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Source: Journal of Wildlife Diseases, 29(2) : 304-309

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-29.2.304

Journal of Wildlife Diseases, 29(2), 1993, pp. 304–309

SERUM ENZYMES AS INDICATORS OF CAPTURE MYOPATHY IN MALLARDS (*ANAS PLATYRHYNCHOS*)

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ABSTRACT: Serum concentrations of the enzymes creatine kinase (CK) and aspartate aminotransferase (AST) were measured in captive and wild mallards (*Anas platyrhynchos*) as indicators of muscle damage. Baseline values for both enzymes were determined from six captive male mallards. During winter 1990 to 1991, six diets (including controls) representative of food available in the Mississippi alluvial valley were fed to captive female mallards housed in an outdoor aviary at the White River National Wildlife Refuge, Arkansas County, Arkansas (USA). Controlled handling of penned mallards resulted in elevated serum CK ($\bar{x}$ = 1,352 IU/liter; SD = 1,212) and AST ($\bar{x}$ = 101 IU/liter; SD = 95) concentrations consistent with myopathies. These serum enzyme elevations were not affected ($P > 0.3$) by dietary selenium concentrations in the six diets or by energy malnutrition suffered by birds fed soybeans. Capture of wild mallards with an entanglement type rocket net resulted in serum CK and AST concentrations ($\bar{x}$ = 12,035 and 330 IU/liter; SD = 8,125 and 171, respectively) that were higher ($P < 0.001$) than those reported after capture with an enveloping type rocket net. Baseline values, controlled handling values, and entanglement rocket net values for serum CK and AST all differed ($P < 0.0001$).

Key words: Capture myopathy, creatine kinase, aspartate aminotransferase, mallard, normal values, rocket net, *Anas platyrhynchos*.

INTRODUCTION

Waterfowl are routinely captured and transported. Capture and transport can affect physiological status of birds and influence postcapture survival. Capture myopathy is an acute degeneration of muscle resulting from intense muscular exertion and trauma caused by restraint and transport (Hulland, 1985). Extreme metabolic acidosis resulting from lactic acid buildup (primarily in muscle tissue) produces the clinical signs of capture myopathy, such as muscle stiffness, paralysis, weakness, and locomotive abnormalities. Subsequent increased permeability of cell membranes and cell lysis result in release of intracellular enzymes such as creatine kinase (CK) and aspartate aminotransferase (AST) (Bollinger et al., 1989). If capture myopathy does not result in acute death the muscle lesions may repair; yet skeletal muscle and myocardial scars may remain and contribute to death weeks after capture (Hulland, 1985). Because death from capture myopathy may be delayed or indirect, it may bias waterfowl population studies which assume "natural" mortality among individuals released after capture (Bollinger et al., 1989).

Capture myopathy has been noted in many wild species (Windingstad et al., 1983; Spraker et al., 1987; Carpenter et al., 1991). In mallards (*Anas platyrhynchos*), Bollinger et al. (1989) reported serum CK and AST concentrations were elevated after capture with bait traps, decoy traps, and enveloping rocket nets during summer. He also found that exertion time against a net was positively related to CK and AST concentrations. Identification of the causes of high variability in muscle damage among and within capture methods may allow investigators to avoid capture situations which increase capture myopathy.

The study of capture myopathy and subsequent survival probability is complicated because nutritional myopathy and lead poisoning also cause increased serum CK concentrations (Lewandowski et al.,

1986). Nutritional myopathy is caused by deficiency of vitamin E and selenium, nutrients required to protect cells from free radicals. Starvation or poor food quality can cause vitamin E and selenium deficiencies that result in the same clinical signs and enzyme release as capture myopathy (Hulland, 1985). Based on the patterns of food availability and food choice in some wintering mallards, the mallards may suffer nutritional deficiencies (Jorde et al., 1983; Loesch and Kaminski, 1989). Agricultural grain diets are rich sources of carbohydrates for energy, but are deficient in amino acids, vitamins, and minerals (Baldassarre et al., 1983). Thus, some mallards consuming monotypic diets of agricultural grains could be deficient in vitamin E or selenium. Such dietary deficiencies could exacerbate conditions which result in muscular damage during capture.

Our objectives were to determine values for serum AST and CK concentrations in unstressed and well fed mallards, determine the effects of diet and a controlled handling routine on the serum AST and CK values of mallards, and measure CK and AST concentrations of mallards after capture during winter with an entanglement type rocket net.

MATERIALS AND METHODS

Experiment 1

To establish baseline values for AST and CK, 10 male mallards were obtained from Oakridge Gamebird Farm (Gravette, Arkansas, USA) and maintained in outdoor pens (1.8 × 2.4 m), at five birds per pen (two replicates). Ducks were fed poultry layer feed (SFA Cooperative, Fayetteville, Arkansas) from 25 June until 3 July 1991 and then changed to a whole corn diet. Food, water, and grit (stream pebbles) were provided ad libitum. Handling each week included capture with a dipnet, placement in a plastic poultry cage, and individual weight determination. All birds were handled similarly to standardize bird to bird handling stress factors (Trust et al., 1990).

Ducks were bled 31 July, within 15 min of capture, via brachial venipuncture into evacuated glass tubes (Monoject brand, Sherwood Medical, St. Louis, Missouri, USA) and blood was allowed to clot at 20 to 23 C. Clots were centrifuged for 10 min at approximately 2,500 rpm and serum was frozen at −20 C until assayed. Hemolyzed samples were discarded. Creatine kinase and AST concentrations were determined by the Oklahoma State University Veterinary Special Medicine Lab using a Roche Cobas Mira Chemistry Analyzer (Hoffmann-LaRoche, Nutley, New Jersey, USA) and Sigma reagents (Sigma Chemical, St. Louis, Missouri). A Mann-Whitney *U*-test (Norusis, 1990) was used to analyze differences between the two replicates.

Experiment 2

We tested the effects of food quality on CK and AST concentrations from December to February at White River National Wildlife Refuge, Arkansas County, Arkansas. Sixty mature mallard females were obtained from Oakridge Gamebird Farm and were housed in outdoor pens (1.8 × 3 m) providing the same ambient weather conditions experienced by wild birds. Grit (stream pebbles) and water were provided ad libitum in 1.2-m-diameter plastic swimming pools.

There were six diet treatments, ten birds per treatment. Two replicates were created for each treatment by maintaining half the birds (five birds) in different pens. Corresponding diet treatment replicates were randomized and interspersed (Hurlbert, 1984). Diet groups included a pelleted high protein (23%) balanced diet, and a pelleted low protein (7%) diet (University of Arkansas Feed Mill, Fayetteville, Arkansas); corn; rice; soybean; and a natural diet, composed of one third (by weight) each of crushed willow oak acorns (*Quercus phellos*), Pennsylvania smartweed seeds (*Polygonum pensylvanicum*), and Japanese millet seeds (*Echinochloa crus-galli*) based on food habits data of mallards foraging in the Mississippi alluvial valley (Heitmeyer, 1985). Selenium content of the grains and seeds was determined by the Oklahoma Animal Disease Diagnostic Laboratory (Stillwater, Oklahoma, USA) using the Zeeman atomic absorption graphite furnace technique (Edwards and Blackburn, 1986) and a Zeeman atomic absorption spectrometer (Perkin-Elmer Corporation, Norwalk, Connecticut, USA). All groups were maintained for 10 days on the pelleted rations during a pen acclimation period and then changed to their respective diets. The mean of the daily feed consumption of the pelleted rations by all replicates during this acclimation period determined the daily diet allowance of each replicate. This allowance was adjusted approximately biweekly to equal the mean daily consumption (for two sampling days) of ad libitum feeding by the two high protein balanced replicates. This feeding scheme in-

volved restricted feed quantity as well as reduced quality in some diet treatments. Wild birds consuming lower quality food items may be able to increase their food intake to meet minimum daily nutrient requirements (Perry et al., 1986a). Thus our experiment may represent more limiting conditions than encountered by wild birds.

Handling included capture with a dipnet, temporary confinement to a plastic poultry cage, transfer from the poultry cage to a wire mesh transport cage normally used to hold wild waterfowl, transportation 4.8 km in a truck, and blood sampling approximately 45 min postcapture via the occipital sinus (Campbell, 1988). All birds were sampled after 9 wk on their respective diet treatments. One replicate (pen) of each diet group was sampled on one of two subsequent days. Birds were handled five times (biweekly) prior to blood sampling (steps 1 and 2) providing some acclimation to this portion of the handling regimen. The CK and AST concentrations were determined by the Washington Regional Medical Hospital Lab using a Beckman CX4 chemistry analyzer and Beckman reagents (Beckman Instruments, Inc., Fullerton, California, USA). A Kruskal-Wallis *H*-test (Norusis, 1990) was used to test for differences between replicates and diet groups.

Experiment 3

Thirty free-ranging mallards (17 males, 13 females) were captured with an entanglement type rocket net on 21 November 1990. This net was constructed of 6.35 cm mesh. Handling included impact of the net on the birds, bird removal from the net by hand within 20 minutes, bird transfer to a wire holding cage, cage transportation approximately 12.9 km, and blood sampling via medial metatarsal vein (Campbell, 1988). All birds were euthanized with carbon dioxide in an enclosed chamber (Gullet, 1987) following blood sampling. Blood sampling was initiated approximately 45 min postcapture and serum CK and AST concentrations were determined by the same methods as in experiment 2. Thus, this experiment built on experiment 2 by performing the same comparable trauma from handling but adding the additional effects of a capture device. A Mann-Whitney *U*-test (Norusis, 1990) was used to analyze differences in serum CK and AST concentration between sexes. Coefficients of variation (CV) (Ott, 1988) were calculated for the pen and wild birds and for both assays.

RESULTS

The two replicates from experiment one did not differ for either AST or CK ($P > 0.5$); consequently they were pooled for further comparisons (Table 1). Selenium concentrations of the diets fed in experiment 2 ranged from 0.418 to 0.875 ppm (Table 2). Neither AST nor CK differed among diet treatments or any of the replicates in experiment 2 ($P > 0.3$). Thus, these values also were pooled (Table 1). Serum CK and AST concentrations did not differ ($P \geq 0.3913$) between sexes captured in the entanglement rocket net, hence we consider subsequent comparisons between sexes to be valid. In addition, serum AST and CK values from birds captured by our entanglement net differed from birds in experiments 1 and 2 ($P < 0.0001$; Table 1). Birds captured in entanglement rocket nets exhibited less variability (AST CV = 0.52, CK CV = 0.68) for both serum CK and AST concentrations than birds subjected to controlled handling (AST CV = 0.93, CK CV = 0.90).

DISCUSSION

Serum CK and AST baseline values for male mallards in our study are close to or lower than the values previously reported for mallards and other waterfowl species (Franson, 1982; Perry et al., 1986b; Spano et al., 1987; Fairbrother et al., 1990). Small differences in concentration between our data and those reported by others for mallards probably reflect variations in assay techniques, restraint methods, and time after initial restraint. Although we used different sexes and blood sampling sites, these variables should not affect the serum enzymes measured (Zimmerman and Dhillon, 1985; Fairbrother et al., 1990).

All grain and seed diets tested contained at least two times higher selenium levels than are apparently sufficient for mallards (Hoffman and Heinz, 1988; Anonymous, 1987; Hoffman et al., 1989). Accordingly, serum enzyme levels consistent with muscular damage following the controlled handling were not affected by the selenium concentrations of diets fed to mallards in this study. Myopathy, however, also may be induced by vitamin E deficiency (Hul-

TABLE 1. Comparison of the serum concentrations of creatine kinase (CK) and aspartate aminotransferase (AST) of mallards captured with minimal handling and minimal time for enzyme leakage (experiment 1), controlled handling with time for leakage (experiment 2), and with an entanglement rocket net (experiment 3) in Arkansas during summer 1990, and winter 1990 to 1991.

	Concentration of enzyme (IU/liter)					
	AST			CK		
Handling treatment	Sample size	$\bar{x}$	SD	Sample size	$\bar{x}$	SD
Experiment 1	6*	19	7	6*	225	52
Experiment 2	51*	101	95	51*	1,352	1,212
Experiment 3	30	330	171	30	12,035	8,125

* Sample is lower than number of birds used in each experiment because of sample hemolysis and bird mortality.

land, 1985). The vitamin E requirements of mallards is unknown (McDowell, 1989), but mule ducks require 11.4 IU of vitamin E per kg of feed (Shen, 1991). The vitamin E concentrations of the seeds and grains was not determined, but it is probable that the control diets contained adequate concentrations of both selenium and vitamin E. The two pelleted rations contained 13 IU of vitamin E per kg of feed supplied through a commercial vitamin premix (Dabbert, unpubl.). Some concentrations of dietary selenium above minimum requirements may prevent the complications of vitamin E deficiency; conversely, an excess of dietary vitamin E may compensate for a shortage of selenuim (Combs and Combs, 1984). Hence, from our data, it seems that the seed and grain diets fed in experiment 2 provided sufficient nutrients for similar antioxidant protection as the pelleted diets. Additionally, birds fed soybeans were catabolizing body fat and protein, as determined by serum chemistry (Dabbert, 1991). Thus, a negative energy balance also did not affect serum CK and AST concentrations following controlled handling. It is probable that mallards wintering in the Mississippi alluvial valley would encounter food items of comparable nutritional quality because grains used in this study were grown on or near

TABLE 2. Comparison of the selenium concentrations of the 6 diets fed to captive mallards at White River National Wildlife Refuge from December 1990 to February 1991.

Diet or constituent	Se (ppm)
Corn	0.418
Rice	0.875
Soybeans	0.710
Smartweed seeds	0.780
Japanese millet seeds	0.600
Willow oak acorns	0.796
23% protein control	0.427
7% protein control	0.501

the refuge. Some populations, however, may be subject to poor quality diets for >9 wk (Jorde et al., 1983).

The mean serum CK and AST values resulting from the controlled handling of mallards in experiment 2 are close to, and in some cases higher than, many of the values previously reported for mallards captured by bait trap, decoy trap, and rocket net (Bollinger et al., 1989). The effect of handling and exertion which results in serum CK levels of this magnitude on survival probability is unknown. But 10-wk-old turkeys had plasma CK values 30 times normal, following handling and rigorous exercise, and yet survived without apparent effects (Tripp and Schmitz, 1982). All birds used in experiment 2 survived the initial transport from the breeder facility to the study site (approximately 6 hr) and five dipnet captures (including restraint) without any observable complications consistent with capture myopathy in birds such as weakness, locomotive abnormalities, or paralysis.

Using a separate variances t-test (Ott, 1988), mean AST and CK concentrations of birds captured by the entanglement type rocket net were higher ($P < 0.001$) than mean concentrations of both enzymes reported 1 hr postcapture for birds caught with an enveloping type rocket net (Bollinger et al., 1989). Many variables such as season, bird physiology, and nutritional status could contribute to this difference. The most probable variable, however, is

the larger mesh size of the entanglement rocket net. Enveloping rocket nets used by Bollinger et al. (1989) generally were made from a much smaller mesh size (38 mm) (G. A. Wobeser, pers. comm.). Larger openings of the entanglement rocket net allow wings and necks to be held in unnatural positions, promoting more struggle and muscle trauma. Additionally, increasing serum CK values appear to be positively related to time spent struggling against a net (Bollinger et al., 1989). Birds caught in our entanglement rocket net struggled ≤20 min and exhibited a mean serum CK value of 12,035 IU/liter (Table 1). Five birds captured in Canada that struggled against an enveloping rocket net for approximately 26 min had a mean serum CK value of only 980 IU/liter (Bollinger et al., 1989).

Because there were higher serum CK and AST values among birds caught with the entanglement rocket net than birds hand-netted in experiment 2, rocket net capture probably adds considerable muscle trauma above general handling by humans. Handling trauma was comparable between experiment 2 and the entanglement rocket net capture. Thus the higher serum CK and AST values among the rocket net captured birds likely were caused by the effects of the rocket net. Based on the higher variability in the serum CK and AST values after controlled handling (experiment 2) than during rocket net capture, we believe that muscle damage in response to capture techniques varies greatly among individual mallards. During attempts to haze or catch waterfowl, some clearly were more easily coaxed or caught than others. It is probable, however, that the amount of muscle damage inflicted by a capture attempt also is highly affected by variables such as proximity to the capture device, bird size, and body position.

More experiments are needed to determine the ranges of CK and AST levels that are associated with capture myopathy and predict subsequent effects on mallard survival. Additionally, the factors which cause high variation in these enzyme levels within one capture attempt should be determined.

ACKNOWLEDGMENTS

We received invaluable help from L. Bisswanger, C. Burrell, T. Cunningham, J. Denman, C. J. Free, R. Gray, J. Harris, M. Hurdle, J. B. Lee, K. Litzenberger, and H. Sansing of White River National Wildlife Refuge and J. Jones. T. Moser helped with blood collection. P. Waldroup, University of Arkansas Department of Poultry Sciences, graciously provided the pelleted rations. Thanks also go to J. K. Ringelman and G. A. Wobeser who provided helpful comments on the manuscript. This study was partially funded by the Arkansas Game and Fish Commission.

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Received for publication 3 February 1992.