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EFFECTS OF MYOFIBROGRANULOMA ON SERUM CALCIUM LEVELS IN WALLEYE (STIZOSTEDION VITREUM)

Craig A. Shoemaker and Harry L. Holloway, Jr.

Abstract.—The effect of myofibrogranuloma (skeletal muscle degeneration) on serum calcium levels in spawning walleye (Stizostedion vitreum) was examined. Mean serum calcium levels for healthy male walleye (11.7 ± 1.5 mg/100 ml serum) were significantly lower (P < 0.05) than calcium levels for healthy female walleye (15.4 ± 1.8 mg/100 ml serum). Significant increases (P < 0.1) in serum calcium were seen between healthy male and myofibrogranuloma-diseased male walleye (13.6 ± 2.1 mg/100 ml serum) and between healthy and myofibrogranuloma-diseased female walleye (20.2 ± 3.7 mg/100 ml serum). Elevations seen in mean serum calcium levels suggest the muscle degeneration and subsequent granuloma formation in later stages of myofibrogranuloma have a significant effect on serum calcium.

Key words: calcium, walleye, myofibrogranuloma, colorimetric determination, Stizostedion vitreum.

Calcium (Ca) levels of osteichthyan extracellular fluids are regulated to a finer degree than those of more primitive fishes (Dacke 1979). Hormones from the pituitary, ultimobranchials, stannius corpuscles, and gonads affect osteichthyian Ca metabolism (Dacke 1979). Since Ca concentration is essential for cell membrane permeability to water, neuromuscular irritability and blood clotting, growth and enzyme reactions, it is less variable than other ions in serum (Urist and Van de Putte 1967). Myofibrogranuloma (MFG) is a unique form of skeletal muscle degeneration recognized only in adult walleye (Stizostedion vitreum; Mayes 1976, Economon 1978, Holloway and Smith 1982). This myopathy is characterized by profound alterations of the trunk musculature produced by extensive hypertrophy of the muscle fibers (Economon 1978). Holloway and Smith (1982) noted 2 degenerative processes. The 1st and most pronounced lesion consisted of coagulation necrosis of muscle fibers accompanied by an inflammatory response and the formation of granulomas (muscle tumors). The 2nd was noninflammatory and characterized by focal areas of acute myolysis. Mineralization or calcification was evident in the central portion of some fibers in the more granular stage of degeneration (Economon 1978). Kelly et al. (1987) showed an increase in muscle Ca associated with myofibrogranuloma (55 times normal muscle fibers). Histochemical staining indicated relatively more Ca-positive fibers in normal muscle of MFG-positive fish and MFG-positive tissue than in muscle from healthy walleye (Kelly et al. 1987). Holloway and Shoemaker (1993) used X-ray technology to demonstrate increased opacity, presumably due to concentrated Ca in MFG-diseased tissue. The cause of MFG is unknown. Fisheries managers and anglers are concerned about the disease because the involved muscle has a yellow color with sandy texture. Anglers discard these fish.

Adult osteichthyans (bony fishes) exhibit seasonal variations in plasma Ca levels associated with breeding cycles (Dacke 1979). Sex differences in plasma Ca levels of fish were first reported by Hess et al. (1928). Booke (1964) found differences in the Ca levels of spawning male and female brook trout (Salvelinus fontinalis). Hunn (1972) presented values of various fishes, including spawning male walleye, from the upper Mississippi River. Our objective was to establish Ca values for spawning male, female, and MFG-diseased walleye using a simple colorimetric procedure.

Materials and Methods

Spawning walleye were collected by gill and frame net from Merrit Reservoir, Nebraska (1991 and 1992), and Lake Sakakawea, North Dakota (1991). Only walleye greater than 500 mm total length were sampled to eliminate
killing smaller fish that would not show macroscopic signs of disease. Blood was sampled via cardiac puncture with heparinized 10-ml syringe and 21-gauge needle. Centrifugation was carried out within 24 h to minimize sample hemolysis. Serum was placed in dry ice for transport and stored at –80°C until analyzed. Heavily hemolyzed samples were discarded. Walleye were filleted and examined with unaided eye for MFG. MFG-diseased fish were determined by observing yellow-colored, sandytexured muscle.

The procedure for measuring serum Ca concentration was modified from Fales (1953) by Oser (1965). We used the latter protocol, but serum and reagent volumes were halved due to small sample volume. A 1% stock Ca standard solution was prepared by dissolving 2.497 g calcium carbonate in 6 N hydrochloric acid and evaporating on a steam bath to dryness. The residue (CaCl₂) was then dissolved in distilled water to make 100 ml. A Ca standard (0.1 mg/ml) was prepared by diluting 1 ml of the stock Ca to 100 ml with 1.4 N sodium chloride. A light-sensitive stock solution of murexide was prepared by dissolving 0.25 g ammonium purpurogallin in 5 ml distilled water and 25 ml 95% ethanol. The working murexide solution was prepared by adding stock murexide solution to 100 ml 0.05 N sodium chloride until an OD of 0.39–0.48 was reached at 620 nm. The titrant was prepared by mixing 18 volumes of the working murexide solution, 1 volume of 0.14 N sodium chloride, and 1 volume of stock disodium dihydrogen ethylene-diaminetetraacetic acid (EDTA) solution (0.005 M EDTA). Titrant was mixed prior to each set of determinations, kept in brown bottles, and used within 1.5 h.

The principle of the procedure as described by Oser (1965) is that the Ca in serum mixed with murexide forms a red-colored complex in equilibrium with free Ca ions. Titration of the Ca-murexide complex with EDTA chelates free Ca ions, causing the release of Ca ions from the complex. As the murexide ion: calcium murexide ratio increases, there is a shift in color from red to purple (endpoint). The procedure followed is described briefly: 0.25 ml of 0.14 N sodium chloride was placed in a blank cuvette, and equal amounts (0.25 ml) of serum and working Ca standard were placed in test and standard cuvettes, respectively. Then, 2.25 ml of working murexide and 0.5 ml of titrant were added to each cuvette and mixed. The spectrophotometer (Bausch and Lomb Spectronic 20) was set at 50% transmittance at 620 nm with the blank, and the test and standard cuvettes were then read. After this, 0.25 ml of titrant was added to each cuvette and the spectrophotometer reset at 50% transmittance with the blank, before reading the test and standard. This step (titration) was repeated and followed colorimetrically until the endpoint was reached (all serum Ca bound to EDTA). Calculations were then carried out using the following formula as described by Oser (1965) to determine serum Ca level:

\[
\text{Volume of titrant for serum} \times 10 = \text{mg Ca/100 ml serum}
\]

A Student’s t test compared mean serum Ca levels of healthy male and female walleye. Significance was determined at \( P < 0.05 \). Due to small sample size and differences between variances, healthy male walleye and MFG-positive male walleye as well as healthy female walleye and MFG-positive female walleye were compared by unequal variance t test (Sokal and Rohlf 1981). The critical significance level was \( P < 0.10 \) for tests between MFG-positive and healthy male and female walleye because of the low numbers of MFG-positive fish (6 males and 4 females).

RESULTS AND DISCUSSION

Selected serum Ca titration curves (standard, spawning female [NB201], spawning male [0066], MFG-positive spawning female [0043], and MFG-positive spawning male [0252]) are shown in Figure 1, and serum Ca concentrations are described in Table 1 for healthy and MFG-positive walleye. A significant difference was found between serum Ca levels of male and female walleye \( (P < 0.05) \). Serum Ca levels of male walleye differed significantly from mean serum Ca levels of MFG-positive male walleye \( (P < 0.10) \). A significant difference was found between serum Ca levels of healthy female walleye and MFG-positive female walleye \( (P < 0.10) \).

Serum Ca values were higher in this study than in others (Table 2). One explanation may be species differences. Hunn (1972) examined spawning male walleye from the upper Mississippi River and found serum Ca lower (9.52
mg/100 ml) than our mean value (11.68 mg/100 ml) for spawning male walleye. This may be a result of the blood-sampling procedure (caudal peduncle puncture vs. cardiac puncture), which is known to influence other blood parameters (e.g., serum enzyme levels; Hille 1982), or simply differences between river and reservoir fish. Low serum Ca levels in paddlefish (Polyodon spathula) suggest physiological hypocalcemia, and in this respect paddlefish show phylogenetic affinity to sturgeons (Grant et al. 1970). The low levels seem to support Urist and Van de Putte’s (1967) suggestion that hypocalcemia is related to the absence of a bony skeleton; however, gravid female white sturgeon (Acipenser transmontanus) exhibit increased serum Ca. Low serum Ca determined for channel catfish (Ictalurus punctatus; 9.2 mg/100 ml) may be a result of obtaining fish from culture ponds rather than from natural waters (Warner and Williams 1977).

Various techniques were used to determine serum Ca levels, which may have resulted in differences noted. Methods of Ca determination include automated methods (Warner and Williams 1977), colorimetric methods (Field et al. 1943, Shell 1961, Booke 1964), and atomic absorption spectrophotometry (Grant et al. 1970, Hunn 1972). The colorimetric method described by Falcs (1953) determined total serum Ca (ionic plus protein-bound), and he found excellent agreement with the method of Clark and Col-lip (1925), which also measured total Ca. In addition, the method is inexpensive (low cost of equipment [Spectronic 20] and reagents).

The difference between serum Ca levels of healthy male and female walleye was due to egg production. Sex differences in Ca levels in osteichthyan fish were first reported by Hess et al. (1928), who found a range of 9–12.5 mg/100 ml serum in male cod (Gadus morhua), while mature females had a range of 12.7–29 mg/100 ml serum. Dacke (1979) suggested that hypercalcemia is related to the influence of estrogen and ovarian follicle maturation, which stimulate synthesis of yolk protein and hence an increase in protein-bound plasma Ca. Urist and Van de Putte (1967) found similar results for spawning sturgeon. Spawning brook trout females also exhibited increased levels of Ca (Booke 1964). Hunn et al. (1992) found Ca levels were significantly higher in gravid female than in male golden trout (Oncorhynchus aquabonita).

Dacke (1979) stated that Ca levels throughout the osteichthyan subphylum are regulated within narrow limits. This is accomplished, in part, by exchange with Ca in the aquatic environment and by exchange with bone Ca. Oser (1965) stated slight variations in normal levels of serum Ca are indicative of pathology such as bone abnormalities and muscle tumors. Even though our sample sizes of MFG-positive fish were small, elevations were seen in Ca levels of MFG-positive male and female walleye. These elevations suggest Ca is highly regulated in walleye and that MFG has a significant effect on serum Ca levels, probably resulting from acute myolysis (Holloway and Smith 1982) leading to increased extracellular Ca.

ACKNOWLEDGMENTS

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Table 1. Serum Ca (mg/100 ml serum) of healthy and MFG-positive spawning male and female walleye (Stizostedion vitreum) from Merrit Reservoir, NE, and Lake Sakakawea, ND.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Sample size</th>
<th>Mean ± s[^1]</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy female</td>
<td>20</td>
<td>15.4 ± 1.8[^4]</td>
<td>11.9-19.5</td>
</tr>
<tr>
<td>Healthy male</td>
<td>18</td>
<td>11.7 ± 1.5[^4]</td>
<td>9.1-14.6</td>
</tr>
<tr>
<td>MFG-positive female</td>
<td>4</td>
<td>20.2 ± 3.7[^4]</td>
<td>17.1-25.4</td>
</tr>
<tr>
<td>MFG-positive male</td>
<td>6</td>
<td>13.6 ± 2.1[^4]</td>
<td>11.5-16.8</td>
</tr>
</tbody>
</table>

[^1]Means with different alphabetical letter superscripts were significantly different between sexes at P < 0.05. The means marked with different symbols were significantly different within the same sex (i.e., healthy female compared to MFG-positive female and healthy male compared to MFG-positive male) at P < 0.10.

Table 2. Published serum Ca values (mg/100 ml serum) for freshwater fishes.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Sample size</th>
<th>Species</th>
<th>Mean ± s</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warner and Williams (1977)</td>
<td>107</td>
<td>Ictalus punctatus</td>
<td>9.2 ± 2.65</td>
<td>3.9-4.5</td>
</tr>
<tr>
<td>Field et al. (1943)</td>
<td>5-10</td>
<td>Cyprinus carpio</td>
<td>11.5</td>
<td>9.45-14.77</td>
</tr>
<tr>
<td>Shell (1961)</td>
<td>30 (pooled sample)</td>
<td>Micropterus dolomieui</td>
<td>—</td>
<td>10.2-19.5</td>
</tr>
<tr>
<td>Grant et al. (1970)</td>
<td>3 female</td>
<td>7.46</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Grant et al. (1969)</td>
<td>14 males</td>
<td>12.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Grant et al. (1969)</td>
<td>6 gravid females</td>
<td>18.4</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Urish and Van de Putte (1967)</td>
<td>4 male/4 gravid female</td>
<td>7.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Huns (1972)</td>
<td>9 male</td>
<td>Stizostedion vitreum</td>
<td>9.52 ± 0.7</td>
<td>—</td>
</tr>
<tr>
<td>Booke (1964)</td>
<td>females</td>
<td>5.54-27.79</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>males</td>
<td>6.34-10.65</td>
<td>—</td>
<td>—</td>
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LITERATURE CITED


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