

**Desempenho produtivo e perfil de ácidos graxos de alevinos de carpa húngara
alimentados com ração enriquecida com Spirulina**
**Productive performance and fatty acid profile of hungarian carp fingerlings fed with
Spirulina enriched feed**
**Rendimiento productivo y perfil de ácidos grasos de alevinos de carpa húngara
alimentados con ración enriquecida con Spirulina**

Recebido: 01/12/2019 | Revisado: 06/12/2019 | Aceito: 18/12/2019 | Publicado: 21/02/2020

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Resumo

O incentivo para o consumo de pescado pela população aumentou a produção de peixes em cativeiro e conseqüentemente aumentou a demanda por rações. Este estudo objetivou avaliar a utilização da microalga *Spirulina* como fonte proteica, em substituição a levedura *Saccharomyces cerevisiae*, sobre o desempenho produtivo e o perfil de ácidos graxos das carcaças de alevinos de carpa húngara (*Cyprinos carpio*). Para realização deste experimento utilizou-se uma ração basal formulada com farelo de soja e levedura desidratada (controle), e outras rações formuladas com a microalga *Spirulina* substituindo a levedura em níveis de 25, 50, 75 e 100%. Foram utilizados 280 alevinos de carpa húngara com 50 dias e peso vivo inicial médio de $2,87 \pm 0,02$ g. As rações enriquecidas com a microalga aumentaram linearmente o ganho de peso e taxa de crescimento específico dos alevinos. Os ácidos graxos saturados representaram mais de 50% do conteúdo lipídico. Em relação aos ácidos graxos poliinsaturados observou-se aumento crescente conforme aumentou-se a substituição de proteína da ração pela microalga.

Palavras-chave: Aquicultura; Lipídios; Microalga.

Abstract

The incentive for fish consumption by the population increased its captive breeding production and consequently, increased the demand for feeds. This study aimed at evaluating the use of *Spirulina* microalga as source of protein, in place of the *Saccharomyces cerevisiae* yeast, on the productive performance and fatty acid profile of Hungarian carp fingerlings (*Cyprinos carpio*). In order to carry out this experiment, a basal ration formulated with soybean meal and dehydrated yeast (Control), and other rations formulated with the *Spirulina* microalga substituting yeast at 25, 50, 75 and 100% levels. 280 Hungarian carp fingerlings with 50 days and average initial live-weight of 2.87 ± 0.02 g were used. The microalgae enriched rations increased linearly the weight gain and the specific growth rate of the fingerlings. The saturated

fatty acids represent more than 50% of the lipid content. In relation to the polyunsaturated fatty acids, an increasing rise was observed as the protein substitution of the ration by the microalgae increased.

Keywords: Aquaculture; Lipids; Microalgae.

Resumen

El incentivo para el consumo de pescado por parte de la población aumentó la producción de peces cautivos y, en consecuencia, aumentó la demanda de alimento. Este estudio tuvo como objetivo evaluar el uso de la microalga *Spirulina* como fuente de proteína, reemplazando la levadura *Saccharomyces cerevisiae*, sobre el rendimiento productivo y el perfil de ácidos grasos de alevines de carpa húngara (*Cyprinos carpio*). Para este experimento, se utilizó una dieta basal formulada con harina de soja y levadura deshidratada (control), y otras dietas formuladas con la microalga *Spirulina* que reemplazan la levadura en niveles de 25, 50, 75 y 100%. Se utilizaron 280 alevines de carpa húngara con 50 días y un peso vivo inicial promedio de $2,87 \pm 0,02$ g. Las dietas enriquecidas con microalgas aumentaron linealmente el aumento de peso y la tasa de crecimiento específico de los alevines. Los ácidos grasos saturados representaron más del 50% del contenido de lípidos. Con respecto a los ácidos grasos poliinsaturados, se observó un aumento creciente a medida que aumentaba la sustitución de la proteína de alimentación por microalgas.

Palabras clave: Acuicultura; Lípidos; Microalgas.

1. Introduction

The world per capita consumption of fish increased from an average of 9.9 kg in 1960 to 17.0 kg in the 2000s to 18.9 kg in 2010. This increase has been as a result of the combination of population growth, income rise and urbanization associated to strong expansion of fish production and modern means of distribution (FAO, 2014).

Fish play an important role in global food security and in nutritional necessities of the people in developing and developed countries (FAO, 2014). Their nutritional benefits such as the high content of unsaturated fatty acids, as well as high biological proteins value, result in large participation of fish in the food market (Widjaja et al., 2009).

One of the most used protein sources in the preparation of rations for aquatic organisms is the soybean meal, however, this source presents antinutritional factors such as proteases inhibitors and lectins, and sulfur deficiency in the amino acids (Furuya et al., 2004). An

alternative ingredient in the substitution of soybean meal in fish feed is *Saccharomyces cerevisiae* yeast, which has a protein content ranging from 37 to 45% crude protein (Lazzari et al., 2007).

Another alternative for protein source substitution in feeds is the *Spirulina* microalgae which presents a high amount of protein and amino acid content, similar to those recommended by FAO (Food and Agricultural Organization). This microalga is considered as a source of carotenoids and essential fatty acids, with emphasis on gamma linolenic acid (Holman & Malau-Aduli, 2013). The biomass of the *Spirulina* microalgae has a GRAS (General Recognized as Safe) certificate, issued by the FDA (Food and Drug Administration) recognizing this microorganism safe for consumption, enabling it to be used in food, pharmaceutical and medical areas (Soccol et al., 2013; Mendonça et al., 2012). In addition, in the natural environment, microalgae serve as basis for feeding the fish larvae food chain as well as the zooplankton in the food chain and in aquaculture to feed fish, crustaceans and molluscs (Brown et al., 1997).

The fish as well as the rest of the animals are unable to endogenously produce ω -9, ω -6 e ω -3 fatty acids, therefore, they must be supplied by the feed. Although the influence of environmental parameters in determining lipid requirements for fish is important, feeding is the major factor that contributes to the fatty acid profile of these animals. Studies have shown that some farmed fish contain a greater amount of ω -3 fatty acids in their meat than in natural fish (Suárez-Mahecha et al., 2002).

This work had the objective of evaluating the utilization of *Spirulina* microalgae as protein source, in place of *Saccharomyces cerevisiae*, on the productive performance and the fatty acids profile of the carcasses of Hungarian carp fingerlings (*Cyprinos carpio*).

2. Material and Methods

2.1 Elaboration of rations

The experimental diets were isoproteic, containing an average of 40% brutal protein. Soybean meal associated to *Saccharomyces cerevisiae* or *Spirulina sp.* LEB-18 was used (Morais et al., 2008). The substitution of yeast by the microalgae took place at levels 0 (control), 25, 50, 75 and 100 % (Table 1).

To elaborate the formulations, the ingredients were first of all ground and sieved. The diets were mixed in a y-type mixer, pelleted and dried at 50 °C for 18 h in an air circulating dryer. After which, they were ground and sieved to obtain a diameter between 1.0 to 2.38 mm. To each formulated ration, humidity, ash, proteins (AOAC., 2012), digestibility (Sgarbieri, 1996) and lipids (Bligh & Dyer, 1959) were determined.

Table 1. Formulations and ingredients used in the diets.

Ingredients	<i>Spirulina</i> sp. LEB18 biomass concentration (%)				
	0	25	50	75	100
Yeast	36	27	18	9	0
Soybean meal	36	32	30	28	27
Wheat meal	10	10	7	6	2
Maize	9	11	15	17	19
<i>Spirulina</i>	0	9	18	27	36
Soybean oil	7	9	10	11	14
Premix ¹	1	1	1	1	1
Common salt	1	1	1	1	1
Total	100	100	100	100	100

¹ Vitamin premix and mineral composition (per kg): Vitamin A: 160,000 UI; Vitamin D₃: 50,000 UI; Vitamin E: 3,000 mg; Vitamin C: 5,000 mg; Vitamin K₃: 200 mg; Riboflavin: 400 mg; Pantothenic acid: 1,000 mg; Niacin: 2,000 mg; Vitamin B₁₂: 6,000 mcg; Folic acid: 120 mg; Thiamine: 400 mg; Pyridoxine: 300 mg; Copper: 12,000 mg; Iron: 1,200 mg; Manganese: 300 mg; Iodine: 10,000 mg; Selenium: 2,000 mg; Zinc: 1,000 mg; Calcium: 6 g; Cobalt: 2,000; Choline 85 g; Phosphorus: 2 g; Lysine 30 g; Magnesium: 1 g; Sodium: 2.5 g; Threonine: 25 g;

2.2 Biological tests

For biological tests, 280 Hungarian carp fingerlings (*Cyprinos carpio*) were used with 50 days and initial live weight of 2.87 ± 0.02 g, distributed in a completely distributed in a completely randomized design with 5 treatments and 4 replicates. The experiment lasted for 30 days and each experimental unit consisted of 20 aquariums with 50 L of water, each with an individual and constant aeration, containing 14 fingerlings.

The animals were feed at a rate of 5% of the biomass, divided twice a day. The physical and chemical variables, being the temperature and dissolved oxygen (YFF-55 oximeter), pH (PHS-3B digital pH meter), total ammonia (Alfakit AT 10P photo colorimeter microprocessor) and alkalinity of water were evaluated volumetrically three times a week (ALPHA, 1998). It affected the daily cleaning of the bottom of the aquariums through syphoning and exchange of 30 % of water after the first feeding.

From the beginning to end of the 30 days experiment, biometrics of the animals were taken, evaluating the weight, total length and the growth rate (TCE), calculated through the $TCE = (\ln PF - \ln PI) / t \times 100$ equation, being: $\ln PF$ = Negative logarithm of the final weight; $\ln PI$ = Negative logarithm of the initial weight; t = experiment time (d). The total length was obtained by measuring the anterior region of the head to the end of the upper lobe of the caudal fin. The survival was obtained at the end of the experiment by the difference between the number of animals initially stocked and the number of surviving animals in each treatment.

2.3 Fish body chemical composition analysis

At the end of the experimental period, 5% of the animals from each treatment were euthanized and frozen (-18 °C). The analysis of the body chemical composition was performed with the whole fish, determining crude protein, lipids, ash and moisture (AOAC., 2012). The determination of the fatty acids profile was performed from the fish remains, using the method proposed by Bligh & Dyer (1959) for the extraction of total lipids. The methylation of fatty acids was performed by the method of Metcalfe et al. (1966). The determination of fatty acids was performed in a Varian - 3400CX, a gas chromatograph equipped with a flame ionization detector and a fused silica column containing stationary phase of polyethylene glycol, 30 m long and 0.32 mm in diameter. The entrainment gas was nitrogen at 0.5 mL min⁻¹. The injector and detector temperatures were 250 and 280 °C, respectively. The initial column temperature was 100 °C followed by an increase of 8 °C min⁻¹ to 230 °C, maintaining it constant for 20 minutes. Fatty acids were identified by comparing the retention times with standards and quantified by area normalization. The chemical composition and digestibility results of the rations were subjected to the variance analysis and tukey test, and for the data performance and fish body composition, ANOVA and regression analysis.

3. Results and Discussion

In Table 2, the chemical composition and the digestibility of the rations are presented. The ration with 100% protein substitution by the microalgae presented a low humidity (3.2%) in relation to the others. The lipid content was low in the control diet (3.2%) and the diets enriched with microalgae presented a growing increase in lipids content from 4.0 to 6.1%. The diets were isoprotein as expected, varying from 41.3 to 42.3% of protein, and the diets with low amount of *Spirulina* sp. LEB-18 were more digestible since this microalga is easily digested because its cell wall is composed of mucopolysaccharides, simple sugars and proteins (Tomaselli, 1997).

Table 2. Ration chemical composition and digestibility.

<i>Spirulina</i> (%)	Humidity (%)	Ashes* (%)	Protein* (%)	Digestibility* (%)	Lipids* (%)
0	3.6 ± 0.11 ^a	6.7 ± 0.07 ^a	41.3 ± 0.15 ^a	74.8 ± 0.02 ^a	3.2 ± 0.01 ^a
25	4.0 ± 0.11 ^b	7.0 ± 0.03 ^b	41.9 ± 0.60 ^a	81.7 ± 0.28 ^b	4.0 ± 0.11 ^b
50	3.5 ± 0.05 ^a	6.8 ± 0.03 ^{ab}	41.2 ± 0.50 ^a	82.9 ± 0.35 ^c	4.2 ± 0.08 ^b
75	4.1 ± 0.06 ^b	7.2 ± 0.07 ^c	41.9 ± 0.61 ^a	85.5 ± 0.16 ^d	5.2 ± 0.16 ^c
100	3.2 ± 0.04 ^c	7.2 ± 0.08 ^c	42.3 ± 0.28 ^a	87.2 ± 0.50 ^e	6.1 ± 0.12 ^d

The same letters in column did not differ from each other, while different letters presented a significant difference at 95% confidence level by the Tukey test.

* results expressed as dry basis.

The performance results (Table 3) of the fingerlings demonstrate that the substitution of yeast by *Spirulina* sp. LEB-18 had an increasing effect on the average weight gain of Hungarian carp fingerlings.

The specific growth rate increased with the increase in *Spirulina* concentration, and this effect can be attributed to the better nutritional quality, which was verified by Olivera-Novoa et al. (1998), who evaluated the substitution of fish meal with *Spirulina maxima* meal in the diet of *tilapia* (*Oreochromis mossambicus*).

The survival rate of the fingerlings was 100% for all the treatments. Studies by Watanuki et al. (2006), using carps (*Cyprinus carpio*) fed with *S. plantensis* showed that the survival index were maximized, suggesting that diets with *Spirulina* have immunostimulatory effects for the species studied.

In relation to the body chemical composition, there was a reduction in the crude protein ($p \leq 0,01$) with an increase of the *Spirulina* flour level in the diet (Table 4). Similar results were

observed by El-sayed (1994) for goldlined seabream fingerlings (*Rhabdosargus sarba*) fed with *Spirulina* flour instead of fish flour. Olvera-Novoa et al. (1998) state that protein reduction in fish muscle as the *Spirulina* inclusion level is increased can be attributed to the increase in the hardness of the food, reducing its intake by the animals.

Table 3. Initial weight (IW), weight gain (WG), specific growth weight (SGW), total length (TL) and survival (Sur.) of Hungarian carp fingerlings fed with increasing level of *Spirulina* in substitution to yeast.

Variables	Levels of yeast substitution by <i>Spirulina</i> %					P*
	0	25	50	75	100	
IW (g)	2.87 ± 0.02	2.91 ± 0.01	2.86 ± 0.02	2.90 ± 0.01	2.88 ± 0.02	0.75
WG (g)	0.42 ± 0.07	0.39 ± 0.15	0.56 ± 0.10	0.49 ± 0.10	0.62 ± 0.12	0.01*
SGW (%)	0.46 ± 0.08	0.42 ± 0.16	0.59 ± 0.11	0.51 ± 0.08	0.65 ± 0.10	0.02**
TL (cm)	5.82 ± 0.23	5.81 ± 0.26	5.96 ± 0.24	6.02 ± 0.17	5.57 ± 0.35	0.08
Sur. (%)	100	100	100	100	100	-

Mean values ± standard deviation. *P = level of significance ($P \leq 0.05$). *Increasing linear regression ($y = 0.40 + 0.002x$, $R^2 = 0.20$), ** Increasing linear regression ($y = 0.43 + 0.001x$, $R^2 = 0.25$).

The levels of ether body extract exceeded (in percentage) the difference in soybean oil levels of the experimental diets, and probably the addition of *Spirulina* may have determined this elevation. Takeuchi et al. (2008) observed the increase in the deposition of linoleic and γ -linolenic acids in juvenile tilapia fed with *Spirulina* compared to the control diet.

Table 4. Body chemical composition of Hungarian carp fingerlings fed with increasing level of *Spirulina* in substitution to yeast.

Levels of <i>Spirulina</i> (%)	Proteins (%) ¹	Lipids (%) ²	Ashes (%)	Humidity (%) ³
0	18.6 ± 0.48	2.1 ± 0.10	1.9 ± 0.04	79.9 ± 0.05
25	18.3 ± 0.01	6.1 ± 0.09	1.7 ± 0.01	79.5 ± 0.03
50	18.1 ± 0.06	7.0 ± 0.20	1.7 ± 0.05	79.8 ± 0.61
75	18.3 ± 0.30	6.6 ± 0.01	1.8 ± 0.03	79.2 ± 0.07
100	18.4 ± 0.25	6.6 ± 0.20	1.9 ± 0.07	78.7 ± 0.46
P*	0.001	0.001	0.60	0.001

¹PB: Linear regression: $y=95.05 - 0.005x$ $R^2=0.74$; ²EE: Polynomial regression: $y=12.08 + 0.71x - 0.0054x^2$ $R^2=0.74$; ³UMID: Polynomial regression: $y=79.80 + 0.0008x - 0.0001x^2$ $R^2=0.68$. *P = Level of significance ($P \leq 0.05$).

Table 5 presents the main fatty acids found in the carcass of the Hungarian carp and it can be observed that the saturated fatty acids account for more than 50% of the lipid content. The polyunsaturated fatty acids varied from 4.3 to 38.5%, registering a growing increase of polyunsaturated fatty acids, with the increase of the feed protein substitution by microalgae. These values are greater when compared to those found by Andrade et al. (1995) in the fish species *Cyprinus carpio* (5,4%), *Pseudoplatystoma corruscans* (20,7%), *Brycon cephalus* (13,8%) e *Brycon orbignyianus* (7,2%).

Table 5. Fatty acids profile in Hungarian carp (%).

Fatty acids	Substitution levels of <i>Spirulina</i> by yeast (%)				
	0	25	50	75	100
C14:0	14.1	5.8	Nd	Nd	Nd
C14:1	Nd	Nd	7.6	9.3	Nd
C16:0	52.8	48.2	43.0	45.4	Nd
C16:1	12.6	30.9	21.2	18.7	Nd
C18:0	10.1	3.1	5.7	5.1	9.9
C18:1	2.9	4.7	6.6	4.0	Nd
C18:2 ω-6	0.6	0.4	5.9	6.9	Nd
C18:3 ω-3	1.0	1.1	2.4	1.9	17.1
C20:1	1.8	0.5	Nd	Nd	Nd
C20:2	2.2	0.2	1.1	1.0	7.0
C20:3	0.4	2.9	6.7	Nd	2.5
C20:4 ω-6	Nd	0.7	Nd	6.2	1.0
C20:5 ω-3	Nd	Nd	Nd	0.3	7.0
C21:0	1.4	0.3	Nd	Nd	14.6
C22:0	Nd	0.3	Nd	1.1	0.9
C22:6	Nd	0.3	Nd	Nd	Nd
% saturated	78.5	58.3	48.6	51.5	58.1
% monounsaturated	17.2	36.1	35.4	32.0	3.5

% poly-unsaturated	4.3	5.6	16.0	16.4	38.5
* AGPI/AGS	0.1	0.1	0.3	0.3	0.7
ω -6/ ω -3	0.6	1.0	2.5	6.0	<0.1

nd= not detected. * AGPI/AGS: polyunsaturated and saturated fatty acid ratio

Palmitic acid (C16: 0) was the predominant fatty acid, followed by palmitoleic acid (C16: 1). For the fingerlings that consumed ration with 100% protein substituted by the microalga, the predominant fatty acid was arachidic fatty acid (C20:0), followed by α -linolenic acid (C18:3 ω -3) and heneicosanoic acid (C21:0). A predominance of these fatty acids in the fish remains could have been as a result of the microalga influence in the diets. Colla et al. (2004) found that the highest percentage of fatty acids present in *Spirulina* strains was palmitic acid (C16:0), followed by linolenic acids (C18:3) and linoleic (C18:2). According to Palmegiano et al. (2008) in experiments realized with “white sturgeon” fingerlings (*Acipenser transmontanus*) using diets containing *Spirulina*, the predominant fatty acids were oleic acids (C18: ω -9), palmitic acid (C19:0) linoleic acid (C18:2 ω -6) and α -linolenic (C18:3 ω -3), showing that in the diets containing *Spirulina*, the fatty acids C16:0, C18:2 e C18:3 stand out.

According to the Department of Health of England, diets that present AGPI/AGS ratio above 0.45 (DHSS, 1984) and ω -6/ ω -3 ratio below 4.0 (DH, 1994), are considered healthy under the human nutritional point of view. The fish subjected to treatment with 0, 25, 50, 57% yeast substitution with microalga presented an APGPI/AGS ratio below recommendation. However, in ration to the ω -6 and ω -3, only the treatment with 75% yeast substitution by the microalga exceeded the recommended maximum.

In this work, only the fish subjected to 100% yeast substitution treatment with *Spirulina* microalgae presented values as recommended by the Department of Health of England, with AGPI/AGS of 0.66 and ω -6/ ω -3 relation of 0.04, indicating that *Spirulina* sp. LEB-18 can constitute a nutritional source for the preparation of fish rations (Table 5).

The physical and chemical characteristics of water did not present significant variation between the treatments, having a mean temperature of 21 ± 1.2 °C, dissolved oxygen 7.3 ± 1.2 mg/L, pH 7.1 ± 2 , total ammonia 0.3 ± 0.1 mg/L and alkalinity 48 ± 5 mg of CaCO₃/L, these being adequate conditions for the cultivation of common carp fingerlings according to Watanuki et al. (2006) and according to the recommended pisciculture limits (CONAMA, 2005). Makinouchi (1980) affirms that the best carp growth is within a temperature range of 24 °C to 28 °C. Although during the experiment, the temperature was lower than the one

recommended by the author, it can be assumed that these temperatures did not influence negatively since behavioral observations, feed intake, growth and weight remained satisfactory throughout the experimental period.

4. Conclusions

The partial or total substitution of the yeast with *Spirulina* sp. LEB-18 microalga caused an increase in linear weight gain of the fingerlings, proving that the microalga concentrations in the rations used satisfy the necessities of the Hungarian fingerlings carp.

The saturated fatty acids represented more than 50% of the lipid content of the carcasses and for the polyunsaturated fatty acids, a growing increase was observed as the protein ration was substituted by microalga.

Considering that the lipid composition of fish reflects directly on the amount of their diet, the AGPI/AGS and ω -6/ ω -3 ratios observed in the Hungarian carp fed with 100% *Spirulina* sp. LEB-18 ration indicate their nutritional value, making this microalga a protein source for the preparation of fish rations.

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