOVA with a post-hoc Tukey's test determine differences of blood biomarkers and daily feed consumption among treatments. We assigned behaviors into either calm or alert categories and analyzed frequency of behaviors over time using a hypothesis-based linear mixed effects model with treatment and day of observation as fixed effects and individual deer as the random effect. All analyses used an alpha value of <0.05 to determine statistical significance.

RESULTS

Deer in treatments Chem5 and Chem15 showed immediate reaction to exposure of the CEW by rigidly extending or kicking their legs briefly until muscles became rigid and multiple deer raised their head and emitted loud vocalizations. Stimulus from CEW exposure counteracted the effect of chemical immobilization in 2 of 5 (40%) deer in the Chem5 treatment and 5 of 5 (100%) deer in the Chem15 treatment which allowed for deer to stand up and become mobile. Chemical immobilization effects returned all deer back to a state of complete sedation within 5 minutes except for 1 deer in the Chem5 treatment that was still standing after 20 minutes when reversals were administered. All but one deer in treatments CEW5 and CEW15 were immediately immobilized upon exposure to the CEW and immediately regained muscle control and footing when the CEW exposure ceased. One deer in the CEW5 treatment was not adequately immobilized for the 5-second exposure period. The CEW operator noted that the CEW did not appear to fire normally, but probes were attached to the deer in both hemispheres. The 3 additional deer exposed to the CEW only in the rear body hemisphere showed immediate immobilization in rear legs but retained function of their forelegs and were able to drag themselves short distances using their forelegs. During all effective CEW exposures, neck and leg muscles of deer became noticeably rigid, the neck was extended, and the legs locked into extension causing deer to lunge forward and fall. Deer emitted loud vocalizations ranging from one short vocalization when initially exposed to the CEW to vocalizations lasting the entire exposure period.

Blood biomarker levels sampled in the welfare assessment treatments, Chem5 and Chem15, were similar across the 3 sampling periods within each treatment except for CK and AST (Table 3.4). The CK levels for both Chem5 and Chem15 dramatically increased at the day 2

sample period but returned to baseline levels by day 5. Levels of AST increased, however less drastically than CK, and returned to baseline measurements by day 5. Overall, feed consumption rates did not change for any treatment group before to after the assigned treatment (Table 3.5) except for deer in the CEW15 treatment while housed in individual barn stalls. The CEW15 group increased consumption from 0.71 (SE = 0.11) kg/deer/day before treatment to 1.07 (SE = 0.08) kg/deer/day after treatment while in individual barn stalls.

Eighteen of 20 (90%) probes remained attached to deer in the Chem5 and Chem15 treatments 5 days post exposure. Mild soft tissue swelling developed at the site of the hindquarter probe insertion on 2 of 5 (40%) deer in the Chem15 treatment. Probes were located more distal on deer that experienced swelling. Swelling occurred by day 2 post exposure for both deer but reduced to normal conditions 5 days post exposure. For deer in the CEW5 treatment, we removed 10 of 21 (48%) probes 18 days post exposure. For deer in the CEW15 treatment, we removed 16 of 18 (89%) probes 25 days post exposure. Probes that we did not remove had already been removed, either by naturally falling out or being pulled out by deer. Deer in the CEW5 and CEW15 treatments had minor scabbing at all probe sites, and often localized and insignificant (e.g., ~6mm-diameter) swelling around the probe insertion site. No deer had evidence of significant tissue damage or infection as a consequence of probe insertion.

Mean immobilization scores were 2.5 (Range = 0.0-11.5) for deer in the CEW5 treatment. The deer with the highest immobilization score in the CEW5 treatment potentially did not receive any exposure to the CEW. Mean immobilization scores were 3.0 (Range 1.5-5.0) for deer in the CEW15 treatment. The mean immobilization score of the 3 deer exposed to the CEW only in the rear hemisphere was 5 (Range = 3-7). Immobilization scores indicate a desirable immobilization effect when probes contact both hemispheres of the deer's body.

Mean recovery scores were -0.4 (Range = -1.5-0.0) for deer in the CEW5 treatment. Mean recovery scores were 0.4 (Range = 0.0-1.0) for deer in the CEW15 treatment. The mean recovery score of the 3 deer exposed to the CEW only in the rear hemisphere was 0.2 (Range 0-0.5). Recovery scores indicate a desirable recovery to a CEW regardless of if probes contact both hemispheres of the deer's body.

There was no relationship between the treatment a deer received and the frequency at which calm or alert behaviors were observed according to the hypothesis-based model (Table 3.6). The timing of receiving a treatment did not alter the frequency of observing behaviors across time according to the hypothesis-based model. Frequency of calm behaviors ranged from 87% to 90% for Chem5, 93% to 95% for Chem15, 87% to 89% for CEW5, 90% to 92% for CEW15, and 93% to 95% for the control. Frequency of alert behaviors ranged from 10% to 13% for Chem5, 4% to 7% for Chem15, 10% to 13% for CEW5, 7% to 10% for CEW15, and 5% to 7% for the control (Figure 3.4).

DISCUSSION

We conclude that short-term CEW exposure (≤15 seconds) safely immobilizes white-tailed deer and detrimental physiological effects did not occur among our measurements. The 15-second immobilization period provided sufficient immobilization to make resolving certain deer related wildlife emergencies feasible. The combination of no detrimental physiological effects and sufficient immobilization would allow wildlife managers to further restrain or euthanize white-tailed deer in emergency situations.

To determine physiological effects of CEW exposure to deer, we chemically immobilized each deer in Chem5 and Chem15 to collect blood samples. In real-world scenarios the combination of CEW exposure and chemical immobilization are not likely to be combined but

the powerful stimulation of CEW was demonstrated by 7 of 10 sedated deer becoming aroused and ambulatory after CEW deployment. In deer exposed to a CEW for 5 or 15 seconds CK and AST enzymes increased but returned to baseline measurements within 5 days, indicating transient muscle injury. Furthermore, daily clinical observation for 15 days post-treatment indicates there are likely no long-term detrimental effects of short-term CEW exposure on white-tailed deer.

Some CEW probes in this study remained attached to the deer for ≤25 days before being removed by researchers. Probes from the hindquarter were more likely to be missing when remaining probes were manually removed after the 15-day observation period. Missing probes could have been removed potentially by the deer during grooming leading to possible ingestion. However, deer may have pulled the probes out and then simply dropped them. If deer were to ingest CEW probes nutrient absorption may decrease, internal injuries may be sustained, and death may occur (Sheferaw et al. 2014). However, study deer were monitored daily during and after the study for assessment of general health and well-being and we observed no evidence of ingestion or adverse health consequences.

All deer were immobilized immediately after CEW exposure when probes were applied to both hemispheres of the body. Proper placement of the CEW probes into large muscle groups of both hemispheres of the deer's body is critical for effective immobilization and may not be possible in all emergency situations. For example, deer may have limited or full mobility in many situations and poor or missed shots with a CEW could lead to a more dangerous situation. If placement of probes only causes CEW exposure to a small muscle group, the animal will be able to move other muscle groups normally (Lewis et al. 2012). Scenarios of partial

immobilization should be avoided because the ability for the deer to have any mobility creates additional risk for the animal and personnel involved.

Based on our observations, we believe that CEW use does not provide an appropriate situation for direct handling of the animal during active CEW exposure. The insulation surrounding the copper wire leads can break allowing exposure to the electrical current without being contacted by the probes (Lewis et al. 2012). We believe there is a potential for non-direct handling during an exposure that would allow for direct handling after the CEW exposure has ended. For example, a net could be placed over the deer to facilitate manual restraint immediately after the CEW is disengaged. If personnel touch the exposed animal between where the probes made contact, that individual would also be exposed to the CEW because the electrical current is flowing directly between the probes through the body tissues (Sun and Webster 2007). In addition, avoiding direct handling during the CEW exposure could decrease chance of injury to individual deer because the muscles of the exposed individual become very rigid (Ho et al. 2012). Muscle groups became rigid in all deer exposed to a CEW during our experiment and we believe forcing the exposed muscle groups to move against the neuromuscular stimulation may increase chance of injury.

Defined policies for use and best practices for well-trained individuals are a critical component of use of CEW during wildlife management activities. For instance, determining if and when CEW usage is appropriate and having a plan of action in case full immobilization does not occur will ensure safety of personnel and wildlife involved. It is important to highlight that use of CEW produces partial or full immobilization that the public could find distressing. Hands-on trainings and policies should guide wildlife managers to interact with the public and explain reasoning and necessity of exposing deer to a CEW.

Two potential applications of CEWs are deterring wildlife habituation and creating a safer atmosphere for euthanasia of animals in emergency situations (Lewis et al. 2012). Young deer are commonly taken from the wild because the general public believe the fawn is orphaned and human intervention is needed for survival which can lead to negative deer-human interactions (Beringer et al. 2004; VerCauteren and Hygnstrom 2011). Deer may become accustomed to humans providing food leading to a variety of safety concerns including pathogen transmission and aggressive deer. (McCance et al. 2015). Also, female and male deer may become defensive and attack anyone in close proximity during fawning or breeding season, respectively (Grovenburg et al. 2009; Hubbard and Nielsen 2009). Wildlife exposed to a CEW generally avoid interactions with humans or are much more cautious in the vicinity of humans after an exposure (Lewis et al. 2012). The use of operant conditioning through CEW use may decrease need for euthanasia in some instances. Another potential application for CEW is to facilitate safe euthanasia. Euthanasia by gunshot is a common tool used by wildlife managers but may be a cause for concern in some settings (e.g., urban areas) because of the lack of shooting into a safe backdrop (Caudell et al. 2009). To create a safer euthanasia atmosphere, a deer may be exposed to a CEW causing it to fall and become immobile which would allow for a wildlife manager to approach and euthanize the deer while shooting safely in a downward direction.

We demonstrated that CEWs can be used in a safe and effective manner on white-tailed deer. However, agencies should develop policies and trainings for CEW use to maximize animal welfare outcomes and the safety of personnel. However, use of CEWs or other electro-immobilization devices is not recommended for routine capture (American Veterinary Medical Association 2008, 2010). We agree that CEWs should not be routinely used as a tool for capture

deer or other wildlife and use should be focused on emergency situations. Policies should be enacted to ensure proper use of CEWs and should outline justification for use of CEWs when questioned by stakeholders. Several states currently have a CEW policy that provides guidelines on what species can be exposed, for how long, and under what circumstances (Alaska Department of Fish and Game 2010; California Department of Fish and Wildlife 2013). Some observers may find witnessing an animal being exposed to a CEW an unpleasant situation because of the immediate physical response of muscle paralysis and loud vocalizations that typically occur. Science-based policies can give valid reasoning of CEW use to the public or lead to avoidance of CEW usage when not appropriate.

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Table 3.1. Ethogram for classifying focal animal behaviors exhibited by captive white-tailed deer (*Odocoileus virginianus*) for evaluation of immobilization with chemical immobilization and 5-second conducted electrical weapon (CEW) exposure, chemical immobilization and 15-second CEW exposure, no chemical immobilization and 5-second CEW exposure, no chemical immobilization and 15-second CEW exposure, and no chemical immobilization or CEW exposure at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA, during fall 2021.

Behavior Group	Behavior	Definition
Calm	Bedded, relaxed	Deer is bedded and appears to be calm
	Standing, relaxed	Deer is standing and appears to be calm
	Calm movement	Deer's entire body is moving in a calm manner (e.g., walking)
	Foraging	Deer is actively drinking, eating, or searching for food
	Grooming	Deer is grooming itself or another deer
	Groomed	Deer is being groomed by another deer
Alert	Bedded, alert	Deer is bedded with head up and appears attentive
	Standing, alert	Deer is standing and appears attentive
	Rapid movement	Deer's entire body is moving in a rapid manner (e.g., running)
Censored	Undefined	Deer is in the act of a behavior not defined above
	Out of view	Deer is out of view of observer

Table 3.2. Scoring to determine immobilization effect of a conducted electrical weapon (CEW) for a 5-second exposure (n=10) and a 15-second exposure (n=10) on captive white-tailed deer (*Odocoileus virginianus*) at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA, during fall 2021.

Score	Immobilization Effect	Head Movement	Leg Movement	Vocalizations
0	Immediately falls and remains on ground	Locked position, no movement	Locked position	None
1	Falls and remains on ground	Unnatural position, mostly rigid, some movement	Labored kicking with tense muscles	Sporadic
2	Falls, but regains footing	Head upright, some rigidity, some movement	Rapid kicking, relaxed muscles	Half of exposure
3	Staggers	Head upright, little rigidity, continuous movement	Buckling knees	Majority of exposure
4	Able to Stand	Natural movement	Natural movement	Continuous

Table 3.3. Scoring chart to determine recovery of captive white-tailed deer (*Odocoileus virginianus*) after exposure to a conducted electrical weapon (CEW) for a 5-second exposure (n=10) and a 15-second exposure (n=10) at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA, during fall 2021.

Score	Immobilization Reversal Description
3	Extremely prolonged, takes more than 30 seconds to regain full body control
2	Prolonged, takes up to 30 seconds to regain full body control
1	Quickly regains body control, may stagger and fall
0	Quickly regains body control, calm and aware, slowly stands
-1	Some excitability, does not stand quickly, some leg and head movement
-2	Major excitability, stands quickly and walks, collapses repeatedly
-3	Extreme excitability, immediately stands and jumps, can run easily

Table 3.4. Mean level of alanine transaminase (ALT), aspartate aminotransferase (AST), bilirubin, blood urea nitrogen (BUN), creatine kinase (CK), creatinine, and lactate dehydrogenase (LDH) in captive white-tailed deer (*Odocoileus virginianus*) chemically immobilized and exposed to a conducted electrical weapon for 5 seconds (Chem5) or 15 seconds (Chem15) prior to exposure (day 0), 2- and 5-days post exposure at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA, during fall 2021.

	Chem 5					Chem 15				
	Day 0	Day 2	Day 5	F Score	P Value	Day 0	Day 2	Day 5	F Score	P Value
Measurement (unit)	Mean (SE)	Mean (SE)	Mean (SE)			Mean (SE)	Mean (SE)	Mean (SE)		
ALT (U/L)	36 (5.9)	49 (5.4)	42 (5.9)	$F_{2,12}$=1.40	0.28	30 (4.2)	41 (3.1)	37 (4.2)	$F_{2,12}$=1.94	0.19
AST (U/L)	92 (9.8)	275 (36.0)	178 (20.6)	$F_{2,12}$=13.86	<0.01	134 (30.6)	320 (30.8)	256 (43.0)	$F_{2,12}$=7.15	<0.01
Bilirubin (mg/dL)	0.7 (0.2)	0.8 (0.2)	0.6 (0.1)	$F_{2,12}$=0.50	0.62	0.6 (0.1)	1.0 (0.4)	0.6 (0.1)	$F_{2,12}$=0.46	0.64
BUN (mg/dL)	28 (1.6)	25 (2.0)	26 (3.3)	$F_{2,12}$=0.64	0.54	30 (1.7)	25 (2.0)	26 (4.2)	$F_{2,12}$=0.76	0.49
CK (U/L)	278 (64.2)	1261 (123.3)	239 (45.3)	$F_{2,12}$=47.03	<0.01	188 (38.0)	1142 (212.0)	232 (37.7)	$F_{2,12}$=18.19	<0.01
Creatinine (mg/dL)	1.3 (0.1)	1.3 (0.1)	1.2 (0.1)	$F_{2,12}$=0.19	0.83	1.3 (0.1)	1.2 (0.1)	1.2 (0.1)	$F_{2,12}$=0.27	0.77
LDH	510 (130.4)	488 (116.5)	453 (110.8)	$F_{2,12}$=0.06	0.94	670 (139.7)	677 (141.1)	600 (134.7)	$F_{2,12}$=0.10	0.91

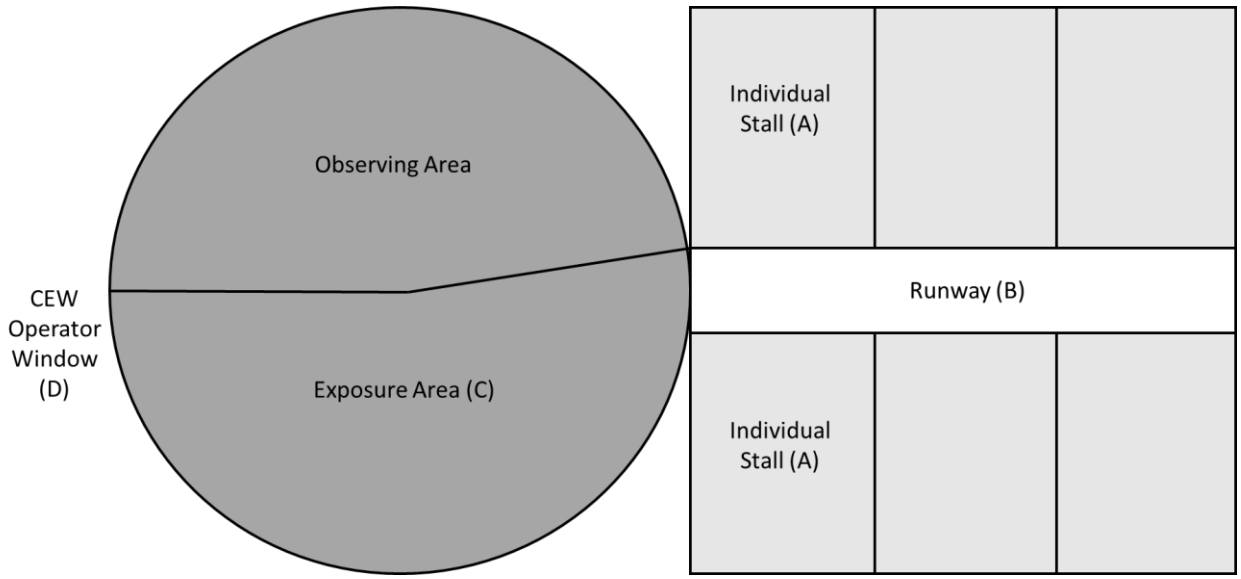
Table 3.5. Feed consumption rates (kg/day/deer) of deer chemically immobilized and exposed to a conducted electrical weapon (CEW) for 5 seconds (Chem5, n=5), chemically immobilized and exposed to a CEW for 15 seconds (Chem 15, n=5), only exposed to a CEW for 5 seconds (CEW5, n=10), only exposed to a CEW for 15 seconds (CEW15, n=10), and a control (n=10) while group-housed in outdoor paddocks or housed in individual barn stalls before and after treatment.

Treatment	Location	Before Treatment (SE)	After Treatment (SE)	F Score	P Value
Chem5	Paddock	0.83 (0.10)	0.76 (0.09)	$F_{1,16}=0.24$	0.63
Chem5	Stall	0.73 (0.10)	0.82 (0.17)	$F_{1,33}=0.19$	0.67
Chem15	Paddock	0.68 (0.12)	0.64 (0.08)	$F_{1,14}=0.10$	0.76
Chem15	Stall	0.74 (0.09)	0.82 (0.14)	$F_{1,37}=0.23$	0.63
CEW5	Paddock	1.25 (0.10)	1.24 (0.09)	$F_{1,17}=0.00$	0.96
CEW5	Stall	0.68 (0.08)	0.76 (0.07)	$F_{1,84}=0.58$	0.45
CEW15	Paddock	1.10 (0.14)	0.83 (0.06)	$F_{1,13}=4.07$	0.06
CEW15	Stall	0.71 (0.11)	1.07 (0.08)	$F_{1,77}=7.80$	0.01
Control	Paddock	1.33 (0.16)	1.63 (0.10)	$F_{1,16}=2.71$	0.12
Control	Stall	1.20 (0.07)	1.31 (0.08)	$F_{1,88}=1.10$	0.30

Table 3.6. Behavioral differences of deer within treatments chemically immobilized and exposed to a conducted electrical weapon (CEW) for 5 seconds (Chem5, n=5), chemically immobilized and exposed to a CEW for 15 seconds (Chem 15, n=5), only exposed to a CEW for 5 seconds (CEW5, n=10), only exposed to a CEW for 15 seconds (CEW15, n=10), and a control (n=10) during the 30-day observation period, based on mean estimates from the hypothesis driven generalized linear mixed model conducted at Whitehall Deer Research Facility in Athens, GA, USA, during fall 2021. Shown are regression coefficients (β), standard error (SE), *t*-scores, and *P*-values.

Behavior	**Predictor**	***β***	**SE**	***t***	***P***
Calm	Control	0.93	0.03	32.37	$<0.002^{-13}$
	Chem5	-0.06	0.05	-1.25	0.222
	Chem15	0.00	0.05	0.01	0.994
	CEW5	-0.06	0.04	-1.60	0.118
	CEW15	-0.03	0.04	-0.87	0.389
	Day	0.00	0.00	1.23	0.218
Alert	Control	0.08	0.03	2.58	0.013
	Chem5	0.06	0.05	1.21	0.233
	Chem15	-0.00	0.05	-0.01	0.994
	CEW5	0.06	0.04	1.47	0.150
	CEW15	0.02	0.04	0.59	0.557
	Day	-0.00	0.00	-1.48	0.140

A


Observing Area
Exposure Area (C)
CEW Operator Window (D)
Individual Stall (A)
Runway (B)
Individual Stall (A)

B

C

D

E

Figure 3.1. Graphics of areas used at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA including: A) overhead diagram of the barn, B) individual barn stalls used to house deer for behavioral observations, C) runway used to move deer into the exposure area, D) CEW exposure area, and E) the CEW operator window and small observer windows.

Figure 3.2. Laser indicators of estimated probe placement produced by the CEW for targeting multiple hemispheres to produce complete immobilization in captive white-tailed deer (*Odocoileus virginianus*) at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA during spring 2021.

Figure 3.3. Laser indicators of estimated probe placement produced by the CEW for targeting a single hemisphere to produce partial immobilization in captive white-tailed deer (*Odocoileus virginianus*) at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA during spring 2021.

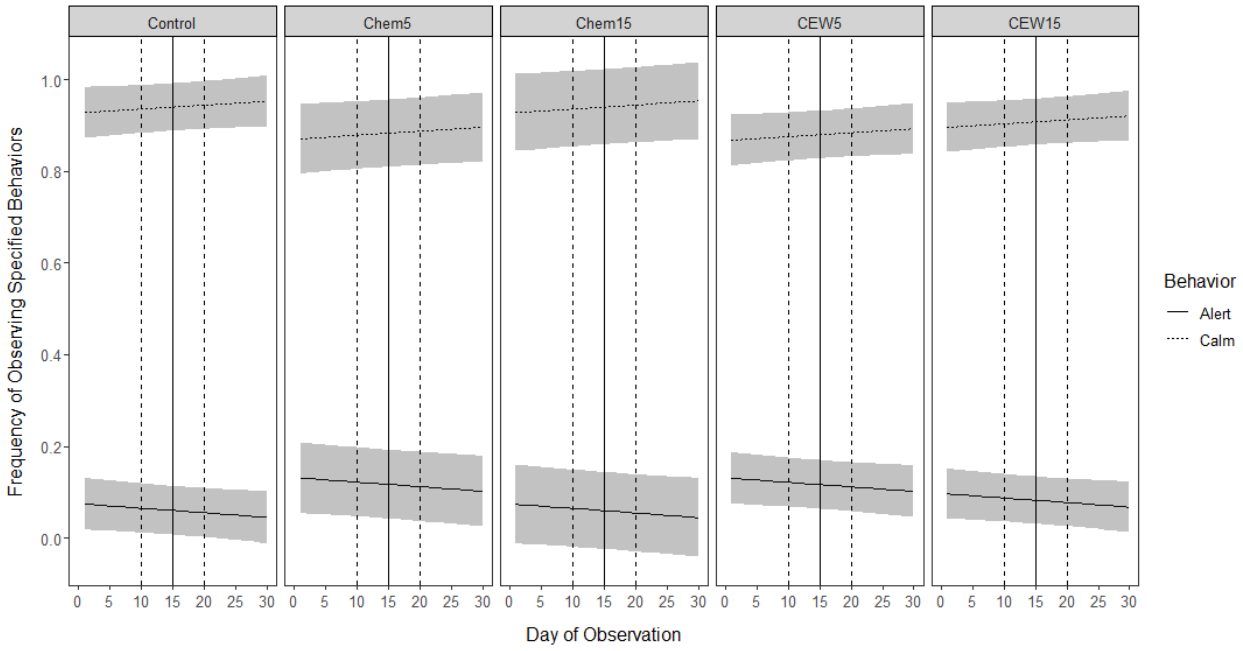


Figure 3.4. Mean frequency of observing calm and alert behaviors in captive white-tailed deer (*Odocoileus virginianus*) across the 30-day observation period for deer in treatments chemically immobilized and exposed to a conducted electrical weapon (CEW) for 5-seconds (Chem5, n=5), chemically immobilized and exposed to a CEW for 15-seconds (Chem15, n=5), only 5-second exposure to a CEW (CEW5, n=10), only 15-second exposure to a CEW (CEW15, n=10), and a control (n=10) at the Whitehall Captive Deer Research Facility, Athens, Georgia, USA during spring 2021. Dashed lines indicate when deer were moved between outdoor paddocks and isolated barn stalls. The solid line indicates day of treatment.

CHAPTER 4

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

CONCLUSIONS

Conclusions drawn from the results of these studies include:

Chapter 2 – Immobilization Efficacy of Butorphanol-Azaperone-Medetomidine (BAM) and Nalbuphine-Medetomidine-Azaperone (NalMed-A) in Captive White-tailed Deer

1) Doses of 1.5 mL BAM, 1.5 mL and 2.0 mL NalMed-A provided adequate immobilization for deer, demonstrated by relatively short times to immobilization stages and the lack of response to stimulus during the immobilization period.
2) Physiological effects of deer in all three treatments were similar and detrimental effects were controlled by standard immobilization safety measures (e.g., cool water enema, supplemental oxygen).
3) Behavioral changes did not occur for deer in any of the three treatments, from before to after treatment, indicating that the stress of chemical immobilization does not alter the behavior of captured deer.
4) Feed consumption rates of deer did not change in any of the three treatments, from before to after treatment, indicating that chemical immobilization should not affect the nutritional status of captured deer.
5) I recommend additional research on the use of BAM and NalMed-A in wild populations of white-tailed deer not only for immobilization effectiveness, but to determine physiological effects on deer not routinely exposed to humans.

Chapter 3 – Immobilization Efficacy of Conducted Electrical Weapons (CEW) in Captive White-tailed deer

1) Conducted electrical weapons quickly, effectively, and safely immobilize deer for an exposure period ≤15 seconds.
2) Exposure to a CEW produced some short-term physiological effects (i.e., muscle damage), however within 5 days of being exposed to the CEW physiological parameters returned to baseline measurements.
3) Changes in behaviors from before to after CEW exposure did not occur indicating that stress experienced during the treatment did not have lasting effects on deer.
4) Direct handling of deer during the CEW exposure would be difficult because personnel involved in handling have a high likelihood of being exposed to the CEW and muscle rigidity in the deer caused by the CEW would make it difficult for personnel to move the deer's limbs without causing injury.
5) Non-direct handling of deer (e.g., placing netting over deer) during the CEW exposure may decrease the likelihood of personnel being exposed to the CEW and direct handling of the deer could occur after CEW exposure ceased.
6) I recommend that if CEW usage does occur on wildlife that data describing the exposure should be recorded and, if possible, the animal should be monitored after exposure to gain field evidence of effective CEW usage.

MANAGEMENT IMPLICATIONS

Chapter 2 – Immobilization Efficacy of Butorphanol-Azaperone-Medetomidine (BAM) and Nalbuphine-Medetomidine-Azaperone (NalMed-A) in Captive White-tailed Deer

1) Use of NalMed-A can be used to effectively immobilize white-tailed deer in situations where BAM has commonly been used in the past.
2) Federal regulations involved with the use BAM as a schedule IV drug do not apply to NalMed-A, which is not a federally scheduled drug, allowing for more agency personnel to use, transport and store NalMed-A.
3) Physiological effects presented by the use of BAM and NalMed-A are similar, so focus can be turned to capture techniques (e.g., free darting, Clover trapping) to reduce overall stress effects of capture.

Chapter 3 – Immobilization Efficacy of Conducted Electrical Weapons (CEW) in Captive White-tailed deer

1) Use of CEWs can be used to mitigate or deter human-deer interactions and wildlife emergencies where other techniques may not be suitable.
2) Policies and trainings should be implemented by agencies to ensure safety of personnel and wildlife involved along with scientific backing for the need to use CEWs on wildlife.
3) Sick or injured deer in urban areas commonly need to be euthanized, but euthanasia by gunshot can be dangerous, especially in urban areas. Use of a CEW would allow for personnel to approach the deer during exposure and safely euthanize the deer by shooting into the ground.
4) Wildlife emergencies (e.g., deer stuck in fence, entry into building) and habituated deer are potential scenarios where a CEW could be used to resolve the emergency or deter deer from interacting directly with humans.