Effects of dietary protein level on growth performance, nitrogen and energy budget of juvenile hybrid sturgeon, *Acipenser baerii* ♀ ×*A. gueldenstaedtii* ♂

Zhiqiang Guo [a,b], Xiaoming Zhu [a,*], Jiashou Liu [a], Dong Han [a], Yunxia Yang [a], Zeqiao Lan [c], Shouqi Xie [a]

[a] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, Hubei 430072, PR China
[b] Graduate School of the Chinese Academy of Sciences, Beijing 100039, PR China
[c] Hubei Tianxia Sturgeon Co., Ltd, Yichang, Hubei 443300, PR China

ARTICLE INFO

ABSTRACT

An 8-week growth trial was conducted to estimate the optimal dietary protein level for juvenile hybrid sturgeon, *Acipenser baerii* ♀ ×*A. gueldenstaedtii*♂ (initial body weight, 25.1 ± 0.12 g). Effects of protein levels on nitrogen and energy budget of the fish were studied. Seven isoenergetic diets were formulated to contain seven levels of crude protein (250, 300, 350, 400, 450, 500 and 550 g kg^{-1}) and each diet was fed to triplicate groups of fifty fish at 21.4 ± 0.6 °C in twenty one 1 m^3 concrete tanks.

The results showed that specific growth rate (SGR) increased when dietary protein levels increased from 250 to 400 g kg^{-1} and then decreased significantly. Feed intake (FI) decreased steadily with increasing dietary protein levels. Fish fed 250 g kg^{-1} protein diets showed the lowest feed efficiency (FE), and those fed 500 and 550 g kg^{-1} protein diets showed significantly higher values than the other diets except 450 g kg^{-1}. Protein retention efficiency (PRE) was highest in 250–300 g kg^{-1} groups, followed by 350–500 g kg^{-1} groups and was significantly lower in 550 g kg^{-1} group. Proportions of nitrogen intake (CN) allocated to excretory nitrogen (UN) were lowest in 250–300 g kg^{-1} protein diets, followed by 350–500 g kg^{-1} protein diets and highest in 550 g kg^{-1} protein diet, whereas recovered (growth) nitrogen (RN) followed the inverse pattern with UN. Percentages of gross energy intake (IE) used for excretory energy (UE) increased steadily when dietary protein levels increased. Fish fed 400–550 g kg^{-1} protein diets showed significantly higher proportion of metabolizable energy (ME) than those fed 250–350 g kg^{-1} protein diets. The lowest and highest percentages of recovered (growth) (RE) were observed in 250 and 550 g kg^{-1} groups. Nitrogen and energy budget equation of the fish fed 350 g kg^{-1} protein diet was $100CN = 11.72FN + 58.78UN + 29.50RN$ and $100 IE = 35.08FE + 4.53UE + 34.16ME + 26.23RE$.

Five-parameter saturation kinetic model and second-order polynomial regression analysis indicated that the optimal dietary protein level for maximal growth of the fish was 340 and 370 g kg^{-1}. Diets with excessive protein contents not only resulted in inferior growth performance but also high proportions of protein and energy used for excretion.

1. Introduction

Sturgeon is considered to be a potential candidate in aquaculture all over the world owing to their high growth rate, large body size and strong adaptability to environments (Bronzi et al., 2011). China has become the largest sturgeon culture region since 2000 and hybrid sturgeon has been widely farmed. During 2007–2009, 38% total productions and 35% caviar productions were from farmed hybrid sturgeon in overall sturgeon industry across the country (Wei et al., 2011). The dominant farmed hybrid sturgeons are *Acipenser baerii* ×*A. schrencki*, *A. schrenckii* ×*Huso dauricus* and *A. baerii* ×*A. gueldenstaedti* (Wei et al., 2011).

An optimal dietary protein level in diets is important for fish growth and maintenance of good farming environments. If excessive protein is supplied in diets, only part of it will be used to synthesize new tissues for growth and the surplus proportion will be metabolized as energy source. Diets with excessive protein contents usually lead to extra energy costs, increased nitrogenous excretions and, occasionally, retarded fish growth (Abdel-Tawwab et al., 2010; Lee et al., 2001; Mohanta et al., 2008; Monentcham et al., 2009; Yang et al., 2002). Earlier studies on *A. transmontanus*, *A. sinensis* and *A. persicus* suggested that the dietary protein requirement for sturgeon was about 400 g kg^{-1} (Mohseni et al., 2007; Moore et al., 1988; Xiao et al., 1999). However, the available information on dietary protein requirement of hybrid sturgeon was scarce, especially the new and domestic species in China.

Nitrogen and energy budget of fish gives an insight to protein metabolism, energy partition and overall evaluation of diets utilization (Chakraborty et al., 1995; Cui, 1989; Cui and Wootton, 1988; Green

and Hardy, 2008). Dietary protein level or protein to energy ratio (P/E) was found to have substantial effects on protein utilization and energy expenditure in aquatic species, such as *Cyprinus carpio*, *Paralichthys lethostigma*, *Anguilla australis australis*, *Litopenaeus stylirostris* and *Gadus morhua* (Chakraborty et al., 1995; Engin and Carter, 2001; Gao et al., 2005; Gauquelin et al., 2007). These studies mainly focused on the relationship between protein intake and ammonia excretion, oxygen consumption or heat increment of feeding (HiE) (Chakraborty et al., 1995; Taboada et al., 1998; Yang et al., 2003). Cui et al. (1996) studied the effect of ration and body size on the energy budget of juvenile white sturgeon, *A. transmontanus*. Effects of dietary protein levels on protein metabolism and energy partition of sturgeon remain to be studied.

The purpose of present study was to estimate the optimal dietary protein level for juvenile hybrid sturgeon and investigate effects of dietary protein levels on growth, feed utilization and body composition. Nitrogen and energy budget equation of the fish were established firstly.

2. Materials and methods

2.1. Experimental diets

Formulation and chemical composition of experimental diets were shown in Table 1. Seven isoenergetic (gross energy 17.8 kJ g^{-1}) diets were formulated to contain crude protein levels of 250, 300, 350, 400, 450, 500 and 550 g kg^{-1}. Fishmeal and soybean meal (100 g kg^{-1}) were used as protein sources. Chromic oxide (Cr_2O_3) was added as inert marker for digestibility determination. Diets were made into pellets (2 mm, diameter), dried at 60 °C and stored at 4 °C.

2.2. Culture facility, experimental fish and growth trial

This study was conducted in a recirculation system in Hubei Tianxia Sturgeon Co., Ltd. (Yichang, Hubei, P R China). The system consisted of 21 concrete tanks (2.7×0.8×0.45 m). Water renewal rate was four times per hour. During the experiment, water temperature and dissolved oxygen was 21.4±0.5 °C and 7.7±0.9 mg L^{-1} measured in situ by a handheld dissolved oxygen meter (YSI Model Pro20, Ohio, USA). pH was 7.9±0.5 measured in situ by a handheld pH meter (YSI Model EcoSense pH10A, Ohio, USA). Ammonia-N was 0.30± 0.08 mg L^{-1} determined by a standard colorimetric method using Nessler reagent to produce a color from pale yellow to brown (APHA et al., 1995). The experiment was subjected to natural photoperiod (May to June).

The juvenile hybrid sturgeon was obtained from Hubei Tianxia Sturgeon Co., Ltd. Before the experiment, all fish were fed the equal mixture of seven test diets for 2 weeks. At the beginning of feeding trial, all fish (about 2000 individual) were starved for 24 h and pooled in a big tank. Fifty fish with uniform body size (initial body weight, 25.1±0.12 g fish^{-1}) were randomly transferred to 21 tanks. At the beginning, middle (after fish transferred to 10th tank) and end (after fish transferred to 21th tank) of this procedure, triplicate groups of 6 fish were sampled randomly by three times for initial body composition. Each test diet was fed to three replicates. Fish were fed to apparent satiation twice a day (8:00 and 15:00) for 8 weeks. Uneaten diets were siphoned 1 h after feeding and then dried and re-weighted. Four hours after siphoning out the uneaten diets, apparently fresh and intact feces were siphoned out to a white plate (50 cm×20 cm×6 cm) and then the intact feces was picked out using suction pipe to small metal box (5 cm×3 cm×2 cm). The feces in small metal box were oven-dried at 60 °C to constant weight and kept at −20 °C until analysis. Feces collection was conducted from the 15th to 45th day of feeding trial (Zhang et al., 2008). At the end of feeding trial, fish were batch-weighted after deprivation of feed for one day. Dorsal muscle of three fish from each tank was sampled and pooled. Two fish of each tank were sampled to determine the whole body composition.

2.3. Chemical analysis

Proximate composition analysis of all samples was conducted using the methods described by AOAC (1984). Crude protein content (N×6.25) was determined by Kjeldahl method after acid digestion using an Automatic Kjeldahl System (2300 Kjeltec Analyzer Unit, FOSS Tecator, Haganas, Sweden). Crude lipid content was determined by ether extraction in a Soxtec System HT (Soxtec system HT6, Tecator, Haganas, Sweden). Dry matter content was determined by oven dried at 105 °C to constant weight. Crude ash content was determined by incineration in a muffle furnace (550 °C for 12 h). Gross energy was determined by combustion in an adiabatic microbomb calorimeter (Phillipson microbomb calorimeter, Gentry Instruments Inc., Aiken, USA). Content of Cr_2O_3 was determined used the method described by Bolin et al. (1952).

2.4. Data calculations

Growth performance and feed utilization of the fish were calculated as following:

Survival rate (SR,%) = 100 × number of final fish / number of initial fish;

Specific growth rate (SGR,% day^{-1}) = 100 × [ln(final body weight) − ln(initial body weight)] / days;

Feed intake (FI,% body weight day^{-1}) = (100 × total feed intake) / [days × (initial body weight + final body weight) / 2];

Feed efficiency (FE,%) = 100 × wet weight gain / feed intake;

Protein efficiency ratio (PER) = wet weight gain / protein intake;

Protein retention efficiency (PRE,%) = 100 × protein retained in fish body / protein intake;

Energy retention efficiency (ERE,%) = 100 × energy retained in fish body / energy intake;

Table 1
Formulation and chemical composition of experimental diets.

Ingredients	Dietary protein level (g kg^{-1})						
	250	300	350	400	450	500	550
Fishmeal[a]	282.0	353.5	425.0	496.4	567.8	639.3	710.7
Soybean meal	100.0	100.0	100.0	100.0	100.0	100.0	100.0
α-starch	376.5	330.0	280.0	220.0	170.0	110.0	60.0
Fish oil	41.0	32.8	25.4	20.1	12.7	7.5	0.0
Soybean oil	41.0	32.8	25.4	20.1	12.7	7.5	0.0
Mineral premix[b]	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Vitamin premix[c]	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Choline chloride	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Cellulose	64.0	55.4	48.7	47.9	41.3	40.2	33.8
Cr_2O_3	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Carboxymethyl cellulose	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Chemical composition (g kg^{-1} on dry matter)							
Dry matter	978.5	975.9	982.6	983.1	981.0	965.80	983.0
Crude protein	250.4	305.2	342.9	390.1	436.2	486.1	530.8
Crude lipid	113.0	99.4	97.5	91.7	80.0	80.8	75.2
Ash	103.7	116.8	134.5	144.7	155.0	170.9	183.9
Gross energy (kJ g^{-1})	18.3	18.3	17.7	17.9	17.8	18.2	17.3
P/E ratio (mg kJ^{-1})[d]	13.7	16.7	19.4	21.9	24.5	26.7	30.7

[a] Imported from Seafood Company (USA) by Coland Feed Industry Co., Ltd. (Wuhan, PR China) (crude protein: 699.8 g kg^{-1}, crude lipid: 94.9 g kg^{-1}).
[b] Mineral premix (mg kg^{-1} diet): NaCl, 500; MgSO$_4$·7H$_2$O, 7500; NaH$_2$PO$_4$·2H$_2$O, 12,500; KH$_2$PO$_4$, 16,000; Ca(H$_2$PO$_4$)$_2$·H$_2$O, 10,000; FeSO$_4$, 1250; C$_6$H$_{10}$CaO$_6$·5H$_2$O, 1750; ZnSO$_4$·7H$_2$O, 176.5; MnSO$_4$·4H$_2$O, 81; CuSO$_4$·5H$_2$O, 15.5; CoSO$_4$·6H$_2$O, 0.5; KI, 1.5; Starch, 225.
[c] Vitamin premix (mg kg^{-1} diet): vitamin A, 1.83; vitamin D, 0.5; vitamin E, 10; vitamin K, 10; niacin, 100; riboflavin, 20; pyridoxine, 20; thiamin, 20; D-calcium pantothenate, 50; biotin, 0.1; folacin, 5; vitamin B$_{12}$, 20; ascorbic acid, 100; inositol, 100.
[d] Protein to energy ratio.