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EFFECT OF CREATINE SUPPLEMENTATION ON *LEPOMIS MACROCHIRUS* (BLUEGILL SUNFISH)

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Introduction

Creatine, a naturally occurring amino acid derivative, has long been used as a human muscle supplement. When supplemented, creatine draws water into muscle cells causing an increase in cell volume, total body water and total muscle volume (Hultman et al., 1996). Studies have been conducted on supplementing creatine to swine and horses (Berg and Allee, 2001, Berg et al., 2003, D'Angelis et al., 2005 and Stahl et al., 2001), but little has been done with supplementation to fish.

Bluegill (*Lepomis macrochirus)* were chosen for this study because of their popularity as food fish and sportfish. Bluegills are commonly raised by government and private producers for stocking into recreational ponds. Bluegills used for stocking are often small, fingerling (5-7 cm) sized fish. In the Midwest United States, research on using bluegill as a food fish is ongoing (Wang et al., 2000). Bluegill need to reach at least 227 g to be used in the food fish market (Brunson and Morris, 2000). When raised in ponds in the Midwest U.S., bluegills take more than two years to reach the 227 g market size (Hayward and Wang, 2001). Fish that have an extended grow-out period of more than two years are not ideal culture species. Since bluegill do not reach market weight within two years, it is necessary to finds ways to increase gain in the same or a shorter amount of time.

Creatine was chosen as a supplement due to its ability to increase muscle mass by drawing more water into muscle cells. The increased muscle mass would increase the fish's overall gain, allowing fish to gain more in the same amount of time. Creatine was also chosen because it is a naturally occurring peptide, so foreign substances would not be introduced to the fish.

Objectives

Hypothesis: Fish supplemented creatine will weigh more than controls, will have more fat free tissue and moisture than controls, and also will have higher critical swimming speed.

Objective: To determine if creatine supplementation to bluegill would increase gain, increase the amount of fat free mass and moisture, and increase critical swimming speed in the fish.

Methodology

Live Animals

One hundred Bluegill sunfish (*Lepomis macrochirus*) of two different age groups were obtained from Osage Catfisheries (Lake Ozark, MO, USA) and held in the fisheries facilities at the University of Missouri-Columbia. Age group one (age-1) fish were approximately one year old and age group three (age-3) fish were approximately 3 years old. Fish were allowed to acclimate to the laboratory conditions before being sorted for size. Twenty four fish of the largest fish were selected from each age group to be used in the study. The starting mean weight of age-1 fish was 3.16 g ± 0.74 (mean ± SD) and the age-3 fish starting mean weight were 30.39 g ± 3.36. Fish were randomly assigned to individual ten liter chambers (38 x 20 x 30 cm) within three different recirculating tanks. Sixteen chambers were held in each 1000 liter recirculating tank. Fish were acclimated to the chambers before the feeding trial began. Two balanced diets were formulated and manufactured by Purdue University. The treatment diet contained 2.5% creatine, and the control diet contained no creatine. Age-1 fish were fed three equal amounts daily, totaling 10% of their mean body weight. Age-3 fish were fed three equal amounts daily, totaling 4% of their mean body weight. Fish weights (g) were recorded the day before the feeding trial began. Twelve fish from each size group were randomly assigned to each diet. Fish were arranged in a randomized complete block design, with one fish from each size and treatment in each block and four blocks per tank. Fish were fed the experimental diets for 40 days, then measured and weighed on day 41. Feed was withheld for approximately 18 hours ± 1, prior to being weighed on day 41. Feeding was resumed once fish were weighed. Relative amount of gain, which is grams of gain of per gram of fish, was figured using the following equation:

Relative gain = (final weight – initial weight) / [(final weight + initial weight)/2]

Swim Testing

On day 42, swim capacity testing was started utilizing a Blazka flow through style swim tube. The tube is a cylindrical chamber with a propeller at one end, which can be programmed to create specific rates of water flow. The tube was used to obtain the bluegills' critical swimming speed (Ucrit), or the highest water flow velocity that the fish can swim in before becoming exhausted. Ucrit can be determined using the following equation:

$$U_{crit} = V_{ls} + (t_s/t_i)V_i\,,$$

where V_{ls} (cm/sec) is the velocity of the last swimming period prior to exhaustion, t_s is the time (min) spent swimming at the final velocity, t_i is the time increment of each swimming period, and V_i is the velocity increment (cm/sec). All velocity measurements were then converted to body lengths per second (BL/sec) to account for differences in

fish size. Fish were placed in the swimming tube and allowed to acclimate for twenty minutes at a minimal rate of flow (0.25 BL/sec). After acclimation the water velocity was increased 0.25 BL/sec (V_i) every five minutes (t_i) until the fish could no longer swim in the flow. Fish were considered exhausted when they became impinged against the rear panel of the swim tube. Once exhausted, fish were removed from the tube and replaced in their chamber. V_{ls} and t_s was recorded and U_{crit} for each fish was calculated.

Tissue Analysis

Fish were euthanized using a clove oil and water solution, then whole fish were grinded and homogenously mixed to allow for tissue analysis. Percent fat and moisture was obtained using a CEM Smart Trac System 5 (CEM Corporation, Matthews, NC, USA). Age-3 fish tissue was analyzed in duplicate with a sample size of 4.14 g ± 0.15 (mean ± SD). Age-1 fish did not provide enough tissue to analyze in duplicate, so single 4.02 g ± 0.12 g tissue samples were analyzed. The following equation was used to obtain percent fat-free mass:

Percent fat-free mass = [final weight (g) – (final weight * % fat)] / (final weight)

Statistical Analysis

The GLM procedure of SAS (SAS Inst. Inc.,Cary, NC) was utilized to test for differences between relative gain of fish fed either treatment or control diets. The dependent variable in the model was treatment, which was creatine, with the independent variable being relative gain.

The GLM procedure was again used to test for differences between Ucrit of treatment and control fish and also between the two age groups. The dependent variable was treatment, with the independent variable being Ucrit.

The GLM procedure was also utilized to test for differences between percent fat and moisture of fish fed either the treatment or control diets. The dependent variable was treatment, which was creatine, and the independent variable was either fat or moisture.

Results & Discussion

40 Day Growth Results

Fish were weighed at the beginning of the feeding trial and again weighed at the end of the trial. Age-1 fish mean starting weight was 3.16 g ± 0.74 and mean end weight was 30.39 g ± 3.36. The mean relative gain for age-1 fish fed the treatment diet, containing creatine, was 1.03 g ± 0.52, versus 1.21 g ± 0.11 for age-1 fish fed the control diet, with no creatine (refer to Table 1). The mean relative gains for age-1 fish did not significantly differ. The lack of difference and also the large deviation for the creatine supplemented fish could be attributed to the same cause. Two of the creatine fed fish ceased to eat shortly after the trial began, resulting in loss of weight in one fish and nearly no gain in the second fish.

Age-3 fish mean starting weight was 30.39 g ± 3.36 and the mean end weight was 73.89 g ± 4.47. The mean relative gain for age-3 fish fed the treatment diet was 0.84 g ±

0.04 compared to 0.84 g ± 0.08 for age-3 fish fed the control diet. The mean relative gains are observably not significantly different. Additionally, the relative gain of age-1 fish was not significantly different from the relative gain of age-3 fish.

The relative gain results did not support the hypothesis of the experiment. The lack of increased gain due to creatine supplementation could be attributed to the environment the fish were housed in. The recirculating tanks allowed for water flow through among the chambers, but the circulation did not create a significant flow. The lack of flow or water movement resulted in a low activity rate in the fish. In addition to the lack of flow, there were also no predators to increase the bluegills' activity or swimming. Creatine functions to aid in reenergizing muscle after contraction. Due to environmental factors, the bluegills were not active, resulting in little muscle use. The lack of muscle use could explain the lack of effect from creatine.

U_{crit} Results

The mean Ucrit of age-3 fish (3.41 BL/sec) versus age-1 fish (4.04 BL/sec) differed significantly ($P<0.0001$). Furthermore, the age-3 fish receiving creatine supplementation possessed a significantly lower Ucrit than age-1 fish supplemented creatine (3.46 vs. 3.98 BL/sec, respectively). However, the fish age group*creatine interaction was not significant ($P=0.184$). The Ucrit results suggest that small fish may have a greater capacity to yield results from creatine supplementation.

Tissue Analysis Results

Tissue samples were analyzed for percent fat and moisture. The mean percent fat-free mass did not differ for age-1 treatment fish (92.89% ± 1.22) versus control fish (92.76% ± 2.07). The two means were not found to be significantly different. The mean percents fat-free mass for age-3 fish were 92.74% ± 0.59 and 93.07% ± 0.62, for treatment and control fish, respectively. The means were also not significantly different. Age-1 treatment fish had mean percent moisture of 71.39% ± 1.14, and control fish percent moisture was 71.10% ± 2.63. The mean treatment and control proportional moisture content for age-1 fish were not significantly different. The mean percent moisture did not differ for age-3 treatment fish (70.84% ± 0.77), versus control fish (70.73% ± 0.87).

The tissue sample analysis also did not support the hypothesis of the experiment. A characteristic of creatine is to increase total muscle volume by increasing intramuscular water. Based on this, it was hypothesized that percent moisture in the fish would increase. Additionally it was expected that percent fat-free mass would increase due to the increase in muscle volume due to creatine. The lack of significant differences in the percent fat-free mass and moisture could be attributed to the low activity level of the fish. The low activity level, explained by the aforementioned environmental effects, could have resulted in decreased intramuscular creatine uptake and storage.

Table 1. Relative gains, percent fat-free mass and moisture, and U_{crit} results

	Mean relative gain[f]	Mean % fat-free mass[g]	Mean % moisture[h]	U_{crit}[i]
Age-1 treatment fish	1.03 g ± 0.52[a]	92.89% ± 1.22[b]	71.39% ± 1.14[c]	3.98 BL/sec ± 0.25[d]
Age-1 control fish	1.21 g ± 0.11[a]	92.76% ± 2.07[b]	71.10% ± 2.63[c]	4.09 BL/sec ± 0.19[d]
Age-3 treatment fish	0.84 g ± 0.04[a]	92.74% ± 0.59[b]	70.84% ± 0.77[c]	3.46 BL/sec ± 0.31[e]
Age-3 control fish	0.84 g ± 0.08[a]	93.07% ± 0.62[b]	70.73% ± 0.87[c]	3.36 BL/sec ± 0.22[e]

[a,b,c,d,e] Means with letters in common are not significantly different ($P<0.05$).

[f] Relative gain = grams of gain/gram of fish = (final weight – initial weight) / [(final weight + initial weight)/2]

[g] % fat-free mass = [final weight (g) – (final weight * % fat)] / (final weight)

[h] % moisture values are obtained by running samples in CEM machine

[i] $U_{crit} = V_{ls} + (t_s/t_i)V_i$,where V_{ls} (cm/sec) is the velocity of the last swimming period prior to exhaustion, t_s is the time (min) spent swimming at the final velocity, t_i is the time increment of each swimming period, and V_i is the velocity increment (cm/sec), all values are reported as BL/sec.

Conclusions

Despite the lack of significantly different results in relative gain and tissue samples, this experiment still provides useful information. The individual chambers the bluegills were confined to allowed for accurate gain measurements, but as noted, lowered activity level. Additionally, bluegills naturally inhabit freshwater lakes or ponds that do not have strong currents, resulting in an inherently lower activity level than fishes that live in flowing water (streams or rivers). The activity level of fish is an important aspect of creatine supplementation that offers opportunities for additional experiments. Bluegills could be used again in a similar experiment, but with an increased level of activity. An alternate option would be to use a species of fish that naturally occupy flowing water and house them in tanks that facilitate constant swimming.

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