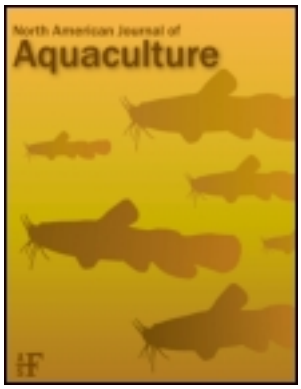

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Effect of Altering Dietary Protein: Energy Ratios on Juvenile Pallid Sturgeon Growth Performance

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ARTICLE

Effect of Altering Dietary Protein: Energy Ratios on Juvenile Pallid Sturgeon Growth Performance

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Abstract

Pallid Sturgeon *Scaphirhynchus albus* are endemic to the large, freshwater, river systems of North America and have long been prized for caviar production. Declining wild populations and fishing prohibitions have created interest in the culture of these fish. Protein and energy are often the first nutrients examined when attempting to identify diets appropriate for a novel culture species. Therefore, the present study evaluated the effects of different dietary protein: energy ratios on Pallid Sturgeon growth and body composition. Twelve semipurified diets composed of four crude protein (CP) levels (32, 39, 46, and 53%) and three gross energy (GE) levels (3,600, 4,200, and 4,800 kcal/kg) were fed to 180 juvenile Pallid Sturgeon for 18 weeks. Diets were offered to triplicate groups of five fish each in separate tanks. Fish were fed 2% body weight daily and sampled every 3 weeks. Mean weight gain and body composition were significantly affected by GE content of the dietary treatment ($P \leq 0.05$), but not by dietary CP or protein and energy interactions ($P > 0.05$). The most energetically dense diets yielded significantly larger fish than the least energetically dense diets and the intermediate diets were not significantly different from either extreme. These results suggest that Pallid Sturgeon are able to perform similarly across a wide range of protein: energy ratios (79–147 mg protein/kcal), as long as adequate dietary energy (≥3,800 kcal GE/kg) is provided and essential amino acids are not limiting. This research is the first to evaluate dietary protein: energy ratios for *Scaphirhynchus* sturgeon culture.

Research developing the artificial propagation, husbandry, and nutrition of sturgeon species has developed greatly since the 1950s (Conte et al. 1988; Celikkale et al. 2005). This increase in research activity can be correlated with the sharp worldwide decline of Acipenseriformes throughout the 1980s, caused in part by habitat alteration and overharvest of wild stocks (Keenlyne 1997; Catarci 2004; Moghim et al. 2006; Seibert et al. 2011). Throughout the northern hemisphere, sturgeon species are prized for their meat and unfertilized roe, which is processed and marketed as the delicacy, caviar (Catarci 2004). Currently, many sturgeon species are cultured in captivity for both stock enhancement and commercial purposes (Conte et al. 1988; Fajfer et al. 1999; Ballestrazzi and Garavello 2003; Wei et al. 2004; Celikkale et al. 2005; Mohseni et al. 2006).

Literature describing the biology and nutritional requirements of Acipenseriformes is incomplete. Singer and Ballantyne (2004) stated that the study of metabolic and hormonal regulatory systems in Acipenseriformes provides valuable information regarding vertebrate evolution, and developing a better understanding of this information is increasingly imperative given the decline of wild populations. Fortunately, a sizeable body of research has accumulated regarding the anatomy, physiology, and nutrition of sturgeon species (Dabrowski et al. 1987; Doroshov et al. 1997; Hung and Deng 2002; Singer and Ballantyne 2004; Garcia-Gallego et al. 2009). For example, essential amino acid requirements were investigated by evaluating whole-body, specific tissue, and egg composition of White Sturgeon *Acipenser transmontanus* (Ng and Hung 1994, 1995) and also via whole-body analysis of Siberian Sturgeon *A. baerii* Brandt (Kaushik et al. 1991). Additional research has focused on feedstuff digestibility (Kaushik et al. 1989; Stuart and Hung 1989; Medale et al. 1991; Herold et al. 1995; Liu et al. 2009), dietary crude

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protein (CP) requirements (Moore et al. 1988; Mohseni et al. 2007), digestive enzyme activities (Buddington and Doroshov 1986a, 1986b; Lin et al. 1997; Furne et al. 2005; Babaei et al. 2011), thermal regimes (Hung et al. 1989a; Mayfield and Cech 2004; Kappenman et al. 2009), and feeding rate and frequency (Hung et al. 1993; Cui et al. 1997; Deng et al. 2003; Mohseni et al. 2006), as well as the effects of culture densities (Fajfer et al. 1999; Celikkale et al. 2005). It is important to note, however, that these previous examples used many different sturgeon species, including White Sturgeon (Buddington and Doroshov 1986a, 1986b; Hung et al. 1989a, 1989b, 1993; Cui et al. 1997; Lin et al. 1997; Ballestrazzi and Garavello 2003; Deng et al. 2003), Siberian Sturgeon (Medale et al. 1991; Liu et al. 2009), Atlantic Sturgeon *A. oxyrinchus* (Mohler et al. 1996; King et al. 2004), Adriatic Sturgeon *A. naccarii* (Furne et al. 2005), Chinese Sturgeon *A. sinensis* (Xiao et al. 1999), Russian Sturgeon *A. gueldenstaedtii* (Celikkale et al. 2005), Persian Sturgeon *A. persicus* (Mohseni et al. 2007; Babaei et al. 2011), Lake Sturgeon *A. fulvescens* (Fajfer et al. 1999), Green Sturgeon *A. medirostris* (Mayfield and Cech 2004), Beluga Sturgeon *Huso huso* (Mohseni et al. 2006), and Shovelnose Sturgeon *Scaphirhynchus platorynchus* (Kappenman et al. 2009). Despite the breadth of previous research objectives, limited information is available regarding the optimization of protein: energy ratios for the grow out of juvenile sturgeon species. Additionally, no literature is available describing specific nutritional requirements of the uniquely freshwater *Scaphirhynchus* genus of sturgeon.

The *Scaphirhynchus* genus comprises the Pallid Sturgeon *S. albus*, Alabama Sturgeon *S. suttkusi*, and Shovelnose Sturgeon. These fish are endemic to the Mississippi and Missouri river basins of North America that flow into the Gulf of Mexico (Keenlyne1997; Tripp et al. 2009a). As with other sturgeon species, the Pallid and Shovelnose Sturgeon are prized for their meat and roe (Tripp et al. 2009b; Seibert et al. 2011). In addition to commercial harvest pressure, lack of recruitment due to impoundment and channelization led to the U.S. federal listing of the Pallid Sturgeon as an endangered species in 1990 (USFWS 1990). Subsequently, in 2010 the Shovelnose Sturgeon was granted limited protection as a threatened species under the U.S. Endangered Species Act of 1973 in regions where it cohabitates with the Pallid Sturgeon (USFWS 2010). As a result, interest in sturgeon culture has developed to supplement and replace harvest of dwindling wild stocks protected by increasingly tight harvest restrictions. Optimization of the dietary protein: energy ratio for Pallid Sturgeon will aid in the development of sustainable sturgeon culture diets, which minimize waste and maximize growth and condition. The present research sets the groundwork for developing juvenile *Scaphirhynchus* sturgeon diets by providing the first evaluation of the dietary protein: energy ratios.

METHODS

Dietary formulations.—Twelve semipurified diets were formulated using a 4 × 3 factorial design. Four CP levels (32, 39, 46, and 53%) were assigned to three gross energy (GE) levels (3,600, 4,200, and 4,800 kcal/kg). Limited information was available regarding digestible energy of feedstuffs in juvenile sturgeon; therefore, diets were assayed for GE, which was used to calculate the protein: energy ratios. As each CP increment was associated with each GE increment, the resulting protein: energy ratios overlapped between treatments (Table 1).

TABLE 1. Protein: energy ratios (mg protein/kcal) of experimental diets fed to Pallid Sturgeon determined based upon assayed mean dietary crude protein and gross energy.

Crude protein (mg/g)	Protein: energy ratio(mg/kcal)		
341	96.8	88.8	78.9
400	113.5	104.1	92.6
489	138.8	127.3	113.2
566	160.6	147.3	131.0
Gross energy (kcal/kg):	3,524	3,842	4,321

Feeds were produced by homogenizing ingredients in a cutter–mixer (model CM450, Hobart, Troy, Ohio), formed into approximately 2-mm pellets by using a meat grinder (1.5-hp electric grinder; Cabela's, Sydney, Nebraska) and then air dried at 100°C to approximately 10% moisture by using a commercial food dehydrator (Harvest Saver R-5A, Commercial Dehydrator Systems, Eugene, Oregon). Selections of dietary CP and GE levels, as well as specific ingredients, were based upon previous studies conducted in other sturgeon species (Moore et al. 1988; Hung et al. 1990; Herold et al. 1995; Mohler et al. 1996; Cui et al. 1997). Amino acid profiles were maintained at or above the requirements reported by Ng and Hung (1995) for White Sturgeon by the addition of feed-grade arginine, which was first limiting. Utilization of crystalline amino acids by White Sturgeon is poor relative to amino acids from intact protein (Ng et al. 1996). For this reason, feed-grade arginine was incorporated in the feeds at levels exceeding the requirements determined for White sturgeon. All experimental diets were designed to incorporate an identical basal formulation of fish meal (FM), blood meal, brewer's yeast, dehydrated fish solubles, crystallized arginine, betaine hydrochloride, vitamins, and minerals (Table 2). Contents of CP and GE were varied by increasing or decreasing casein, wheat feed flour, fish oil (FO), dextrose, and cellulose. The semipurified diets included casein and dextrose to allow for the formulation of a wide range of CP and GE.

Growth trial.—An 18-week feeding trial was conducted using 180 juvenile Pallid Sturgeon having a mean ± SE weight of 73.3 ± 1.2 g. Fingerlings originated from wild parents spawned at the U.S. Fish and Wildlife Service (USFWS) Gavins Point National Fish Hatchery and Aquarium, Yankton, South Dakota. Prior to the initiation of the experiment, individuals were stocked into thirty-six 150-L aquaria and allowed to acclimate to the experimental environment for 3 months. The experimental system incorporated two sets of tanks. One set of 24 tanks was linked

TABLE 2. Dietary formulations (g/kg) and determined proximate composition (g/100 g) of experimental diets for Pallid Sturgeon. Diets are named according to their protein (%) to energy (kcal/kg) ratio; e.g., 34:3524 = 34% protein and 3,524 kcal/kg energy.

	Experimental diets											
Ingredients (g/kg)	34:3524	40:3524	49:3524	57:3524	34:3842	40:3842	49:3842	57:3842	34:4321	40:4321	49:4321	57:4321
Fish meal[a]	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245.0	245
Casein	70.8	152.2	234.0	315.0	70.8	152.2	234.0	315.0	77.6	159.0	240.4	321.7
Blood meal	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6	68.6
Yeast, brewers	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Fish solubles[a]	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6	19.6
Fish oil[a]	154.0	144.8	135.5	126.3	154.0	144.8	135.5	126.3	237.8	219.3	210.1	200.8
Dextrose	219.0	146.8	75.0	2.5	219.0	146.8	75.0	2.5	215.6	152.7	80.6	8.4
Wheat flour	88.2	88.2	88.2	88.2	88.2	88.2	88.2	88.2	39.2	39.2	39.2	39.2
Cellulose	39.2	39.2	39.2	39.2	39.2	39.2	39.2	39.2	1.0	1.0	1.0	1.0
Alginate HV[b]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20
Crystallized arginine	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9	4.9
Betaine hydrochloride	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Dicalcium phosphate	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7
Sodium phosphate	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7	14.7
Choline	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
Mineral premix[c]	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Vitamin premix[d]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Stay C[e]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Proximate composition (g/100 g dry matter):												
Crude protein	33.1	39.4	45.9	55.8	33.6	40.0	50.9	57.0	35.5	40.6	49.9	57
Crude lipid	13.6	10.3	9.4	9.1	19.0	11.1	17.9	16.9	27.6	26.3	27.4	24.4
Ash	9.7	10.1	10.1	10.2	9.1	9.6	8.6	9.7	8.5	8.9	9.4	9.3
Gross energy (kcal/kg)	3372	3442	3563	3718	3838	3655	4032	3842	4242	4568	4187	4289
Protein: energy (mg protein/kcal)	98.3	114.5	128.7	150.0	87.5	109.4	126.2	148.3	83.7	89.0	119.1	132.9

[a]Omega Protein, Houston, Texas.

[b]Manucol high viscosity alginate; FMC Corp., Philadelphia, Pennsylvania.

[c]Contribution, mg/kg of diet: zinc, 215.36; iron, 38.06; manganese, 24.57; copper, 6.67; iodine, 2.40; selenium, 0.30; cobalt, 0.17; potassium, 0.000072.

[d]Contribution per kilogram of diet: vitamin A, 5,000 IU; vitamin D-3, 50 IU; vitamin E, 84 IU; vitamin K (as menadione), 8.432 mg; thiamin hydrochloride, 11.488 mg; riboflavin, 75.000 mg; niacin, 125.000 mg; pantothenic acid, 57.506; folic acid, 4.500 mg; pyridoxine, 20.575 mg; biotin, 1.250 mg; vitamin B-12, 50.000 mg; ascorbic acid, 87.500 mg.

[e]DSM Nutritional products, Basel, Switzerland.

to a large mechanical and biological filter and another set of 12 tanks was linked to similar filters. Both systems were linked together at a junction in the return lines to provide mixing of system water in an effort to minimize block effect. The system as a whole was treated as a randomized complete block design such that the former 24-tank block contained two replicates of all treatments and the latter 12-tank block contained one replicate of all treatments.

Makeup water was supplied by treating municipal water with sodium thiosulfate ($Na_2S_2O_3$) and sodium bicarbonate ($NaHCO_3$) for dechlorination and maintenance of alkalinity, respectively. Dissolved oxygen (DO) and water temperature were checked daily using a YSI model 550A oxygen meter (Yellow Springs, Ohio). Water temperature was maintained at 21.7°C ± 0.3°C throughout the experiment, following the suggestions of Kappenman et al. (2009), who estimated the optimum temperature for growth in the closely related Shovelnose Sturgeon at approximately 22°C. Dissolved oxygen was maintained at 6.81 ± 0.04 mg/L. Total alkalinity, total un-ionized ammonia (NH_3), nitrite, and pH were monitored weekly using a LaMotte Smart3 colorimeter (La Motte, Chestertown, Maryland) and a S20 SevenEasy pH meter (Mettler Toledo, Columbus, Ohio); total alkalinity, un-ionized ammonia, nitrite, and pH averaged 122 mg/L, 0.57 mg/L, 0.01 mg/L, and 7.84 respectively. Salinity was maintained at approximately 1‰ and was monitored using a salinity refractometer. All fish were maintained on a 12 h light: 12 h dark cycle.

At the start of the growth trial, all fish were captured and sedated with 100 mg/L tricaine methanesulfonate (MS-222; Western Chemicals, Ferndale, Washington). Fish were weighed

and fin-clipped for individual identification, then five fish were randomly distributed to each aquarium. Dietary treatments were subsequently randomized in triplicate following the complete block design. Feeding to apparent satiation was a logistical difficulty due to this species' habit of slow benthic feeding, and as such, feed was offered at 2% body weight/d, split into two equivalent feedings, following the recommendations of Hung et al. (1989a) and Mohseni et al. (2006).

Every 3 weeks, fish were sedated and sampled to adjust feed amounts. Fish were fasted for 24 h prior to each sampling. Performance was evaluated by assessing differences in total weight gain and proximate composition of fish carcasses between dietary treatments.

After the 18th week, all fish in the experiment were euthanized, weighed, and stored at −20°C for proximate analysis. All fish sacrificed in the study were euthanized by an overdose of MS-222 at a concentration above 250 mg/L in a neutral pH buffered solution and remained in the solution for 10 min after the cessation of opercular movement, as suggested by the American Veterinary Medical Association (AVMA 2013). This experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Southern Illinois University, Carbondale (protocol number 10–035).

Proximate analysis.—Diet and fish carcass composition were analyzed using the following standard methodology. Moisture was determined by weight lost upon lyophilization (Labconco Freezone 6, Labconco, Kansas City, Missouri). After dehydration, samples were ground prior to further analytical procedures. Percent CP was determined using a LECO model FP-528 nitrogen determinator (LECO, St. Joseph, Michigan) following the Association of Official Analytical Chemists (AOAC) official method 992.15. Percent crude lipid (CL) was determined using an ANKOM XT10 (ANKOM Technology, Macedon, New York) following the American Oil Chemists' Society (AOCS) official procedure AM 5–04. Percent ash was determined via combustion in a muffle furnace at 600°C following AOAC protocol number 942.05. The GE was determined using a Parr 1425 Semimicro bomb calorimeter (Parr Instrument Company, Moline Illinois).

Statistical analysis.—Data were analyzed by two-way ANOVA for a randomized complete block. Assumptions for homogeneity of variance and normality of the data were tested by examining the correlation between absolute residuals and predicted values and the Shapiro–Wilk test for normality. All data met the assumptions. If the ANOVA was significant, pairwise contrasts using Fisher's least significant difference (LSD) test were performed to identify significant differences between treatments at the alpha = 0.05 level. All statistical analyses were performed using SAS version 9.2 software (SAS Institute, Cary, North Carolina)

RESULTS

Mean weight gain was significantly affected by the GE content of the dietary treatment ($P = 0.05$; Figure 1). The most energetically dense diets, assayed to contain an average of 4,321 kcal/kg, yielded significantly larger fish than the least energetically dense diets, which contained 3,524 kcal/kg. The intermediate diets, 3,842 kcal/kg, were not significantly different from either of the other two. Mean weight gain was comparable ($P > 0.05$) between fish fed protein: energy ratios between 79 and 147 mg protein/kcal, when energy met or exceeded 3,842 kcal/kg. Growth performance was not significantly ($P > 0.05$) affected by dietary CP ($P = 0.24$) or protein: energy ($P = 0.81$) interactions. The CV (SD·100/mean) of fish weight increased over time from 22.3% at initiation to 56.5% at 18 weeks. Initially, fish ranged in weight from 44.5 to 105.4 g. At the end of the study, fish ranged in weight from 35.2 to 313.7 g. Survival was greater than 95% in all treatments and there were no significant treatment effects ($P > 0.05$; data not shown).

Pallid Sturgeon whole-body proximate composition was also affected ($P = 0.01$) by GE content of the diet. Carcass CP was significantly higher in the lowest energy treatment, while CP levels from the intermediate and high energy treatments were not different from one another (Table 3). Carcass moisture, CL, and ash were not significantly affected ($P > 0.05$) by dietary CP, GE, or protein: energy interactions.

DISCUSSION

The results of this study emphasize the importance of dietary energy in formulating Pallid Sturgeon feeds. It is important to note that all diets were formulated to meet the estimated amino acid requirements reported by Ng and Hung (1995) for White Sturgeon. As such, it is not surprising that dietary CP content did not significantly affect weight gain or whole-body composition of fish carcasses. Dietary protein: energy ratios have been evaluated for many fish species (NRC 2011). Most fish species' optima range between 84 and 105 mg digestible protein/kcal digestible energy (NRC 2011).

Using practical FM and FO formulations, Mohseni et al. (2007) estimated the CP requirement of 140-g juvenile Persian Sturgeon at 40% of the diet. Mohseni et al. (2007) reported an ideal protein: energy ratio between 75.2 and 83.6 mg protein/kcal. Kaushik et al. (1989) used practical ingredients to estimate the dietary CP requirement of 90- and 150-g juvenile Siberian Sturgeon to be between 36% and 42%, and predicted an optimal protein: energy ratio of 75.2 mg digestible protein/kcal. The study of Moore et al. (1988) used casein- and dextrose-based formulations to estimate the dietary CP requirement of 145-g juvenile White Sturgeon at 40.5%. Applying the standard energetic values of 4, 4, and 9 cal/g for protein, carbohydrate, and lipid, respectively, the protein: energy ratio of the optimal diet in Moore et al. (1988) was approximately 106 mg protein/kcal.

Data from the present study suggest that juvenile Pallid Sturgeon grow similarly across a wide range of protein: energy ratios. Growth was not different between fish fed diets ranging from 79 to 147 mg protein/kcal when energy approached or exceeded 3,842 kcal/kg. Other researchers have observed certain

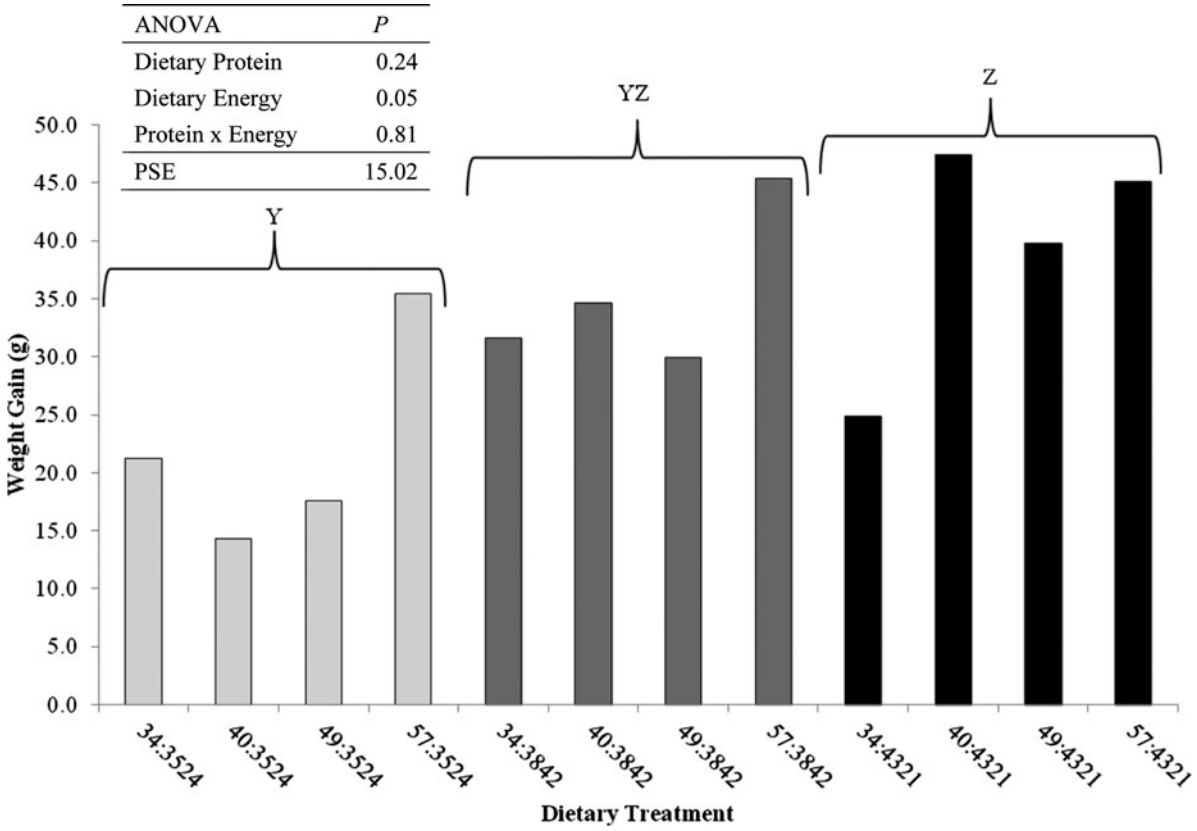

FIGURE 1. Mean weight gain (g) of Pallid Sturgeon fed diets differing in crude protein and gross energy content. Diets are named according to their protein (%) to energy (kcal/kg) ratio (see Table 2). Energy levels (indicated by bar color and brackets) accompanied by different letters are significantly different at $P \leq 0.05$.

fish species perform equivalently across a wide range of protein: energy ratios. Bright et al. (2005) found that Largemouth Bass *Micropterus salmoides* utilized diets with protein: energy ratios between 86 and 137 mg protein/kcal without relative detriment to growth. Also, Nematipour et al. (1992) reported that hybrid Striped Bass (female White Bass *Morone chrysops* × male Striped Bass *M. saxatilis*) grew equally well on protein: energy ratios ranging from 111.1 to 166 mg protein/kcal.

Dietary composition was diverse in the present study in order to formulate the wide range of protein: energy ratios. Even so, purified ingredients, e.g., casein and dextrose, were maintained at near-constant levels within energy treatments across protein levels. Since dietary energy, and not protein, had significant effects on growth and body composition, neither casein nor dextrose appears to have affected performance. Utilization of these ingredients seems to differ between sturgeon species. Stuart and Hung (1989) observed that White Sturgeon performed comparably when fed experimental diets formulated with casein and dextrose or a herring meal and dextrose control. Hung et al. (1989b) reported that White Sturgeon were capable of using simple monosaccharides and disaccharides, including dextrose, though other carbohydrates, e.g., lactose and fructose, were found to be poor sources of energy and significantly reduced lipid utilization. Furthermore, Kaushik et al. (1989) observed that Siberian Sturgeon did not efficiently utilize complex carbohydrates. These studies, together with the present study, support the use of dextrose in experimental feeds for sturgeon.

Our findings suggest that the relatively high dietary CL levels of the 4,321-kcal/kg treatments (26.4% ± 0.7%) are not necessarily optimal for efficient growth. This is supported by the proximate composition of the fish fed diets high in CL. Carcass CP concentration decreased in these fish, and there were trends toward lower ash and higher lipid concentrations. Commercial salmonid diets are often fed to sturgeon and can contain CL above 20%, which may be excessive in sturgeon feeds. Mid-level energy treatments in the present feeding trial contained 16.2% ± 1.8% CL and provided weight gain in the fish comparable with the high energy diets.

The Pallid Sturgeon used in this study demonstrated a high level of variability, with the CV for fish weight increasing from 22.3% at initiation to 56.5% at 18 weeks. Monaco et al. (1981) reported that CVs for total length of White Sturgeon fed artificial diets increased from 29% at 5 months posthatch to over 80% at 10 months posthatch. As size variability increased during the present 18-week feeding trial, a dominance hierarchy appeared to develop within the tanks. As fish grew, the largest fish demonstrated aggressive behavior and appeared to out-compete smaller fish for space and feed. High variability in growth was

TABLE 3. Carcass composition of Pallid Sturgeon after 18 weeks of being fed experimental diets. Values accompanied by different letters are significantly different at $P \leq 0.05$ within a main effect. PSE = pooled standard error.

			Composition (g/100 g dry matter)		
Main Effect	Dietary treatment	% Moisture	Protein	Lipid	Ash
Energy (kcal/kg):					
	3,524	77.7	61.7 z	14.1	15.9
	3,842	75.2	53.8 y	17.5	12.9
	4,321	75.3	53.4 y	18.0	11.9
	P-value	0.18	0.01	0.39	0.11
	PSE	1.05	1.80	2.17	1.33
Crude protein (%):					
	34	76.7	55.9	14.9	13.4
	40	76.7	57.3	14.7	12.8
	49	77.0	58.9	14.3	16.1
	57	73.9	53.0	21.3	11.9
	P-value	0.22	0.25	0.21	0.26
	PSE	1.20	2.08	2.50	1.54
Protein × Energy:					
	34:3524	76.9	57.5	17.2	12.8
	40:3524	77.4	63.0	11.8	14.8
	49:3524	79.5	65.6	11.3	20.0
	57:3524	76.8	60.9	16.0	16.0
	34:3842	76.9	56.7	9.67	15.6
	40:3842	75.6	53.8	16.2	11.7
	49:3842	75.2	54.1	19.6	14.2
	57:3842	73.1	50.4	24.6	10.0
	34:4321	76.2	53.5	17.7	11.9
	40:4321	77.0	55.2	16.1	11.9
	49:4321	76.3	57.0	15.1	14.2
	57:4321	71.6	47.8	23.2	9.5
	P-value	0.85	0.73	0.56	0.70
	PSE	2.06	3.60	4.34	2.67

confounding in the present study and may be related to the interactions of dominance, genetics, and culture stress. In general, juvenile Pallid Sturgeon appear to be able to utilize a wide range of protein: energy ratios, as long as amino acids are not limiting and the diet contains GE approaching or above 3,842 kcal/kg. Additional research evaluating nutrient requirements and ingredient utilization, as well as a better understanding of sturgeon feeding behavior and husbandry, will help lead to a more complete understanding of sturgeon culture requirements.

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