# An Evaluation of Vitamin E and Selenium as a Treatment for Capture Myopathy in Rio Grande Wild Turkeys

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*Abstract:* Capture and relocation is commonly used to reintroduce Rio Grande wild turkeys (*Meleagris gallopavo intermedia*). However, isotonic muscle contraction during the capture and restraint process reduces blood flow to muscles and may induce the stress related disease, capture myopathy. The goal of this study was to determine if intramuscular injections of vitamin E and selenium could be an effective treatment for capture myopathy. Survival rates and enzyme levels did not differ between the control and treatment group. Results suggest that vitamin E and selenium injections do not significantly improve survival of wild turkeys when trapped and relocated under conditions experienced in this study. Factors such as handling time relative to rate of enzyme secretion, trapping techniques employed, and possible nutrient deficiencies may have influenced the results of this study.

*Key words:* capture myopathy, *Meleagris gallopavo intermedia*, Rio Grande wild turkey, creatine kinase, aspartate aminotransferase

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Wildlife management practices often include capture and handling of wild animals for various reasons including research and relocation (Cromwell et al. 1999, Terhune et al. 2006). However, isotonic muscle contractions during the capture and restraint process can reduce blood flow and cause lactic acid build-up in the muscles. This may induce the stress-related disease, capture myopathy (Hulland 1993, Nicholson et al. 2000). The increase in muscle exertion may cause the cardiac and skeletal muscles to scar. Resulting muscle damage may cause immediate mortality or contribute to death several weeks after capture (Dabbert and Powell 1993, Abbott et al. 2005). Affected animals may be slower to escape due to pain or inability to ambulate normally and therefore are more susceptible to predation (Abbott et al. 2005). Cox and Afton (1998) found that flight quality and, subsequently, survival rate declined as handling times of captured northern pintails (*Anas acuta*) increased. Some affected animals may experience partial or complete posterior paresis or paralysis (Beringer et al. 1996).

Accurate and rapid diagnosis of myopathy in captured animals would be beneficial in identifying affected individuals. Visual examination of muscle tissue for damage has been used (Spraker et al. 1987, Davidson and Nettles 1997), but is not feasible for diagnosis in live animals. Evidence of indicator enzymes in blood samples may be a practical alternative. Increased muscle contractions and lactic acid build-up during the capture process result in tissue cell necrosis, causing the release of enzymes creatine kinase (CPK) and aspartate aminotransferase (AST) (Bollinger et al. 1989). Therefore, high levels of these enzymes in serum have been suggested to be an index of muscle damage in mammals (Chalmers and Barrett 1982) and birds (Franson et al. 1985). Dabbert and Powell (1993) and Bollinger et al. (1989) found higher CPK and AST levels in mallards (*Anas platyrhyncos*) that were captured using methods that resulted in more struggling. Creatine kinase activity in domestic turkeys has been found to be extremely sensitive to stress and an indicator of mortality risk in wild turkeys (*Meleagris gallopavo*) (Tripp and Schmitz 1982, Nicholson et al. 2000). Mueller (1999) also evaluated the relationship between CPK levels and survival rate of northern bobwhites and found that the probability of survival to 16 weeks decreased by 14% with each 1,000 u/L increase of plasma CPK.

Currently there are no reliable prophylactic measures or treatments for capture myopathy in wild animals. Prior studies have suggested that animals deficient in vitamin E or selenium are more susceptible to some form of myopathy (Fleming et al. 1977, Peplowski et al. 1981, Hansen et al. 1993). Vitamin E and selenium are beneficial antioxidants that speed recovery and protect muscles from damaging impacts that free radicals produce during exhaustive exercise

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(Vina et al. 2000). This form of treatment has been used to treat myopathies in avian species and domestic livestock (Whanger et al. 1976, Businga et al. 2007). Abbott et al. (2005) examined this treatment in northern bobwhites and found that survival to 45 days was 29% greater in treated individuals than untreated. However, their sample size was insufficient to verify results statistically.

Capture myopathy has been shown to affect the survival of captured wild turkeys (Spraker et al. 1987, Nicholson et al. 2000), thus potential treatments should be evaluated. Spraker et al. (1987) found that wild turkeys are highly susceptible to this disease. Therefore, this species should be a good model to test the effects of vitamin E and selenium as a preventative/treatment for capture myopathy in captured wild animals.

Wild turkeys are captured annually throughout Texas for research and relocation efforts (Feuerbacher et al. 2005). However, some individuals die during or soon after these capture events. These deaths are frequently attributed to natural causes, but may ultimately be due to the effects of capture myopathy (Abbott et al. 2005). The effects of this condition are often overlooked during capture events and may bias research results or impair relocation efforts. The goal of this study was to determine whether a single intramuscular injection with vitamin E and selenium can be used as a treatment for muscle damage resulting from capture myopathy in captured Rio Grande wild turkey hens (*Meleagris gallopavo intermedia*). The objectives of this study were to 1) compare the survival rate of treated and untreated wild turkeys 14 days after capture and relocation, 2) examine the relationship between the survival rate and CPK/AST blood enzyme levels, and 3) compare evidence of muscle damage using indicator enzymes of treated and untreated wild turkeys after 48 hours in captivity.

## Study Site

To assess our objectives, we sought to simulate common wild turkey capture and relocation efforts. We selected multiple capture and release sites in Texas for this study. Capture sites were located on the Alex Fambro Ranch (3,237 ha) in Erath County and the Comanche Star Ranch (1,700 ha) in Comanche County, both in the Cross Timbers ecoregion; and on the Concho Creek Ranch (902 ha) in Concho County and the Colbert Ranch (259 ha) in Childress County, both in the Central Great Plains ecoregion (Griffith et al. 2007). Release sites were located on the Navarro Mills Lake property (2,052 ha) in Navarro County, the Buena Vista Ranch (3,440 ha) in Cameron County, the Alex Fambro Ranch, and the Comanche Star Ranch. The Navarro Mills Lake property is located in the Texas Blackland Prairies ecoregion and is maintained by the U.S. Army Corps of Engineers. The Buena Vista Ranch is located in the Western Gulf Coastal Plain ecoregion (Griffith et al. 2007).

## Methods

### Capture Techniques

Turkeys were captured during early January 2007, 2008, and 2009. Turkeys were captured using rocket nets (Schemnitz 2005), walk-in funnel traps (Davis 1994), and air cannon nets. The air cannon net is a modified version of a black powder-charged rocket net (Silvy and Robel 1968) and uses compressed air to propel weights attached to the net. Each trap site was selected near roost sites, travel corridors, or areas with known turkey activity. Prebaiting (Davis 1994) began in mid December at each selected trap site. Milo and cracked corn were used as bait in elevated buckets with holes in the side. Remote cameras were used to determine presence and flock size at bait sites. Traps were constructed at selected sites in late December. During late winter 2007 and 2008, walk-in funnel traps were used at four sites on the Alex Fambro Ranch. During late winter 2009, walk-in funnel traps were used at four sites on the Comanche Star Ranch, a rocket net was used at one site on the Colbert Ranch, and two walk-in funnel traps and one cannon net was used at three sites on the Concho Creek Ranch. Trapping began in early January.

Funnel traps were set the evening prior to the day of trapping by attaching the funnel. They were checked once between 0800 to 1000 hours. If there were no captures, they were checked twice more throughout the day. The rocket and cannon nets were set up at baited sites one week prior to trapping. On the morning of each trap day, the bait bucket was removed and milo or cracked corn was spread near the net.

A tarp was placed over the funnel traps once birds were captured to keep them calm (Peterson et al. 2003). A blanket was placed over birds trapped in the nets until removed from the net. After removal from the trap or net, birds were hooded and temporarily placed in a National Wild Turkey Federation (NWTF) box until they could be handled (Cardoza et al. undated). Transport boxes were silicon-laced cardboard boxes with holes to allow for ventilation (outer dimension, 53.3 x 30.5 x 63.5 cm).

### Experimental Design

After capture, the body weight, gender, and age of each bird was determined and recorded, and each bird was banded with a Texas Parks and Wildlife (TPWD) numbered leg band. Each bird was randomly-assigned to either a treatment or a control group. Birds in the treatment group received a 0.06-ml/kg intramuscular injection of vitamin E (3 mg/kg as d-alpha tocopherol acetate) and selenium (0.06-mg/kg as sodium selenite) (BO-SE Selenium, manufactured by Schering-Plough Animal Health Corporation, Union, New Jersey) dissolved in sterile saline (Abbott et al. 2005). Birds in the control group received a 0.06-ml/kg intramuscular injection

of sterile saline. Dosages were derived from calculations made by Abbott (et al. 2005).

Turkey hens captured at the Alex Fambro Ranch, the Comanche Star Ranch, and the Concho Creek Ranch were fitted with backpack style radio transmitters (ATS A1540, 73 grams; Advanced Telemetry Systems, Isanti, Minnesota). Radio transmitters were attached to the birds with bungee cords, a modified technique from Norman et al. (undated). Radiomarked birds were located 14 days post-release to determine survival. Nicholson et al. (2000) found that individuals suffering from capture myopathy typically die within 14 days following capture. For birds that died within 14 days post-release, cause of death was recorded as predation and/or natural causes if discernible.

After birds were fitted with radio transmitters, a 1-ml (post-capture) blood sample was collected from the cutaneous ulnar vein. In 2007 and 2008, 4-ml serum separator vacutainers and 20-gauge needles were used. The blood collection technique was changed in 2009 to 1-ml syringes and 20-gauge needles to reduce hemolysis of the samples. Blood samples were centrifuged and serum was pipetted off the blood cells and placed into a micro container. Serum was refrigerated immediately after centrifugation.

After handling, birds were held in NWTF transport boxes until relocated. The birds trapped from the Alex Fambro Ranch and the Comanche Star Ranch were held in a quiet climate-controlled laboratory for 48 hours, a common time turkeys are held for relocation efforts. After the holding period, birds were transported to a designated release site on the property from which they were captured. The release site was at a different roost site to simulate relocation into a novel habitat. The birds trapped at the Concho Creek Ranch and the Colbert Ranch were held for approximately 40 hours and relocated to either the Navarro Mills Lake property or to the Buena Vista Ranch. Prior to release, a second (pre-release) blood sample was collected. Blood samples were submitted for laboratory analysis (Antech Diagnostics, Southhaven, Mississippi) of creatine kinase (CPK) and aspartate aminotransferase (AST) levels.

### Data Analysis

We examined mean two-week survival rates between birds in the treatment and control groups. Serum CPK and AST levels were used to indicate the amount of muscle damage occurring during the trapping process (Abbott et al. 2005). Due to the amount of variation between individuals, CPK and AST levels were base 10-tranformed (Guglielmo et al. 2001). Descriptive statistics and Student's *t*-tests were used to compare survival rates and enzyme levels between treatment groups.

We then compared control and treatment group enzyme log ratios (% increase) between the post-capture and the pre-release samples using a Student's *t*-test. This was an attempt to determine if treatment affected enzyme levels during the transportation process. Normality was assessed using the Kolmogorov-Smirnov test, histograms, and quantile plots (Q-Q plots). For all statistical tests used, $P$-values $< 0.05$ were considered to be significant (SPSS 2008).

## Results

We captured 56 Rio Grande wild turkey hens from 2007 to 2009. Three died from unknown causes during the trapping process. The data from these birds were discarded. Thus, the sample size for this study consisted of 53 hens. We excluded from the survival analysis individual hens that were not fitted with transmitters ($n = 17$) and 1 hen that experienced transmitter failure or detachment. We also excluded hens that were fitted with transmitters, but had unknown fates ($n = 3$). Sixteen hens from each group (control and treatment) were fitted with mortality radiotransmitters to examine survival at two weeks post-capture. Thirteen hens in the control group survived up to two weeks post-capture. Of the three that died, two hens died from natural causes and/or predation ($n = 2$) and one hen died during the transport process. Thirteen hens in the treatment group survived at least 14 days post-capture. Of the three that died, one died due to natural causes and/or predation and two died during the transport process. For both treatment and control groups, 81.3% survived 14 days post-release. Based on our data, there was no difference between survival rates of the control and treatment group.

We excluded birds that had enzyme levels that produced negative values between the post-capture and pre-release samples (Abbott et al. 2005), samples that were inadequate in volume, or samples that were found to be significant outliers as identified using box and quantile plots (SPSS 2008). Adequate blood samples were obtained from 39 birds. Many samples were affected by varying degrees of hemolysis. An analysis of variance (ANOVA) test was used to determine if hemolysis levels had an effect on the enzyme levels observed (log transformed). No differences were found in AST and CPK enzyme levels due to hemolysis. Therefore, samples affected by hemolysis were used in this study.

We did not obtain adequate pre-release enzyme samples from birds that died within two weeks of capture because blood samples were either inadequate in volume or found to be outliers. Therefore, the relationship between survival rates and enzyme levels of the birds in the treatment and control group could not be compared. However, results for enzyme levels from post-capture to pre-release were analyzed (log ratios). The AST log ratios for birds in the control ($n = 19$) and treatment ($n = 20$) group were

**Table 1.** Average aspartate amino transferase (AST) and creatine kinase (CPK) log ratios and percent increase for wild turkey hens in untreated (control) and treated groups trapped in north central Texas during early January 2007–2009.

| | Enzymes | |
|---|---|---|
| **Group** | **AST** | **CPK** |
| Control | | |
| *n* | 19 | 19 |
| Log ratio | 0.50 | 0.80 |
| SE | 0.05 | 0.09 |
| % Increase | 316 | 631 |
| Treatment | | |
| *n* | 20 | 20 |
| Log ratio | 0.55 | 0.88 |
| SE | 0.05 | 0.07 |
| % Increase | 355 | 759 |

0.50 (SE = ±0.05) and 0.55 (SE = ±0.05), respectively. When converted to a percentage increase, the enzyme level of birds in the control group increased by 316% and the enzyme level of birds in the treatment group increased by 355% between the time from post-capture to pre-release ($P = 0.47$). The CPK log ratios for birds in the control ($n = 19$) and treatment ($n = 20$) group were 0.80 (SE = ±0.09) and 0.88 (SE = ±0.07), respectively. When converted to a percentage increase, the enzyme level of birds in the control group increased by 631% and the enzyme level of birds in the treatment group increased by 759% between the time from post-capture to pre-release ($P = 0.49$) (Table 1).

## Discussion

Results of this study suggest that Rio Grande wild turkey hens injected with vitamin E and selenium did not have a significantly higher survival rate or decreased amount of muscle damage based on enzyme levels. However, other studies, using northern bobwhite and greater sandhill cranes (*Grus canadensis tabida*) treated with vitamin E/selenium, show increased survival rates after the capture event (Abbott et al. 2005, Businga et al. 2007). Businga (et al. 2007) also administered additional supportive care such as fluid therapy, anti-inflammatories, nutritional support, and physical therapy. It is important to note that small sample sizes for treatment and control groups may have contributed to the failure to detect any differences. Pollock et al. (1989) suggested that a minimum of 20 individuals per treatment group is necessary for satisfactory survival estimates.

We were unable to determine how treatment influenced muscle damage post-capture. The serum enzymes, AST and CPK, have been used to indicate severity of muscle trauma (Bollinger et al. 1989, Nicholson et al. 2000, Abbott et al. 2005). Results in our study are supported by Nicholson et al. (2000), in that all birds exhibited elevated enzyme levels indicative of muscle trauma. When mean AST and CPK enzyme log ratios were examined, there were no differences between birds in the control and treatment group. It remains unclear how treatment of vitamin E and selenium affected enzyme levels in our captured wild turkeys. Variability in enzyme levels between individuals played a significant role in the failure to detect differences between birds in the two groups (control and treated). Although we tried to minimize the amount of variation within enzyme levels by excluding values that were considered significant outliers, substantial variability still occurred. Abbott et al. (2005) also found it difficult to make such comparisons using AST and CPK enzyme levels because of the variability between each individual.

Other factors that may have influenced the results of this study were handling time relative to rate of enzyme secretion, trapping techniques employed, and possible nutrient deficiencies. Handling time for our study and other studies was based on the time between capture and blood collection of an individual (Bollinger et al. 1989, Dabbert and Powell 1993, Nicholson et al. 2000, Abbott et al. 2005). Nicholson et al. (2000) suggested that an average handling time of 74 minutes may not allow enough time for enzyme activity to become significantly elevated. Abbott et al. (2005) tried to alleviate this problem by standardizing when blood samples were taken (i.e., 3 hrs post-capture), but still had large amounts of variation in the enzyme levels between individuals. Other studies that have used AST and CPK as muscle damage indicators had average handling times ranging from 45–106 min (Bollinger et al. 1989; Dabbert and Powell 1993). In comparison to these studies, average handling times for this study were 43 and 84 min for walk-in funnel traps ($n = 15$) and rocket nets ($n = 30$), respectively. Businga et al. (2007) found that it took approximately three days post-capture for AST and CPK enzyme levels to peak in greater sandhill cranes. Enzyme levels began to decrease four days after capture. Based on these prior findings, this study might not have allowed enough time for enzyme activity to become elevated and accurately indicate muscle damage.

Capture technique influences were not considered during this study. Previous studies have suggested a relationship between capture technique and enzyme activity, survival rates, and the onset of capture myopathy in trapped birds (Spraker et al. 1987, Bollinger et al. 1989, Nicholson et al. 2000, Holfe et al. 2004). Spraker et al. (1987) found that turkeys caught by bait traps were less susceptible to capture myopthy than birds caught by drop nets. Bollinger et al. (1989) found that mallards (*Anas platyrhynchos*) trapped in walk-in funnel traps had lower enzyme levels than mallards trapped in

rocket nets. Toepfer et al. (1987) found that prairie grouse hens (*Tympanuchus cupido pinnatus*) trapped in walk-in funnel traps had a lower mortality rate than hens trapped in rocket nets. Based on personal observations and findings from these studies, it appears that funnel traps are less stressful on birds during the capture event. Therefore, use of multiple trapping methods during our study may have affected survival rates and enzyme levels.

Researchers also should consider nutrient deficiencies that may be present within a captured population. Fleming et al. (1977) suggested that woodchucks (*Marmota monax*) deficient in vitamin E were more susceptible to white muscle disease. Many authors have suggested supplementing vitamin E and selenium as a preventative against capture myopathy or white muscle disease (Fleming et al. 1977, Peplowski et al. 1981, Hansen et al. 1993). A limitation to this form of treatment is that one must understand individual animal requirements for vitamin E and selenium. Factors such as environmental stressors and availability in soil and plant species constantly influence vitamin E and selenium requirement and intake, respectively, in different species (Ullrey 1981, Fishbein 1983). It also is important to consider whether or not individuals are deficient in vitamin E or selenium. Based on the findings of Peplowski et al. (1981), the use of vitamin E and selenium as a form of treatment for wild turkeys affected by capture myopathy might not be effective because turkeys might already have adequate amounts of vitamin E and selenium.

## Management Implications

Our results suggest that vitamin E and selenium did not significantly improve survival rates or decrease enzyme activity in turkeys affected by capture myopathy. Due to the amount of variability in enzyme levels, further research and modification of the experimental design is recommended to determine the rate at which muscle trauma indicator enzymes are secreted into the blood stream. We suggest collecting muscle biopsies to determine the amount of muscle trauma caused in turkeys during a capture event. To decrease the amount of stress on birds during capture, we recommend that walk-in funnel traps be used rather than rocket or cannon nets. Adequate personnel should be present to reduce handling times of captured turkeys. Vitamin E and selenium availability should also be considered when trapping wild turkeys. It might be beneficial to supplement these nutrients at bait sites prior to trapping.

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