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Effects of dietary protein levels on growth and body composition of juvenile (age-1) Black Buffalo *Ictiobus niger*

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ABSTRACT

Populations of Black Buffalo *Ictiobus niger*, a broadly-distributed catostomid species native to the Mississippi River basin, are in decline, similar to many other catostomids. Artificial propagation and culture are frequently a part of native species recovery plans, and developing formulated diets is a critical component of these plans. However, studies establishing protein requirements for catostomids are limited, particularly for larger juvenile to sub-adult sizes, even though catostomids are commonly reared to these sizes. Therefore, we conducted a 10-week growth study to evaluate optimal protein levels in juvenile (age-1) Black Buffalo (mean ± SE: total length = 218.7 ± 0.8 mm, weight = 148.5 ± 1.6 g). Five practical diets were formulated to contain 30, 34, 38, 41, and 45% crude protein with each diet fed to four replicate tanks (400-L) containing 10 fish each. Fish fed a diet containing 41% crude protein had greater biomass gain (total weight gain for all fish in the tank) when compared to other diets. Individual weight gain (percent increase) was greater in fish fed diets containing 41% and 45% protein compared to lower protein diets. Results from this study suggest a diet with 41% crude protein would produce optimal growth for juvenile (age-1) Black Buffalo.

1. Introduction

Catostomidae is a diverse family of fishes distributed across North America with an increasing number of species being recognized as at-risk (Cooke et al., 2005; Day et al., 2017). Of the 75 catostomid species native to North America, Harris et al. (2014) reports 35% are recognized as imperiled (threatened, endangered, or vulnerable). Conservation for native species is aided by artificial propagation in 42% of state and federal agencies (Jackson et al., 2004; Day et al., 2017) and is a major component to preserving biodiversity and preventing extinction of at-risk species (Day et al., 2017). However, classification as a non-game species has led to few resources focused on catostomids (Cooke et al., 2005) resulting in little information known about culture techniques, including nutritional requirements.

Information on nutritional requirements of catostomids is important in developing prepared feeds formulated to optimize growth in aquaculture systems. Protein is one of the first nutrients tested when developing a diet for a new species (Sealey et al., 2013), because it provides amino acids necessary for muscle formation and energy (Yang et al., 2002). Additionally, protein is the most expensive component of a diet (Yuan et al., 2010), and minimizing excess protein is important for reducing costs (Sealey et al., 2013). Previous catostomid nutritional studies focused on larval life stages and recommended diets with high protein content for larval June Sucker *Chasmistes liorus* (Hansen, 2002) and larval Robust Redhorse *Moxostoma robustum* (Higginbotham and Jennings, 1999), while diets containing > 50% crude protein have been recommended for larval Razorback Sucker *Xyrauchen texanus* (Tyus and Severson, 1990).

The size and developmental stage of fish being reared is important for consideration when developing formulated diets because optimal protein levels decrease with increasing size and age in fish (Sealey et al., 2013). Sealey et al. (2013) recommended a diet > 40% protein for juvenile (3–6 months old) June Sucker which is reduced from the recommended feeding of a commercial diet with > 45% protein for larval June Sucker at first feeding (Hansen, 2002). Further, catostomids are often reared to larger sizes to increase survival after being stocked in the wild (Day et al., 2017). Thus, understanding how nutritional requirements differ among catostomid life stages is beneficial for conservation.

The Black Buffalo *Ictiobus niger* is a large catostomid species native to the Mississippi River basin which could benefit from developing culture methods, including assessment of optimal dietary requirements,

Abbreviations: RAS, recirculating aquaculture system; K, condition factor; FCR, feed conversion ratio

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Table 1
Ingredient composition and mean proximate composition (standard error) of diets used to evaluate optimal protein levels for juvenile (age-1) Black Buffalo *Ictiobus niger*.

Diet composition	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
			Ingredient (%, as fed)		
Fish meal, menhaden	22.75	25.50	28.15	30.95	33.75
Corn gluten meal	10.00	15.00	20.00	25.00	30.00
Shrimp meal	5.00	5.00	5.00	5.00	5.00
Squid meal	5.00	5.00	5.00	5.00	5.00
Wheat flour	41.60	34.45	27.55	20.40	13.10
Vitamin premix	0.50	0.50	0.50	0.50	0.50
Vitamin C (L-ascorbyl phosphtate, 35%)	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.30	0.30	0.30	0.30	0.30
Dicalcium phosphate	2.65	2.25	1.80	1.40	1.00
Trace mineral premix	0.10	0.10	0.10	0.10	0.10
Carboxylmethyl cellulose[a]	2.00	2.00	2.00	2.00	2.00
Soy lecithin	3.00	3.00	3.00	3.00	3.00
Menhaden oil	7.00	6.80	6.50	6.25	6.15
			Analyzed Composition		
Dry Matter (%, as fed)	92.10 (0.05)	93.38 (0.01)	94.16 (0.01)	95.16 (0.01)	95.28 (0.02)
Fat (%, as fed)	14.20 (0.00)	14.58 (0.01)	15.12 (0.22)	15.44 (0.04)	15.10 (0.30)
Ash (%, as fed)	9.97 (0.02)	10.12 (0.03)	10.39 (0.03)	10.77 (0.02)	10.99 (0.01)
Crude Protein (%, 90% dry matter basis)	30.33 (0.07)	34.04 (0.01)	38.02 (0.12)	41.09 (0.23)	45.17 (0.85)

Diet composition was analyzed in duplicate after diet preparation.
Diets resemble a commercially produced Razorback Sucker Diet (2.0 mm Grower; Rangen, Inc., Buhl, ID) with Diet 4 being the most similar.
Ingredients used in diet formulations include vitamin C (35% active, DSM, Parsippany, NJ), choline chloride (Thermo Fisher Scientific, Waltham, MA), dicalcium phosphate (Potash Corp, Aurora, NC), carboxymethyl cellulose (City Chemical, LLC., West Haven, CT), and menhaden oil (Lab Express, Arlington, TN).
All other ingredients were purchased from a commercial supplier (Rangen, Inc.).
[a] Pellet binder for sinking feeds.

due to population declines (Ross, 2001). Trautman (1981) states populations are declining in abundance in the Ohio River while Ross (2001) lists Black Buffalo as a species of concern in Mississippi. While no other study evaluated optimal protein requirements for larval Black Buffalo, it can be inferred from studies involving similar species, such as Razorback Sucker (Tyus and Severson, 1990), Robust Redhorse (Higginbotham and Jennings, 1999), and June Sucker (Hansen, 2002), a diet containing crude protein > 45% may be appropriate for feeding larval Black Buffalo. Although Sealey et al. (2013) evaluated protein and lipid requirements for early juvenile (3–6 months) June Sucker, there are no studies that evaluate protein requirements for larger juvenile to sub-adult catostomids. Therefore, the purpose of this study was to evaluate protein requirements for juvenile (age-1) Black Buffalo.

2. Methods

2.1. Diet formulations

Five practical diets were formulated to contain 30, 34, 38, 41, and 45% protein (Table 1). Diet formulations were similar to a commercially available Razorback Sucker diet (2.0 mm Grower; Rangen Inc., Buhl, ID), one of the few commercially available feeds for catostomid species. The diets were formulated to be practical and cost-effective by using increasing levels of protein sources, fish meal and corn gluten meal, and decreasing levels of the energy source, wheat flour, to balance the diets to meet the target dietary protein levels. Crude lipid (13%), supplemental vitamins, trace minerals, pellet binders, and total phosphorus (1.5%) were maintained at the same levels across all diets.

Diets were prepared as sinking pellets and produced using similar procedures outlined in Li et al. (1993), except diets were dried at higher temperatures to simulate drying processes for extruded diets. Finely ground ingredients (weighed to the nearest 0.1 g) were placed in a V-mixer (Patterson-Kelly Co., East Stroudsburg, PA) and mixed for 20 min. Resulting mixed ingredients were blended in a dough maker (Hobart Corp., Troy, OH), with fish oil added and mixed for 10 min followed by the addition of water (30%) which was mixed for another 10 min. The feed mixture was then passed through a meat grinder (Hobart Corp) and the resulting feed strands were broken into smaller particles by hand until pellets were about 2.0 mm in length. These smaller particles were dried in an oven (Grieve Corp., Round Lake, IL) at 120 °C for 30 min. Dried feed was screened to remove any dust and stored in a freezer at −10 °C until fed. Samples from each diet were analyzed in duplicate for proximate composition (Table 1).

2.2. Fish and tank acclimation

Juvenile Black Buffalo used in this experiment consisted of F1 offspring from wild broodstock induced to spawn following Guy and Allen (Guy and Allen, 2018b). Fry were first stocked into a fertilized pond (0.04 ha), which was fertilized with an inorganic fertilizer (urea, 46-0-0, Woods Farm Supply, Inc., Byhalia, MS, USA), and reared for 17 months. Fish were grown on a commercial starter diet (55% protein, 1–1.7 mm starter; Rangen Inc., Buhl, ID) for 13 months before being transitioned to a commercial sinking diet (45% protein, 2.4 mm EXTR 450; Rangen Inc.). High protein diets were fed to larval and early juvenile Black Buffalo following recommendations for other catostomid species (Higginbotham and Jennings, 1999; Sealey et al., 2013; Tyus and Severson, 1990), although it is uncertain if fish consumed the commercial diet or foraged on natural prey items in the pond.

Tank acclimation began by transferring Black Buffalo to two indoor recirculating aquaculture systems (RAS) and distributing fish equally among 10 tanks (430-L, 15–20 fish per tank) in each system. Acclimation lasted for four months to ensure fish regularly consumed a formulated diet and to reduce fish agitation caused during feeding and tank cleaning procedures. Pressurized aeration kept dissolved oxygen at > 6 mg/L while a heater maintained the water temperature between 21 and 24 °C during acclimation. Salinity was maintained at 3.0‰ to reduce impacts from stress, and Black Buffalo were fed the same diet as in the ponds at a rate of 1% body weight per day. After acclimation, Black Buffalo were weighed and measured to obtain fish of similar size and randomly redistributed back into both RAS. The experiment began four days after sorting and redistribution.

2.3. Experimental design

This experiment follows a randomized complete block design with the two RAS serving as the block and individual tanks within each RAS defined as the experimental unit. Each RAS contained 10 separate 430-L tanks (N = 20) stocked at 10 Black Buffalo (mean ± SE: total length = 218.7 ± 0.76 mm, weight = 148.5 ± 1.6 g) per tank. Diets were randomly assigned to four tanks with two replicate tanks per RAS. Dissolved oxygen was maintained > 6 mg/L by pressurized aeration, and water temperature was maintained between 21 and 24 °C following a feeding study on a closely related species, Bigmouth Buffalo *I. cyprinellus* (Stanley and Jones, 1976). A constant photoperiod (12 h light: 12 h dark) was used for both RAS with lights controlled by a timer.

Fish were fed to satiation daily for 10 weeks by providing excess feed and removing the remaining feed with an aquarium net after 30 min similar to a method described in NRC (National Research Council) (2011). We determined initial feeding rates by examining fish