



## Utilization of Rice Protein Concentrate in Siberian Sturgeon (*Acipenser baerii* Brandt) Nutrition

Benedetto Sicuro<sup>1,\*</sup>, Manuela Piccinno<sup>2</sup>, Franco Daprà<sup>1</sup>, Francesco Gai<sup>3</sup>, Sebastiano Vilella<sup>2</sup>

<sup>1</sup> University of Torino, Department of Veterinary Sciences, Via L. da Vinci 44, Grugliasco (TO), Italy.

<sup>2</sup> University of Salento, Department of Biological and Environmental Science and Technology, strada per Monteroni, Lecce, Italy.

<sup>3</sup> Institute of Sciences of Food Production – National Research Council, Via L. da Vinci 44, Grugliasco (TO), Italy.

\* Corresponding Author: Tel.: 0039.011 6709260; Fax: 0039.0116 709240;  
E-mail: benedetto.sicuro@unito.it

Received 02 February 2015  
Accepted 15 May 2015

### Abstract

The aim of this research is the substitution of fish meal with rice protein concentrate (RPC) in the nutrition of Siberian sturgeon (*Acipenser baerii*) diet. 240 sturgeons of initial mean body weight of 19.1±6.7 g were used. 3 isonitrogenous (CP 42.4%) and isoenergetic (19.9 KJg<sup>-1</sup> DM) diets were formulated with 20% of RPC inclusion (R20), 35% inclusion (R35), 53% inclusion (R53) against a control diet, fish meal based (FM); feeding ratio was 1.5% of BW. All the diets and fish fillets were analyzed to determine the proximate composition. The diet amino acid composition was measured and fillet colour was detected at the end of experimentation. Fish growth showed very good performances of sturgeon fed with high RPC inclusion, but these data were not considered conclusive because of technical problems during the experimentation. However, considering that the highest level of RPC in the sturgeon diets corresponded to an almost complete substitution of fish meal in the fish diet, the result obtained from fish quality analysis showed no adverse effect of experimental diets, with the only limitation of high muscle lipid storage, in fish fed with highest level of RPC inclusion.

**Keywords:** Fish nutrition, alternative feedstuffs, amino acids, fish fillet quality.

### Introduction

The importance of substitution of fish meal in fish feed for aquaculture is paramount (Gatlin *et al.*, 2007). Among fish species for aquaculture, sturgeon is an emerging species and its importance is related both to meat and caviar production (Cataudella and Bronzi, 2001). Italy is one of the first European sturgeon producer and the importance of this sector of aquaculture in Italy and in other countries (Vaccaro *et al.*, 2005) is increasing (Vaccaro *et al.* 2005; Mohseni *et al.*, 2008; Bronzi *et al.*, 2011; Williot *et al.*, 2011.). Sturgeons are a group of 27 species of fish belonging to *Acipenseridae* family and even if they are morphologically similar, the researches on their artificial diets must be focused on each species requirements, as they have different natural diet in the wild. Sturgeon nutrition is a very general term and is not correct definition as new species and hybrids are often included to the list of those potentially useful for aquaculture as Atlantic sturgeon (*Acipenser oxyrinchus* Mitchell) (Lazur *et al.*, 2010), lake sturgeon (*Acipenser fulvescens* Rafinesque) (Moreau and Dabrowski, 1996) and Persian sturgeon (*Acipenser persicus* Boorodin) (Alipour *et al.*, 2010;

Ghelichi *et al.*, 2010). In general sturgeon nutrition is less studied (Deng *et al.*, 2005; Mohseni *et al.*, 2008; Daprà *et al.*, 2009a) compared to rainbow trout or Atlantic salmon which dominate scientific literature, but also in these species the replacement of fish meal with alternative feedstuffs is crucial for the future development of sturgeon farming (Koshio *et al.*, 2001), considering that at this moment the fish feeds utilized are essentially based on rainbow trout feeds formulations. Sturgeon nutrition has been studied during larval phase (Gisbert and Williot, 1997; Deng *et al.*, 2003) and new ingredients for fish feeds have been already tested, as soybean meal, spirulina meal, corn gluten and pea meal (Hung *et al.*, 1997; Palmegiano *et al.*, 2005; Sicuro *et al.*, 2012). Among vegetal feedstuffs, rice protein concentrate (RPC) is promising protein source as already demonstrated in shrimp (Oujifard *et al.*, 2012a; Oujifard *et al.*, 2012b), rainbow trout (Palmegiano *et al.*, 2006; Gai *et al.*, 2012), gilthead seabream (Sánchez-Lozano *et al.*, 2009) and blackspot sea bream (Palmegiano *et al.*, 2007; Daprà *et al.*, 2009b). The aim of this research was the evaluation of the substitution of fish meal with RPC in the nutrition of Siberian sturgeon (*Acipenser baerii*, Brandt) diet.

## Materials and Methods

### Experimental Plan

The experimental design was balanced monofactorial with randomized blocks, four levels of treatment and four replicates (4x4). Fish diet was the experimental factor tested and a progressive inclusion of rice concentrate was tested.

### Growth Trial

The growth trial was conducted at the Experimental Station of Department of Animal Husbandry of the University of Turin. 240 Siberian sturgeon (*Acipenser baerii*) individually weighed and having a mean initial body weight of 19.1±6.7 g were randomly allotted to 16 tanks of 500 L volume, with a water flow rate of 6 L/min. The sturgeons were purchased by Cisliano sturgeon farm (Milan, NW Italy). Growth trial started June, 23<sup>rd</sup> 2008 and finished October 29<sup>th</sup> 2008. During initial 2 weeks, fish were progressively fed with experimental diets. The fish were fed by hand (feeding ratio 1.5% of body weight (BW) six days per week, twice a day, at 9:<sup>00</sup> and 17:<sup>00</sup>). Fish were bulk-weighed fortnightly in order to adjust the feeding rate and individually at the end of the experiment.

### Diets

Three experimental diet were formulated with 20% of rice protein concentrate (RPC) inclusion (RPC20), 35% inclusion (RPC35) and 53% inclusion (RPC53) against a control diet, fish meal based (FM). Diets analyzed by proximate composition according

to standard methods (AOAC, 1995) showed that all diets were isonitrogenous (CP 42.4% DM) and isoenergetic (19.9 KJg<sup>-1</sup> DM) (Table 1).

### Sampling and Chemical Analysis

At the end of experiment, in order to calculate the somatic indexes, 5 fish per tank, with a body weight close to the mean body weight, were sampled and killed by anaesthesia overdose. Hepatosomatic (HSI) and viscerosomatic (VSI) indexes were calculated as follows:

$$\text{HSI} = (\text{liver/body in weight}) \times 100; \text{VSI} = (\text{viscera/body in weight}) \times 100.$$

The dorsal muscle tissues from the same fish were sampled and frozen until the successive chemical determinations. All the diets and fish fillets were analyzed to determine the proximate composition according to standard methods (AOAC, 1995). The gross energy content was determined using an adiabatic calorimetric bomb (IKA C7000, Staufen, Germany). The total nitrogen content was determined using a nitrogen analyzer (Rapid N III, Elementar Analysensysteme GmbH, Germany) according to the Dumas method and the crude protein was calculated as total N×6.25.

### Amino Acid Composition

The amino acid composition was measured by an amino acid analyser via acid hydrolysis using a Beckman System Gold HPLC system, according to standard analytical methods (AOAC, 1995) as described by Cavallarin *et al.* (2005).

**Table 1.** Ingredient and proximate composition of the experimental diets

Ingredients (% dry weight)	Diets				
	RPC*	RPC20	RPC35	RPC53	FM
Herring meal		360	205	20	570
Rice concentrate meal		200	350	530	0
Fish oil		60	60	60	60
Barley meal		245	250	255	235
Corn meal		90	90	90	90
Lignum sulphite		15	15	15	15
Brewer's yeast		20	20	20	20
Mineral mixture <sup>1</sup>		5	5	5	5
Vitamine mixture <sup>2</sup>		5	5	5	5
Crude protein	75.5	42.8±2	42.5±4	408±2	43.5±1
Ether extract	112	11.5±4	11.4±1	11.7±1	11.1±1
Ash	4.3	7.0±0.1	5.8±1	4.5±0.2	9.8±3
Gross Energy (KJg <sup>-1</sup> DM)	23.8	19.7±3	20.2±4	20.4±0.4	19.2±4

<sup>1</sup> Mineral mixture (g or mg/kg diet): bicalcium phosphate 500 g, calcium carbonate 215 g, sodium salt 40 g, potassium chloride 90 g, magnesium chloride 124 g, magnesium carbonate 124 g, iron sulphate 20 g, zinc sulphate 4 g, copper sulphate 3 g, potassium iodide 4 mg, cobalt sulphate 20 mg, manganese sulphate 3 g, sodium fluoride 1g. (Granda Zootechnica, Cuneo, Italy).

<sup>2</sup> Vitamin mixture (IU or mg/kg diet): DL-a tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium panthotenate, 50 mg; choline chloride, 2000 mg (GrandaZootechnica, Cuneo, Italy).

\*from Palmegiano *et al.*, 2006, modified

**Colour Detection on Fish Fillet**

On 5 intact right fillets, withdrawn at the moment of the analysis from whole fish stored at 1°C, colorimetric measurements were made using a bench colorimeter Chroma Meter CR-400 Konica Minolta Sensing (Minolta Sensing Inc, Osaka, Japan) in the CIELAB colour space (CIE, 1976). Measurements were performed at three sites (dorsal, ventral and caudal) and each measure was replicated twice. The lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) were recorded, and the Chroma and Hue indices were calculated as Chroma [ $C^* = (a^{*2} + b^{*2})^{1/2}$ ] and Hue [ $H^0 = \tan^{-1}(b^*/a^*)$ ]. Chroma is related to the quantity of pigments and high values represent a more vivid colour and denote a lack of greyness. Hue is the attribute that permits colours to be classified as red, green, yellow, blue, and so on.

**Statistical analysis**

All data were analysed by one-way ANOVA using R software. After the ANOVA, differences among means were determined by the Duncan test, using the significant level of  $P < 0.05$  (Venables and Ripley 2002).

**Results**

During this experimentation an high final mortality was registered, caused by technical reasons, for this reason productive results were measured but not analyzed. The water temperature during the growth trial was  $17.4 \pm 1.1^\circ\text{C}$  and dissolved oxygen of  $7.5 \pm 0.8$  mg/L. In the RPC53 diet the 96.5% of fish meal was substituted with RPC (Table. 1). The amino acid composition was similar between the diets, showing no deficiency respect to the fish meal diet

**Table 2.** Diet amino acid composition (% on the total)

	FM	RPC20	RPC35	RPC53
Aspartic acid	46.34 ± 2.0	42.97 ± 3.5	42.91 ± 1.6	43.03 ± 6.4
Threonine	18.59 ± 0.4	16.72 ± 1.1	14.42 ± 1.8	17.36 ± 0.5
Serine	18.68 ± 2.9	16.22 ± 1.8	13.94 ± 3.3	18.25 ± 1.9
Glutamic acid	72.22 ± 4.5	71.71 ± 2.6	75.65 ± 7.5	82.54 ± 3.0
Glicine	28.69 ± 2.5	22.72 ± 2.8	20.72 ± 2.0	20.53 ± 1.3
Alanine	29.39 ± 1.7	23.41 ± 0.4	25.53 ± 0.9	22.06 ± 0.7
Cysteine	4.34 ± 1.9	2.93 ± 4.1	3.26 ± 4.6	7.67 ± 1.3
Valine	23.26 ± 0.2	23.22 ± 5.9	18.04 ± 7.2	25.48 ± 4.1
Methionine	14.50 ± 0.3	15.23 ± 4.4	19.50 ± 11.2	11.91 ± 0.7
Isoleucine	20.18 ± 0.7	16.05 ± 0.9	22.22 ± 2.7	16.50 ± 0.5
Leucine	35.85 ± 1.2	32.88 ± 2.1	37.08 ± 2.1	35.34 ± 3.8
Tyrosine	11.74 ± 0.6	13.08 ± 0.8	17.37 ± 3.7	17.36 ± 0.9
Phenylalanine	16.11 ± 2.5	16.91 ± 0.7	21.11 ± 4.5	20.45 ± 0.2
Histidine	12.64 ± 3.2	14.34 ± 2.9	8.72 ± 2.3	13.32 ± 0.6
Lysine	38.00 ± 4.8	29.91 ± 1.2	20.24 ± 5.8	18.98 ± 1.4
Arginine	27.34 ± 8.6	34.95 ± 0.4	43.18 ± 11.4	34.42 ± 3.1
Proline	21.83 ± 5.1	25.32 ± 11.4	15.05 ± 3.3	22.50 ± 5.6
EAA	235.9	223.6	230	215.8
NEAA	203.8	195	188.9	211.9
EAA/NEAA	1.2	1.2	1.2	1.0

EAA = total essential amino acids

NEAA = total not essential amino acids

mean ± s.d on DM; n = 2

**Table 2a.** Comparison between diet amino acid composition and white sturgeon requirements (expressed as a percentage of total indispensable amino acid plus cystine and tyrosine)

	FM	RPC20	RPC35	RPC53	W.S.R.
Threonine	9	8	6	8	9.7
Valine	11	11	8	12	9.7
Methionine	7	7	9	6	6.6*
Isoleucine	9	8	10	8	8.8
Leucine	16	15	17	17	12.5
Phenylalanine	12	14	18	18	15.5**
Histidine	6	7	4	6	6
Lysine	17	14	9	9	15.8
Arginine	13	16	19	16	14

W.S.R. = white sturgeon requirement (Ng *et al.*, 1995)

\* methionine and cystine

\*\* phenylalanine and tyrosine

(Table 2) and comparing with the amino acid requirements of white sturgeon (Ng and Hung 1995) a Lysine deficiency was observed, proportional with RPC dietary inclusion (Table 2a). Proximate and fatty acid composition of RPC (Table 1, Table 5), showed high protein content and high presence of PUFA, in particular linoleic and oleic acid. From the point of view of quality of product, the RPC inclusion caused a noticeable fillet fat deposition in the group with highest inclusion (RPC53) where the fat quantity was double respect to the control group (Table 3). This noticeable difference in fat deposition between experimental groups also influenced the gross energy content of final product. Fillet protein content was affected by RPC inclusion, interestingly the higher protein content was found in the experimental groups with intermediate RPC dietary inclusion, respectively the RPC20 and RPC35 groups (Table 3). Somatic indexes indicated that liver size significantly increased in the RPC53 group (Table 4), this fact is related to a fat deposition similarly to fillet. Colour analyses on fish fillets showed differences in some parameters measured in this experimentation between experimental treatments (Table 5), in particular a\*, b\*, Chroma and Hue\* showed statistically significant differences. Chroma, a\* and b\* differences in

sturgeon fillet were proportional to RPC inclusion in fish diets.

## Discussion

### Diets and Fish Growth

The substitution of fish meal with RPC in Siberian sturgeon followed the encouraging results previously obtained in other fish species (Palmegiano *et al.*, 2006; Palmegiano *et al.*, 2007). RPC contains 75.5% of protein of high biological value and fatty acid composition is characterized by an high percentage of n6 – PUFA (Palmegiano *et al.*, 2006). Other vegetal concentrates have been included in sturgeon diet in the past, as soybean concentrate (Koshio *et al.*, 2001) and considering the importance of amino acid requirements (Kaushik and Seiliez, 2010; Gershanovich and Kiselev, 1993; Ng and Hung, 1995), often the inclusion was coupled with supplemental crystalline amino acids. In this experimentation, dietary amino acid supplementation was not necessary and the diet amino acid composition was comparable with that of previous research with RPC in blackspot sea bream (Palmegiano *et al.*, 2007) and in rainbow trout

**Table 3.** Fish fillet proximate analysis

	Crude protein (% dry weight)	Crude fat (% dry weight)	Ash (% dry weight)	Gross energy (MJ kg <sup>-1</sup> dry weight)
RPC20	16.1±1.1 <sup>a</sup>	3.6±0.1 <sup>c</sup>	11.2±0.5 <sup>a</sup>	22.54±0.1 <sup>b</sup>
RPC35	15.3±1.8 <sup>b</sup>	4.8 ±1.8 <sup>b</sup>	9.2±0.3 <sup>c</sup>	24.32±0.6 <sup>ab</sup>
RPC53	14.3±0.5 <sup>d</sup>	6.4±1.6 <sup>a</sup>	8.7±0.2 <sup>c</sup>	24.83±0.9 <sup>a</sup>
FM	14.9±1.1 <sup>c</sup>	3.2 ±1.1 <sup>d</sup>	10.2±0.1 <sup>b</sup>	20.44±0.4 <sup>c</sup>

In the columns, different letters mean statistical difference at P≤0.05. n = 3; values are means±S.D.

**Table 4.** Principal fatty acid in RPC

Fatty acid (%)	RPC
C16:0	23.3±0.04
C18:1 n-9	34.8±0.1
C18:2 n-6	34.4±0.13
Σ of SFA	26.7±0
Σ of MUFA	34.8±0.1
Σ of PUFA n-3	3.6±0.07
Σ of PUFA n-6	34.4±0.07
n-3/n-6	0.10

\* Palmegiano *et al.* 2006, modified.

**Table 5.** Colour measurement in fillet

Diet	L	a	b	Chroma	Hue
FM	54.62±4.3	1.95 ±1.4 <sup>b</sup>	6.71 ±2.7 <sup>b</sup>	7.06 ±2.8 <sup>b</sup>	73.50 ±9.7 <sup>a</sup>
RPC20	55.48 ±1.7	1.10 ±1.8 <sup>b</sup>	7.67 ±2.4 <sup>b</sup>	7.88 ±2.6 <sup>b</sup>	36.98 ±74.6 <sup>b</sup>
RPC35	55.64 ±2.5	2.28 ±1.5 <sup>b</sup>	10.89 ±2.3 <sup>a</sup>	11.18 ±2.5 <sup>a</sup>	78.75 ±5.8 <sup>a</sup>
RPC53	55.62 ±3.9	3.68 ±2.4 <sup>a</sup>	10.55 ±3.3 <sup>a</sup>	11.34 ±3.5 <sup>a</sup>	71.20 ±10.3 <sup>a</sup>

In the columns, different letters mean statistical difference at P≤0.05. (n=15: mean±SD)

(Palmegiano *et al.*, 2006). Moreover, observing the single amino acid content in the diet, an apparent deficiency of lysine is observable respect White sturgeon (Ng and Hung, 1995), that did not affect Siberian sturgeon growth, thus confirming differences between sturgeon species requirements. Unfortunately, an accidental mortality that was registered during the experimentation made us extremely cautious about the interpretation of fish growth data, however at the end of experimentation a better growth was visible in the fish fed with highest inclusion of RPC. In the previous researches on RPC utilization in fish nutrition conducted by our group in rainbow trout, we found that the progressive RPC inclusion in rainbow trout diets (Palmegiano *et al.*, 2006) caused a proportional decrease in productive parameters and quality of product and in rainbow trout the maximum inclusion level suggested was 20%. Interestingly, in this research the maximum level of RPC inclusion in sturgeon diet was 53 % and at this level the dietary protein was almost completely RPC protein. Even if we decided to not analyze productive results, we interestingly observed that the specific growth rate (SGR) was 5 times better than the case of white sturgeon (*A. transmontanus*) (Gawlicka *et al.*, 2002), and the productive parameters were also better than Beluga sturgeon (Hosseini *et al.*, 2010; Ta'ati *et al.*, 2011), 'AL' hybrid sturgeon (Sicuro *et al.* 2012), hybrid sturgeon (*Acipenser schrenckii* x *Huso dauricus*) (Qiyu X. *et al.*, 2011) and white sturgeon (Lin *et al.*, 1997).

### Quality of Product and Somatic Indexes

Considering fillet proximate composition, it is clear that the high inclusion of RPC caused relevant fat deposition in fillet. Siberian sturgeon fed the diet containing highest RPC inclusion levels had greater deposition of body fat. Dietary RPC resulted in high lipogenic effect in Siberian sturgeon. In general, body composition analyses were in accordance with the results of earlier researches on sturgeon, such as hybrid sturgeon (Guo *et al.*, 2011) and *Acipenser transmontanus* (Hung *et al.*, 1997; Gawlicka *et al.*, 2002). Sturgeon hepatosomatic and viscerosomatic indexes in this study were comparable with that in other researches on sturgeon and other fish. The RPC53 diet caused the greater increase of HSI, but this increase was lower than those obtained, in a previous experimentations, for sturgeons fed a diet containing 30% gelatinized starch (Kaushik *et al.* 1989) and 35% of glucose in the diet (Fynn-Aikins *et al.*, 1993). Considering the clear fish – meal sparing effect of proposed diets, the effect on quality of final product should be more carefully investigated in the future, in fact the fat deposition in sturgeon fed the higher RPC inclusion was more than double respect sturgeons fed the fish meal based diet.

### Colour

Fish fillet colour is of great importance from the commercial point of view, being directly associated with the product acceptance by the consumers (Izquierdo *et al.*, 2005). Several researches have shown that dietary vegetal pigments, in particular those coming from corn based ingredients are deposited into the flesh of fish, causing a yellow coloration (Liu *et al.*, 2004). Higher b\* values in fillets of rainbow trout fed diets containing corn gluten have been reported (Liu *et al.* 2004), compared to fillets of fish fed wheat gluten, soy protein concentrate, or fish meal diets. As regard as RPC inclusion in fish diet, a slight alteration of fillet colour was observed in our previous study in rainbow trout and in this experimentation comparable colour alteration was noticed, in particular in the yellow coloration. In the measurement of fish flesh colour, the a\* value represents the redness and the b\* value represents the yellowness of the sample (Norris and Cunningham, 2004). In this experimentation the intensity of yellow coloration of sturgeon flesh was proportional to RPC inclusion in the diet. Lightness (L\* value) did not show any difference between experimental treatments.

Considering the optimal RPC amino acid composition, the inclusion of RPC in feedstuff for Siberian sturgeon nutrition was successful even if the fish growth results need to be confirmed. In conclusion, considering that the highest level of inclusion of RPC utilized in this experimentation corresponded to an almost complete substitution of fish protein with RPC protein, these results showed interesting perspectives of RPC inclusion in sturgeon artificial feeds, even if the effect on muscle lipid storage should be clarified.

### Acknowledgements

The authors sincerely thank Mrs. Chiara Bianchi and Dr. Lidia Sterpone for their excellent technical assistance during laboratory analysis. We thanks Prof. Zoccarato for giving us the possibility to use the facilities of experimental centre of Animal Husbandry Department, Agriculture faculty of Torino. We thank Dr. Prearo of State Veterinary Institute for the melamine fillet analyses.

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