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Spirulina as a nutrient source in diets for growing sturgeon (*Acipenser baeri*)

Giovanni B Palmegiano[1], Elisabetta Agradi[2], Gilberto Forneris[3], Francesco Gai[1], Laura Gasco[4], Elisabetta Rigamonti[2], Benedetto Sicuro[3] & Ivo Zoccarato[4]

[1]CNR Institute of Science of Food Production, Torino Division, Grugliasco (TO), Italy

[2]Department of Pharmacological Sciences, University of Milano, Milano, Italy

[3]Department of Animal Production, Grugliasco (TO), Italy

[4]Department of Animal Husbandry Sciences, Grugliasco (TO), Italy

Correspondence: G B Palmegiano, CNR Institute of Science of Food Production, Torino Division, Via L. da Vinci 44, Grugliasco (TO), Italy. E-mail: giovanni.palmegiano@ispa.cnr.it

Abstract

The efficiency of diets with the inclusion of Spirulina for Siberian sturgeon weaning has been tested. Three isoproteic and isoenergetic diets were formulated with an increasing level of Indian strain Spirulina (SP 40%, SP 50% and SP 60%); the diets were tested against a control diet without microalgae. The results show that Spirulina inclusion improves growth and that an inclusion level of 50% gave the greatest growth rate, a better favourable feed conversion rate and the highest protein efficiency. The fatty acid composition of fillets showed differences between the experimental and control diets: an increase in the Spirulina level induces increases in palmitic and linoleic acids and a decrease in the myristic acid. The control diet was characterized by high levels of eicosapentaenoic and docosahexaenoic acids. At the end of the experiment, statistical differences appeared in the fatty acid profile of the sturgeon fillet, mainly concerning high content of monounsaturated fatty acid and polyunsaturated fatty acid in the sturgeon fillets. If the problems related to the high production costs are solved, Spirulina could prove a good partial substitute fish meal.

Keywords: Spirulina, sturgeon, productive traits, fatty acids

Introduction

Diets in aquaculture are based on conventional feedstuffs such as fish oil and fish meal, but these are very expensive. The future development of aquaculture will be greatly constrained by the availability of an alternative source of feed ingredients. The proportion of global fish meal production used in fish feeds has increased from 10% to 35% over the last 15 years. Predictions of fish meal requirements for the future are approximately 44% of the 10-year average global fish meal production (Naylor, Goldberg, Primavera, Kautsky, Beveridge, Clay, Folke, Lubchenco, Mooney & Troell 2000).

A large number of studies have been carried out on use of plant protein sources in fish feeding. Gomes, Rema and Kaushik (1995) have evaluated the efficiency of the substitution of fish meal protein with soybean meal on the performances and body composition in rainbow trout and sturgeon. Different plant protein sources have been evaluated on different freshwater fish (Gouveia & Davies 1999; Borlongan, Eusebio & Welsh 2002; Davies, Gouveia & Tekinay 2002). Sturgeon has mainly been farmed for caviar production but in the last decade there has been increased interest in farming for flesh production. Because of the need to expand production by introducing new species, sturgeon farming has seen an increase. In Italy, which is the largest EU sturgeon producing country, production has increased from 280 t year^{-1}, in the 1980s, to the present 500 t year^{-1} (Guarda, Bertoja, Zoccarato, Tartari & Biolatti 1997). There have been few studies on sturgeon feeding and nutrition, but they often do not concern fish meal and fish oil substitution. Gisbert and Williot (2002) reported a fully detailed review on Siberian sturgeon larval rearing. Papers on Siberian sturgeon feeding have been written by Dabrowski, Kaushik and Fauconneau (1985) and later by Médale,

Corraze and Kaushik (1995). Among the other species of sturgeon, white sturgeon (*Acipenser transmontanus*) feeding has also been investigated to some extent (Hung, Herold, Gawlicka & De la Noue 1998).

Single cell biomass is considered to be a good source of protein and energy (Harel, Koven, Lein, Bar, Beherens, Stubblefield, Zohar & Place 2002). Microalgae has received particular interest (Palmegiano, Forneris, Gai, Sicuro & Zoccarato 2002), and simplified production techniques have been set up to obtain high quantities of plant biomass. To date, microalgae has been used as a fresh meal for mollusc bivalve and fish larvae (Harel *et al.* 2002; Lu, Yoshizaki, Sakai & Takeuchi 2002). Among plant biomasses, Spirulina seems to be a good candidate as a source of protein (Nandeesha, Gangadhara, Manissery & Venkataraman 2001). Spirulina is a freshwater microalgae class *Cyanophyceae*, order *Nostocales*. Some botanists classified Spirulina as a cyanobacteria. This microalgae can grow assembled in colonial or in single cell form. Moreover, micro-organisms can be manipulated to produce high quantities and a high quality of protein and lipids (Harel *et al.* 2002). The aim of the present research is to evaluate the efficiency of diets including Spirulina for sturgeon growing.

Table 1 Formulation of the experimental diets (%)

	Diets			
Ingredients	**Control**	**SP 40**	**SP 50**	**SP 60**
Herring meal	54	20	13	6
Spirulina meal*	0	40	50	60
Fish oil	5.5	6	6	6
Corn oil	1	1	1	1
Soybean oil	1	1	1	1
Wheat bran	3.5	0	0	0
Toasted barley	7.5	9	8	7
Corn flake	0	10	9	7
Corn starch	18	10	9	9
Corn gluten meal	3.5	0	0	0
Lignosulphyte	1	0.5	0.5	0.5
Dicalcium phosphate	3	0.5	0.5	0.5
Vitamin mixture†	1	1	1	1
Brewer's yeast	1	1	1	1

*Spirulina meal (crude protein 60% on 92% dry matter and ether extract 6%) produced by Parry Nutraceuticals, TIAM House Annexe, Chennai, India and purchased by (Azienda Agricola Montepaldi, San Casciano Val di Pesa, Italy).

†Vitamin mixture (IU or mg kg^{-1} diet): DL-a tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15 000 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.

Materials and methods

Siberian sturgeon (*Acipenser baeri*) juveniles were obtained from a private hatchery (Azienda Agricola Pisani) and transferred to the facility of the Department of Animal Production of Torino University. In all, 192 juvenile sturgeons (initial individual weight 92.1 ± 3.6 g) were stocked randomly in 16 fibreglass tanks (1 × 1 × 0.35 m). The experimental design is based on a linear model. Three isoproteic (crude protein (CP) 42%) and isoenergetic (21 MJ kg^{-1} dry matter (DM)) diets were formulated with an increasing level of Indian strain Spirulina (SP 40%, SP 50% and SP 60%) produced by Parry Nutraceuticals (TIAM House Annexe, Chennai, India) and imported by Azienda Agricola Montepaldi (San Casciano Val di Pesa, Italy); these diets were tested against a control diet without microalgae (Tables 1 and 2).

Treatments were assigned to the experimental array according to a completely randomized design, each treatment having four replicates per tank.

At the end of the trial, performance indexes (feed conversion ratio (FCR), protein efficiency ratio (PER) and feeding rate (FR)%) and the thermal growth coefficient ((TGC) is a growth index weighed according to the temperature) were calculated as follows (Cho 1992):

$$\text{FCR} = \text{total feed given (g of DM)/weight gain (g)}$$

$$(\text{PER}) = \text{WG (g)/amount of protein fed (g)}$$

$$(\text{FR})(\%) = \frac{(\text{total amount fed (DM)} \times 100/\text{day of trial})}{e^{(\ln \text{final weight} + \ln \text{initial weight}) \times 0.5}}$$

$$\text{TGC} = [\text{FBW}^{1/3} - \text{IBW}^{1/3}]/\Sigma[T \times D] \times 100$$

where T is the water temperature (°C), the cubic exponent should contain at least four decimals to maintain good accuracy.

Moreover, hepato (HSI) and viscerosomatic (VSI) indexes were calculated per tank on three individuals for each replicate as follows:

$$\text{HSI} = (\text{liver/body weight}) \times 100$$

$$\text{VSI} = (\text{viscera/body weight}) \times 100$$

The water temperature and dissolved oxygen (DO) were measured every 15 days. Water flow was maintained at 2 L min^{-1} throughout; DO was between 3.8 and 4.5 mg L^{-1} and the feeding ratio was 1.2% of the wet biomass.

Table 2 Diets composition (% DM) and energy (MJ kg^{-1} DM)

Diets	Control	SP 40%	SP 50%	SP 60%
Crude protein	42.17 ± 1.41	42.36 ± 1.22	42.85 ± 0.89	42.62 ± 0.69
Ether extract	12.64 ± 0.14	12.58 ± 0.08	11.97 ± 0.38	11.87 ± 0.55
Ash	4.60 ± 0.21	4.38 ± 0.01	4.80 ± 0.30	4.44 ± 0.08
Gross energy	20.90 ± 1.85	21.30 ± 1.00	21.41 ± 0.94	21.43 ± 0.95

Values are means ± SD.
DM, dry matter.

The water temperature was set to 18 °C, but because of a lower winter air temperature, it ranged from 18 to 14 °C with a minimum of 9 °C one night, which occurred during the eighth week, when −13 °C air temperature was recorded during the night.

The feed was supplied 6 days per week, twice a day by hand. All the fish were weighed in bulk every 15 days in order to adjust the feeding quantities.

The experiment lasted 12 weeks.

At the end of the trial, four fish were slaughtered for each tank. The diets and dorsal muscles were analysed for DM, ash, CP, ether extract (EE) and gross energy (GE) according to Association of the Official Analytical Chemists (AOAC) (1990). Moreover, liver samples were analysed for lipid and energy. All the fish tissue samples were freeze-dried before analysis in order to standardize the sample quality. The initial fatty acid composition was detected on four sturgeons randomly sampled from the lot of fish used for the trial, while the diets and the final fish flesh were analysed on 16 sturgeons (four fish per diet) randomly caught and killed. The dorsal fillet was skinned, dissected and immediately frozen in liquid nitrogen.

Three pooled samples of muscle obtained from two fish from each dietary group were prepared. The samples were mechanically ground with the addition of dry ice and carefully mixed. The total lipids were extracted from 1 g of the diets and the muscle tissues using the method of Folch, Lees and Stanley (1957). The fatty acid methyl esters were prepared by transesterification using methanol-HCl (3 N) and finally separated and quantified by gas chromatography (Dani 86.10 HT gas chromatograph) using a capillary column (PAG 30 m × 0.25 mm, 0.25-μm Film) made by Supelco (Bellefonte, PA, USA). Hydrogen was used as gas carrier and temperature programming was from 205 °C (7 min) at 4 °C min^{-1} to 220 °C for 45 min. The individual fatty acids were identified by comparison with known standards.

The apparent digestibility coefficients (ADC) of the diets were determined using the indirect method with Cr_2O_3 (1%) as inert marker added to the four experimental diets need for 4 weeks of feeding. *In vivo* digestibility was evaluated in 300-g (initial weight) fish, the feed was distributed to satiation three times a day and the faeces were collected with a settlement column according to the system developed by Cho, Slinger and Bayley (1982), connected to four 100 L cylindro-conical tanks containing five fish each, equipped with a net bottom (10 × 10 mm mesh). The faecal material of each tank was collected over a period of at least 5 days per week and pooled together for chemical analyses. The determination of the inert marker in the faeces was determined according to Kimura and Miller (1957). Proximate composition of the faeces was performed according to AOAC (1990) rules. The ADC of dry matter ($ADC_{dry\ matter}$) and crude protein ($ADC_{protein}$) were calculated according to Maynard and Loosli (1969).

As far as statistical treatments are concerned, results expressed as percentages and ratios were transformed in arcsin of roots; on transformed and normalized data variance analyses were performed; significant differences were ranged according to the Scheffe test. On statistically significant results, homogeneity of variance was assessed using a goodness of fit KS test.

Results and discussion

Single cell proteins (SCP) are widely used to feed larvae of many fresh and marine water fish (Sargent, Bell, McEvoy, Tocher & Estevez 1999; Harel *et al.* 2002) and to feed sturgeon larvae (Dabrowski *et al.* 1985) in order to improve the level of certain fatty acids. Single cell protein, as yeast or microalgae, is of great interest in fish feeding (Peng & Gatlin III 2003; Lu & Takeuchi 2004); however, the high production cost allows the use only in experimental diets for fish, for this reason no data on practical use are available, except for brewers and torula yeast (Peng & Gatlin III 2004).

Table 3 Performance indexes of sturgeon fed on the experimental diets

Diets	FCR*	PER†	TGC‡	FR%§	Total biomass gain¶
Control	1.39 ± 0.02a	1.90 ± 0.59B	0.0583 ± 0.0198b	0.88 ± 0.05A	338.8 ± 112.6b
SP 40%	1.13 ± 0.16b	3.12 ± 0.44A	0.0818 ± 0.0142a	0.86 ± 0.05A	580.0 ± 90.9a
SP 50%	1.00 ± 0.08b	3.35 ± 0.30A	0.0993 ± 0.0141a	0.77 ± 0.05B	697.0 ± 87.2a
SP 60%	1.22 ± 0.14b	2.72 ± 0.28A	0.0743 ± 0.0123ab	0.75 ± 0.03B	552.3 ± 84.1a

In the column, different letters mean statistical difference at $P \leq 0.05$ and in capital $P \leq 0.01$
Values are means ± SD.
*FCR = total feed given (g of DM)/weight gain (g).
†PER = WG (g)/amount of protein fed (g).
‡TGC = $[FBW^{1/3} - IBW^{1/3}] / \Sigma [T \times D] \times 100$, where T is the water temperature (°C).
§FR (%) = (total amount fed (DM) × 100/day of trial)/$e^{(\ln \text{final weight} + \ln \text{initial weight}) \times 0.5}$.
¶Total biomass gain (g) = final biomass weight (g) – initial biomass weight (g).
FCR, feed conversion ratio; PER, protein efficiency ratio; TGC, thermal growth coefficient; FR, feeding rate.

The feed conversion rate, PER, TGC, FR and biomass gain of the sturgeons are reported in Table 3. The performance indexes show that Spirulina promotes growth better than the control diet, and in particular 50% inclusion seems to result in the best performance: a high biomass increase and growth rate, the best feed conversion rate and a high PER.

The best biomass increase resulted from diet SP 50% and generally speaking the diets containing Spirulina exhibit better production traits.

The FCR and PER in Sturgeon feeding Spirulina-based diets were more favourable than those of the control diet. The TGC of Sturgeon feeding SP 50% and SP 40% were higher than those of the control diet ($P \leq 0.05$). The FR decreased with an increase in the inclusion of Spirulina in the diet up to the minimum values of 0.77 for SP 50% and of 0.75 for SP 60%; the FR of the control diet and of SP 40% are statistically different from SP 50% and SP 60% ($P \leq 0.05$). Very low air temperatures occurred in December, reaching a minimum of -13 °C and because of the fact that the tanks were placed outdoors, the heating system did not buffer the set temperatures of 18 °C and water temperature decreased during the night to 9 °C. Thus, the trend of increase in biomass and the TGC values were affected.

The ADC of the experimental diets are shown in Table 4. No statistical differences appear in the treatments; the levels of Spirulina inclusion in the diets seem to result in an increase in the $ADC_{\text{dry matter}}$ and a decrease in the ADC_{protein}. The highest ADC_{protein} and the lowest $ADC_{\text{dry matter}}$ values appeared in the control diet, whereas the lowest ADC_{protein} and the highest $ADC_{\text{dry matter}}$ values were recorded for the diet containing the highest level of Spirulina inclusion (SP 60%). The observed data are in accordance with the results of Medale, Blanc and Kaushik (1991). The results could be because of the digestibility of non-protein nutrients, such as lipids and carbohydrates, of sturgeon fed on Spirulina diets as stated by Lin, Cui, Hung and Shiau (1997) who showed that sturgeon effectively utilizes carbohydrates such as starch.

Table 4 Apparent digestibility coefficients (ADC) (%) of the experimental diets

Diets	$ADC_{\text{dry matter}}$	ADC_{protein}
Control	62.48 ± 1.62	91.03 ± 1.11
SP 40%	64.53 ± 1.29	86.80 ± 0.57
SP 50%	69.77 ± 4.49	84.68 ± 1.61
SP 60%	75.07 ± 2.24	80.52 ± 3.48

Hepato and viscero somatic indexes, the lipid and energy of the liver, and the biomass gain are reported in Table 5. The VSI of fish fed the control diet was lower than that in the others ($P \leq 0.05$), while no statistical differences between treatments were apparent in the HSI, lipid and energy values of the liver.

The HSI values are similar to those recorded by Hung, Moore, Bordner and Conte (1987) and by Fynn-Aikin, Hung, Liu and Li (1992) for sturgeon fed high carbohydrate level, while the values reported by Kaushik, Luquet, Blanc and Paba (1989) are higher. The values of the lipid in the liver are not similar: 29.6–37.2% and 18.3–26.1%, respectively, for Fynn-Aikin and colleagues (1992) and in the present paper, probably because of D-glucose as a consequence of the carbohydrate source in the trial by Fynn-Aikin and colleagues (1992), composed with the more complex carbohydrates of Spirulina in the present experiment.

Table 5 Somatic indexes and liver lipid and energy content of sturgeon fed on the experimental diets

Diets	HSI*	VSI†	Liver lipid (% of dry matter)	Liver energy (MJ kg^{-1})
Control	2.25 ± 0.38	6.56 ± 1.68B	20.66 ± 2.76	24.50 ± 2.54
SP 40%	2.49 ± 0.70	9.38 ± 0.94A	18.35 ± 3.50	23.68 ± 1.52
SP 50%	2.45 ± 0.85	7.28 ± 1.34AB	20.50 ± 4.07	23.38 ± 1.62
SP 60%	3.07 ± 0.86	9.49 ± 0.25A	26.15 ± 2.46	23.50 ± 2.22

In the column, different letters mean statistical difference: A, B at $P \leq 0.01$.
Values are means ± SD.
*Hepatosomatic index (HSI) = (liver/body weight) × 100.
†Viscerosomatic index (VSI) = (viscera/body weight) × 100.

Table 6 Fillet composition

Diets	Initial	Control	SP 40%	SP 50%	SP 60%
Dry matter (DM)	18	21.98 ± 0.91	19.37 ± 1.30	19.34 ± 2.35	19.29 ± 0.98
Crude protein (% DM)	60	68.25 ± 4.16	77.78 ± 0.93	73.27 ± 3.33	60.25 ± 5.48
Ether extract (% DM)	4.45 ± 0.12	15.01 ± 1.80	7.63 ± 0.66	10.15 ± 2.39	12.37 ± 2.97
Ash (% DM)	5	4.33 ± 0.22	4.90 ± 0.50	5.51 ± 1.13	5.19 ± 0.91
Gross energy (% DM MJ Kg^{-1})	16	22.02 ± 1.18	21.08 ± 1.03	22.56 ± 0.39	23.40 ± 0.98

Values are means ± SD.

Table 7 Fatty acid composition of Spirulina and experimental diets (% of total fatty acids)

Fatty acids	Spirulina	Control	SP 40	SP 50	SP 60
C10:0	1.2 ± 0.1				
C11:0	7.6 ± 0.5				
C12:0	1.1 ± 0.4				
C14:0		5.2 ± 0.1	4.3 ± 0.6	4.6 ± 0.5	4.4 ± 0.3
C16:0	30.6 ± 1.0	18.8 ± 1.3	24.3 ± 0.7	25.6 ± 0.4	29.3 ± 0.6
C16:1n-7	5.2 ± 0.8*	7.0 ± 1.0	7.0 ± 0.4	6.5 ± 1.3	6.9 ± 1.1
C18:0		2.6 ± 0.0	2.2 ± 0.1	2.1 ± 0.0	2.4 ± 0.3
C18:1n-9	1.7 ± 0.1*	14.7 ± 0.2	13.1 ± 0.6	12.5 ± 0.0	12.5 ± 0.1
C18:1n-7		5.4 ± 0.1	4.4 ± 0.9	5.5 ± 0.6	2.2 ± 0.1
C18:2n-6	12.0 ± 0.1	12.6 ± 1.0	17.0 ± 1.2	16.3 ± 0.6	18.2 ± 1.5
C18:3n-6	21.4 ± 0.4	0.3 ± 0.5	3.5 ± 0.1	4.2 ± 0.3	5.3 ± 0.3
C18:3n-3	Trace†	1.4 ± 0.0	1.1 ± 0.1	1.1 ± 0.0	1.1 ± 0.0
C18:4n-3		1.8 ± 0.2	1.3 ± 0.1	1.2 ± 0.1	1.1 ± 0.1
C20:1n-9		4.9 ± 0.2	3.7 ± 0.2	3.7 ± 0.1	3.3 ± 0.2
C20:4n-6		0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.4 ± 0.0
C20:4n-3	Trace†	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
C20:5n-3		8.0 ± 0.1	5.9 ± 0.5	5.7 ± 0.2	5.0 ± 0.6
C22:1n-9		6.5 ± 0.4	4.4 ± 0.5	4.2 ± 0.3	3.9 ± 0.6
C22:5n-3		1.1 ± 0.1	0.8 ± 0.1	0.5 ± 0.5	0.5 ± 0.2
C22:6n-3		8.7 ± 1.1	5.6 ± 1.0	5.3 ± 0.4	3.2 ± 0.3
SFA	40.6 ± 2.1	27.0 ± 1.2	30.4 ± 1.2	32.7 ± 1.0	34.7 ± 2.6
MUFA	7.9 ± 2.3	37.9 ± 1.2	32.9 ± 1.0	31.6 ± 1.8	28.1 ± 2.2
PUFA	33.5 ± 0.5	32.7 ± 1.3	35.1 ± 0.6	34.2 ± 1.1	35.7 ± 4.2
n-3		19.6 ± 1.1	13.7 ± 0.8	12.9 ± 0.8	12.6 ± 1.8
n-6		13.1 ± 0.8	21.5 ± 0.6	21.2 ± 1.9	23.1 ± 1.3
n-3/n-6	–	1.5 ± 0.1	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.2
UFA/SFA	1.0 ± 0.1	2.6 ± 0.2	2.2 ± 0.1	2.0 ± 0.1	1.9 ± 0.2
S/P		0.38 ± 0.02	0.45 ± 0.02	0.50 ± 0.02	0.54 ± 0.06

*Sum of the series n-7 and n-9.
† < 0.08 mg g^{-1}.
SFA, saturated fatty acid; MUFA, mono unsaturated fatty acid; PUFA, poly unsaturated fatty acid; UFA, unsaturated fatty acid.

Table 8 Fatty acids of fillet at the beginning and at the end of the trial (% of total fatty acids)

Fatty acids	Initial	Control	SP 40	SP 50	SP 60
C14:0	3.6 ± 0.5B	4.1 ± 0.2A	3.9 ± 0.1AB	3.7 ± 0.1B	2.9 ± 0.2C
C16:0	20.9 ± 1.4B	20.6 ± 1.0B	22.6 ± 0.3A	23.5 ± 0.7A	23.3 ± 0.4A
C16:1n-7	7.4 ± 0.4A	5.5 ± 0.3C	6.9 ± 0.4B	6.9 ± 0.3B	5.1 ± 0.0C
C18:0	2.0 ± 0.4C	3.6 ± 0.3A	2.6 ± 0.1B	2.2 ± 0.1BC	3.6 ± 0.2A
C18:1n-9	18.7 ± 0.5C	24.5 ± 0.5A	20.2 ± 0.1B	20.7 ± 0.4B	22.1 ± 0.2A
C18:1n-7	5.3 ± 0.8	4.2 ± 0.5	3.9 ± 0.6	3.7 ± 0.3	4.2 ± 0.1
C18:2n-6	10.3 ± 1.2B	9.0 ± 0.7C	9.1 ± 0.2C	12.2 ± 0.4A	10.7 ± 0.0AB
C18:3n-6	1.4 ± 0.8B	0.4 ± 0.0C	1.2 ± 0.1B	1.8 ± 0.1A	1.7 ± 0.1A
C18:3n-3	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	0.9 ± 0.0
C18:4n-3	1.0 ± 0.1	0.7 ± 0.2	1.0 ± 0.0	0.8 ± 0.1	0.6 ± 0.0
C20:1n-9	3.1 ± 0.3B	4.9 ± 0.8A	3.5 ± 0.4B	2.8 ± 0.2B	2.8 ± 0.2B
C20:4n-6	1.0 ± 0.2C	1.1 ± 0.0C	1.3 ± 0.0B	1.1 ± 0.1C	1.7 ± 0.0A
C20:4n-3	0.7 ± 0.1	0.7 ± 0.2	0.6 ± 0.0	0.7 ± 0.0	0.6 ± 0.0
C20:5n-3	7.1 ± 0.8a	6.3 ± 0.5b	6.8 ± 0.1a	5.7 ± 0.3c	5.7 ± 0.0c
C22:1n-9	1.2 ± 0.2AB	0.6 ± 0.3C	1.3 ± 0.1A	1.1 ± 0.2BC	0.7 ± 0.1C
C22:5n-3	1.9 ± 0.2	1.4 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.6 ± 0.1
C22:6n-3	12.1 ± 1.7A	9.7 ± 0.9B	11.4 ± 0.3A	9.0 ± 0.1B	10.2 ± 0.2AB
SFA	26.6 ± 1.2	28.3 ± 1.4	29.1 ± 0.5	29.5 ± 0.7	29.8 ± 0.3
MUFA	35.7 ± 0.9b	40.8 ± 1.1a	35.7 ± 0.7b	35.3 ± 0.6b	34.8 ± 0.2b
PUFA	37.7 ± 1.0a	31.0 ± 2.1b	35.1 ± 0.4a	35.2 ± 1.3a	35.4 ± 0.5a
n-3	23.9 ± 2.8	19.7 ± 1.5	22.4 ± 0.4	18.6 ± 0.5	19.5 ± 0.2
n-6	13.8 ± 2.5	11.2 ± 0.6	12.8 ± 0.3	16.6 ± 0.7	15.8 ± 0.3
n-3/n-6	1.8 ± 0.6A	1.7 ± 0.1A	1.7 ± 0.1A	1.1 ± 0.0B	1.2 ± 0.0B
UFA/SFA	2.8 ± 0.2	2.5 ± 0.2	2.4 ± 0.0	2.4 ± 0.1	2.4 ± 0.0
S/P	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.0

A, B, C: $P \leq 0.01$; a, b, c: $P \leq 0.05$.

SFA, saturated fatty acid; MUFA, mono unsaturated fatty acid; PUFA, poly unsaturated fatty acid; UFA, unsaturated fatty acid.

The proximate analysis of sturgeon fillets is shown in Table 6. There were no statistical differences between treatments.

The fatty acid composition of the diets is reported in Table 7; diets that contain Spirulina are characterized by higher amounts of saturated fatty acids, mainly palmitic acid (C16:0) and higher amounts of n-6 fatty acids, mainly linoleic acid. Moreover, the control diet is characterized by higher n-3 fatty acid contents and, in particular, by eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids.

The fatty acid composition of the sturgeon fillets is reported for the beginning and the end of the trial in Table 8. In comparison to the initial values, the myristic acid increased in fillets of fish fed on the control diet and it decreased in those fed on the diets with increasing inclusion of Spirulina. By contrast, the palmitic acid content decreased in fillets of fish fed on diets with increasing inclusion levels of Spirulina in comparison with the starting point. There are increased values of stearic acid in fillets of fish fed with the experimental diets and also in comparison with the intial values. The trends in individual fatty acids support the lack of differences in the sum of saturated fatty acids for all the diets and the starting values. The differences in the monounsaturated fatty acid content, with the lower values being observed in fish fed on the control diet and the initial value are, because of the high content of C18:1n-9. This fatty acid is higher in fillets of all fish fed diets that include Spirulina than in the starting values, but, in turn, the oleic acid content of the SP 40% and SP 50% is lower than that of the SP 60% and the control diet. In comparison with its initial value, total polyunsaturated fatty acid decreases in the control diet, but remaines constant at around 35% in fillets of sturgeon that had been fed on the Spirulina diets and does not differ much from the initial value (37%). This is because of the high amounts of docosahesaenic, γ linolenic and arachidonic acids in the diets.

Irrespective of the diets, the values of the n-3/n-6 ratio are mainly because of the higher levels of linoleic acid, of C18:3n-6 and of arachidonic acid; moreover, the DHA values are lower in Spirulina inclusion diets SP 50% and in the control diets than the starting point and SP 40% (Table 8).

The results of the present paper confirm that sturgeons as other species (Sargent *et al.* 1999) are able to

elongate and desaturate fatty acids from C18:3n-3, C18:4n-3 and EPA to DHA and from linoleic acid to arachidonic acid as already reported by Hung and colleagues (1998).

The results of the present paper show the effectiveness of Spirulina in sturgeon growing. In the future, Spirulina might represent a good opportunity to partially substitute fish meal if the high production cost problems are solved. Future Spirulina utilization, like other new lipid and protein sources for aquaculture, will depend on production costs and its competitiveness with fish-based products. Large-scale production of algal biomass represents a potential high-quality substitute for fish-based ingredients in aquaculture feeds for sturgeon growing.

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