Spanish Journal of Agricultural Research
16 (1), e0606, 7 pages (2018)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2018161-11698
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, O.A, M.P. (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Effect of spirulina (*Arthrospira platensis*) supplementation on tilapia (*Oreochromis niloticus*) growth and stress responsiveness under hypoxia

Ignacio Plaza[1,2], José L. García[2] and Morris Villarroel[1]

[1]*Universidad Politécnica de Madrid, ETSIAAB, Dept. de Producción Agraria, Avda. Puerta de Hierro 2, 28040 Madrid, Spain.* [2]*CSIC, Centro de Investigaciones Biológicas, Biotecnología Medioambiental. C/ Ramiro de Maeztu 9, 28040 Madrid, Spain.*

Abstract

Several recent studies have shown that *Arthrospira* sp. supplementation of feeds has a beneficial effect on fish health and growth, but less is known about its possible effects on stress responsiveness. The present study was designed to evaluate using *Arthrospira platensis* as a feed supplement for *Oreochromis niloticus* fry, reared in recirculating aquaculture systems. Two isocaloric and isonitrogenous fishmeal-based diets were prepared with 0% and 1% *A. platensis* and fed to fry, approximately 10 mg live weight at the beginning of the experimental period (n=16 tanks, 8 tanks per treatment), at a feeding rate of 6% live weight, four meals a day for 50 d. The weight gain per tank, specific growth rate and feed conversion ratio were similar among treatments but *A. platensis* supplementation significantly increased survival ($p<0.05$). Stress responsiveness was measured in all fish from 12 tanks using a non-invasive two-choice test. All fish from one home tank (n=6 tanks per treatment), were placed into a shaded tank where oxygen levels were slowly reduced. A doorway was then opened to a second illuminated tank with normal oxygen levels and the number of fish that left the home tank were counted. The fish fed *A. platensis* stayed significantly ($p=0.001$) longer in the home tank (30.20 min ± 13.22) than controls (17.35 min ± 8.32), suggesting a lower stress responsiveness and a higher tolerance to hypoxia.

Additional keywords: fish; feed additive; two-choice; behaviour; non-invasive.

Abbreviations used: CTR (control fish); FCR (feed conversion ratio); SPR (*Arthrospira platensis* supplemented fish).

Authors' contributions: Conceived and designed the experiments, and drafted the manuscript: IPG, MV, JLG. Performed the experiments, analysed and interpreted the data: IPG and MV. Contributed reagents/materials/analysis tools: MV and JLG.

Citation: Plaza, I.; García, J. L.; Villarroel, M. (2018). Effect of spirulina (*Arthrospira platensis*) supplementation on tilapia (*Oreochromis niloticus*) growth and stress responsiveness under hypoxia. Spanish Journal of Agricultural Research, Volume 16, Issue 1, e0606. https://doi.org/10.5424/sjar/2018161-11698

Received: 11 May 2017. **Accepted**: 23 Mar 2018.

Copyright © 2018 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC-by 4.0) License.

Funding: Cofunded by the Community of Madrid with structural funds from the European Union FEDER (INSPIRA1, Ref. S2013/ABI2783).

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Ignacio Plaza: i.plazagordo@gmail.com

Introduction

Aquaculture provides more than half of the world's fish (FAO, 2016) but better feeding strategies are needed in order to keep it sustainable. One option is to use natural feed additives instead of artificial antibiotics, which cannot be used as growth promoters in the European Union (EC, 2003) since they promote microbial resistance. *Arthrospira platensis* is a natural product that can improve the quality of fish production by providing a rich source of vitamin B_{12} and β-carotene (20 times more than carrots), essential amino acids, fatty acids (ω3 and ω6), minerals (James *et al.*, 2006), protein (approx. 65% dry weight; Phang *et al.*, 2000), as well as anti-oxidant properties. For all of these reasons, it is commonly used as a natural supplement in animal feeds, including for fish (McCarty, 2007). Less is known about how *A. platensis* can improve the condition of weaker fish or their response to stress.

Several studies have considered the effects of *A. platensis* supplementation (less than 10% of diet) in fish. Red swordtail (*Xiphophorus helleri*) given 8% *A. platensis* increased feed consumption, body weight, length, gonad weight and number of offspring (James *et al.*, 2006). In three-spot gourami (*Trichopodus trichopterus*), Khanzadeh *et al.* (2016) found that 5% *A. platensis* (replacing fishmeal) improved feed intake, feed conversion ratio (FCR) and the gonadosomatic index. Rainbow trout (*Oncorhynchus mykiss*) fed with 10 % *A. platensis* (a natural pigment source) deposit more carotenoids in muscle tissue (Teimouri *et al.*, 2013). Adding *A. platensis* to feed for guppies (*Poecilia reticulata*) improved their tolerance to a toxin (methyl red), manifested by a noticeable reduction in the cytotoxic effects on red blood cells (Sharma *et al.*, 2005). In *Oreochromis niloticus*, adding 0.5, 1 or 2% *A. platensis* to feed improves its immune system (Abdel-Tawwab & Ahmad, 2009), has antioxidant effects and promotes growth (Takeuchi *et al.*, 2002), but less is known about its possible effect on behaviour under stressful situations.

Regarding possible behavioural effects, *A. platensis* contains many different nutrients, including tryptophan and B6, which decrease psychological distress in humans (Shor-Posner *et al.*, 1994). Fatty acids ω3, also present in *A. platensis*, can inhibit adrenal activation elicited by a mental stress in dogs (Delarue *et al.*, 2003). Thus, supplementing *O. niloticus* diet with *A. platensis* could change their stress responsiveness. In their natural habitat, *O. niloticus* normally consumes algae (including cyanobacteria) and some invertebrates (Ibrahem *et al.*, 2013).

In the past decade, there have been many studies on fish welfare (Huntingford *et al.*, 2006), and several methods have been proposed to measure stress, including non-invasive behavioural methods to measure stress coping styles using a two-choice test (Laursen *et al.*, 2011). Barreto & Volpato (2011) found that *O. niloticus* can be sorted into two opposing coping styles under stress, namely proactive (dominant) or reactive (passive). It is assumed that the development of a coping style depends on the genetic makeup of the fish and its previous experience (Sørensen *et al.*, 2013), but less is known about possible effects of nutrition. In this study we set out to verify whether a 1% *A. platensis* supplementation affected growth and survival as well as the behavioural response to stress using the two-choice test.

Table 1. Summary of the four different solutions used to prepare the culture medium for *Arthrospira platensis*. Solutions A and B were added to the macroelement solution at the rate of 1 mL/L, and the trace solution at 100 mL/L.

Solutions	
Macroelements	**g/L**
$NaHCO_3$	16.50
NaCl	1.00
K_2HPO_4	0.55
$NaNO_3$	2.50
K_2SO_4	1.00
Solution A	
H_3BO_3	0.28
$MnCl_2\ 4H_2O$	1.81
$CuSO_4\ 5H_2O$	0.08
$ZnSO_4\ 7H_2O$	0.22
MoO_3	0.02
Solution B	
$CoN_2O_6\ 6H_2O$	0.44
$NiSO_4\ 7H_2O$	0.48
$TiSO_4$	0.20
Trace solution	
$C_{10}H_{12}N_2O_8FeNa\ 3H_2O$	0.08
$CaCl_2$	0.04
$MgSO_4\ 7H_2O$	2.00
$FeSO_4\ 7H_2O$	0.01

Material and methods

Algae culture

We used the *A. platensis* strain PCC 9108 obtained from the Culture Collection at the Spanish National Research Council (CSIC). *A. platensis* was grown in plastic bioreactors (120 × 12 cm) in a greenhouse at the Universidad Politécnica de Madrid (40.446353 N, -3.738341 E), from July to August using the natural photoperiod. The cultivation media was composed of four solutions (Table 1) modified from Zarrouk (1966). *A. platensis* was harvested when the culture reached approx. 1 g/L of dry weight. The harvest was performed from 9:30 to 10:30 am when the protein content was higher (Jourdan, 1999). Then the cells were dried on a horizontal sheet at 50ºC for approx. 4-6 h and kept in an opaque container at 4°C to prevent oxidation before inclusion in the fish feed.

Fish and feeds

O. niloticus fry originally purchased from Valenciana de Acuicultura (Puçol, Valencia), were housed in 16 green fiberglass tanks (120 L, 0.46 m high, and 0.64 m in diameter). Each tank was connected to a filter (EHEIM Classic; MOD. 2217, 6 L of capacity, 20 W and water flow 1000 L/h) and aerated with an air pump common to all tanks. This set up allowed us to maintain independent water conditions in each tank so as not to mix sediments and bacteria among treatments. The filters were prepared by inoculating nitrifying bacteria

(EHEIM water care), using the supplier indications. We added 5 ppm of ammonia daily for 3 weeks, testing the evolution of nitrogen compounds, until microbial activity was satisfactory. The distribution of treatments among the tanks was random.

O. niloticus fry were introduced in the tanks randomly and given a two-week acclimatization period. After that, all the fish from each tank were weighed in bulk and their length measured individually using a ruler (day 0). The initial average weight per tank was a 2.64 ± 0.45 mg (mean ± SD; n= 25) and initial fish total length was 1.17 ± 0.10 cm, with no significant differences among treatments. Fish were counted and weighed in bulk at 30 d, and counted, weighed and measured individually at 50 d. To quantify mortality during the fry phase, tanks were checked every day and dead fish were counted and removed. Mortalities were not concentrated on any particular day nor in any particular tank.

To make the experimental diet, commercial feed (Skretting T3) was crushed and sifted to a crumb size of 0.5-1 mm, mixed with 1% of *A. platensis* by weight and passed through the same sieve. The control diet was made in the same way but with no added algae. To analyze the proximal composition of the feed, five samples were taken per treatment and the percentage of dry matter (DM) was obtained by oven drying at 105ºC, to constant weight, and the ash content by incineration at 550ºC. Protein content was measured by Kjeldahl analysis and the ether extract by hydrolysis. The fiber content was obtained by the Van Soest *et al.*´s (1991) method and finally the energy was calculated using a bomb calorimeter. Both feeds were isocaloric and isoproteic and were kept at 4ºC until used (see Table 2). Fish were fed at 6% live weight using an auto-feeder (EHEIM 3581) that provided four meals a day (at 7:00, 12:00, 17:00 and 22:00 h), beginning the trial on 04 Aug until 24 Sept 2015, for a total of 50 days (7 weeks). The filters were turned off for 20 min after each meal to avoid feed loss.

Table 2. Proximate composition of experimental diets.

Composition[1]	Control	*A. platensis*
DM (%)	92.60	92.60
Ash (%)	7.39	7.37
CP (% DM)	44.59	44.83
EE (% DM)	6.39	6.44
NDF (% DM)	26.48	26.23
ADF (% DM)	4.98	5.05
ADL (% DM)	0.86	0.87
CE (kcal /g DM)	5.00	4.98

[1]DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; CE, crude energy).

Water quality

Water quality measurements were taken in all tanks twice a week on Monday and Thursday after the first meal (from 9:30 to 10:00 h). The measurements included dissolved oxygen (DO), electrical conductivity (EC), and levels of ammonia, nitrites and nitrates (Hanna HI83203). Water quality parameters were maintained within normal values for *O. niloticus* growth (mean ± S.E.; temperature 27.3 ± 0.25ºC; pH 7.36 ± 0.33; EC 0.60 ± 0.07 dS; DO_2 7.19 ± 0.17 ppm; NH_4 0.01 ± 0.02 ppm; NO_2 0.01 ± 0.02 ppm; NO_3 13.48 ± 5.78 ppm), and were not significantly different between treatments.

Two-choice test

At the end of the trial (50 d), we performed a two-choice test based on Laursen *et al.* (2011), built using two circular tanks that were identical to the grow-out tanks (120 L) and attached to one another via a closable gate, 16 cm in diameter (Fig. 1). Tank A was tightly covered with black plastic (shaded) and Tank B was illuminated (natural light). Twelve two choice tests were carried out, using six tanks per treatment, discarding the tanks with less than 20 remaining fry (for *A. platensis* fry, where all tanks had over 20 fish, the six tanks were chosen randomly). For each test, all the fish in a tank were placed in Tank A at the

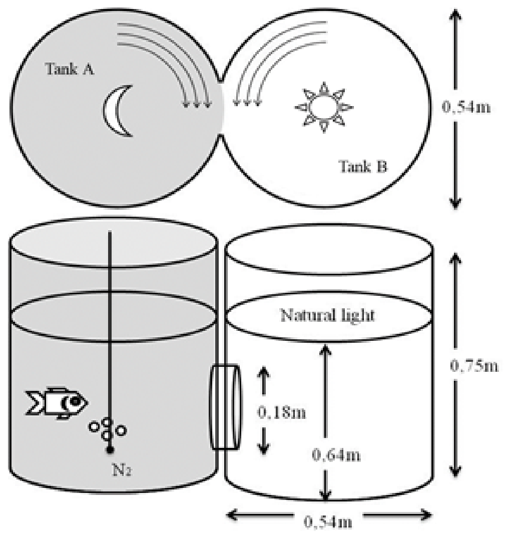

Figure 1. Two-choice scheme with two tanks attached via a closable door. Tank A was in darkness and water was infused with nitrogen to decrease oxygen levels. Tank B was under natural light and had normal oxygen levels. Water flow in the tanks was opposite to avoid mixing water from one tank to another

same time and left to acclimate for 30 min. Then the door to the second tank (Tank B) was opened, and Tank A was bubbled with nitrogen gas to decrease oxygen levels (at a rate of 1 ppm per 10 min). Tank B was oxygenated to maintain normal oxygen levels (approx. 7 ppm) and a counter-current water flow was set up in each tank to avoid water flowing between tanks. Oxygen levels were monitored in both tanks every 5 min. Each test was recorded with a video camera to avoid the effects of any human disturbance. We noted the times when any fish left Tank A. The test ended when the first five fish had left Tank A or after one hour had passed. Then we measured the length of the fish in Tank B.

Statistical analysis

All statistical tests were carried out using R.commander (R Core Team, 2013). According to a Shapiro/Wilk test, the data on growth and survival were normally distributed and were analyzed using a one-way ANOVA. The data from the two-choice tests did not follow a normal distribution and were analyzed using a Kruskal-Wallis test. The level of significance was $p<0.05$ in both cases.

Results

Fish growth and survival

There were no significant growth differences among treatments after 30 or 50 d. Total body length was not significantly different among treatments (cm; mean ± SD; 4.13 ± 1.09 control fish, CTR, 4.22 ± 0.97 A. platensis supplemented fish, SPR), nor the coefficient of variation in total length (mean ± SD; 0.26 ± 0.08 CTR, 0.28 ± 0.08 SPR). However, there were significant differences regarding mortality ($p>0.05$), which was lower in fry that received 1% of *A. platensis* (Table 3).

Two-choice test

The coping styles of the tilapia were significantly different between treatments ($p>0.05$). More SPR fry adopted a proactive coping style, taking a significantly longer time to leave Tank A, despite decreasing oxygen levels (Fig. 2). The CTR fish tended to leave Tank A in groups of two or more fish, while SPR fry tended to leave one by one ($p>0.05$; see Table 4). Also, focusing on fish size, it appeared that fry that left Tank A were smaller, less than 3 cm long in both treatments, but the difference was not significant ($p=0.148$)

Table 3. Mean (±S.E.) initial bulk weight (IBW), bulk weight gain (BWG g), feed conversion ratio (FCR) and % survival of *Oreochromis niloticus* controls and those fed *Arthrospira platensis.*

	Control (n=8)	***A. platensis* (n=8)**	***p*-value**
IBW (d0)	2.42±0.53	2.86 ±0.76	0.20
BWG (d30)	26.21±2.57	26.07 ±2.61	0.82
BWG (d50)	66.93±7.15	70.55 ±8.33	0.32
FCR (d30)	0.73±0.07	0.74 ±0.09	0.89
FCR (d50)	0.83±0.10	0.79 ±0.07	0.40
% Survival (d30)	84.5±7.23 ^{a}	93.5 ±8.80 ^{b}	0.04
% Survival (d50)	84.0±6.76 ^{a}	93.0 ±8.50 ^{b}	0.03
% Survival (d30-50)	99.5±1.54	99.5 ±1.41	0.89

Values followed by different superscript letters in the same row are significantly different ($p<0.05$).

Table 4. Mean exit times (±SD) of the first five fish during the two-choice test, mean oxygen levels when the first five fish left Tank A and the percentage of fish that left Tank A in groups.

	Control (n=6)	***A. platensis* (n=6)**	***p*-value**
Mean exit time (min)	17.35^{a} ±8.32	30.20^{b} ±13.22	0.001
Mean exit O_2 (ppm)	4.01^{a} ±1.35	2.83^{b} ± 1.67	0.001
Group leavers (%)	87^{a}	40^{b}	0.021

Values followed by different superscript letters in the same row are significantly different ($p<0.05$).

Discussion

The main goal of fish nutrition is to provide a balanced mixture of ingredients to support vital functions at an acceptable cost. Following this line of thought, at the moment it would not be cost-effective to use *A. platensis* (≈40 €/kg) as a protein substitute, but it may be useful as a nutritional supplement since it improves feed efficiency, carcass quality, and physiological response to stress in several species of fish (Takechi *et al.*, 2002; Abdel-Tawwab & Ahmad, 2009; Velasquez *et al.*, 2016). In the current study, adding 1% *A. platensis* to feed did not affect production indices (FCR, growth) compared to controls. This contradicts previous studies such as James *et al.* (2006), using *Xiphophorus helleri* (8% supplementation) and Abdel-Tawwab & Ahmad (2009) (0.5% to 1% supplementation) who observed improved growth. However, Takeuchi *et al.* (2002) found that the specific growth rate and feed efficiency of *Arthrospira* sp.-fed *Orechromis* sp. were lower than that of controls. Our findings of a 9% higher survival rate are in accordance with other studies that report 6% higher survival at 1% *Arthrospira* sp. supplementation after a bacterial challenge (Ibrahem *et al.*, 2013). However, Abdel-Tawwab & Ahmad (2009), and Ungsethaphand *et al.* (2010) did not observe differences in survival among fish fed with Arthrospira sp. compared to controls. Our study may be different, however, due to the size of the fish. When the trial began, tilapias were in the fry stage (around 2-3 cm body length), when they begin to change from being more omnivorous to carnivorous, at about 6-7 cm. After that they change back again to more phytoplanktivorous filter feeding (Ibrahem *et al.*, 2013). The change to carnivorous feeding could increase hierarchical stress and cannibalism, reducing the survival of weaker fry (Berrios & Snow, 1983). In this scenario, the high vitamin content of *A. platensis*, among other possibilities, could have played a role, helping to promote growth and decrease mortality.

Two-choice test

Regarding the two-choice test, our results suggest that more of the fry consuming *A. platensis* supplemented feed adopted a proactive coping style, since more of them remained in a stressful environment despite the possibility of escape. As suggested by Barreto & Volpato (2011) the fish that remain in the "stressful" tank have a lower reaction to stress and can be classified as proactive. Although several recent studies have assessed coping styles in fish (*e.g.*, Ferrari *et al.*, 2016), few have considered the effects of nutritional supplementation. Some authors have shown that *A. platensis* affects plasma cortisol and glucose levels. In rainbow trout (*Oncorhynchus mykiss*) cortisol and glucose significantly decreased with increasing levels of inclusion of *A. platensis* at 0 to 10% (Yeganeh *et al.*, 2015). In great sturgeon (*Huso huso*), Adel *et al.* (2016) found that blood glucose increased at a higher inclusion of *A. platensis* (10%). Furthermore, according to Delarue *et al.* (2003), adding ω3 fatty acids to fish diets can inhibit adrenal activation elicited by psychological stress. *Arthrospira* sp. has a high level of unsaturated long chain fatty acids such as ω3 and ω6, which may have provided a similar effect in *O. niloticus*. In this sense, Anzola (2013) found a decrease in psychological stress with higher tryptophan intake in dogs, and Shor-Posner *et al.* (1994) found the same effect in humans with tryptophan and vitamin B6 supplementation. *A. platensis* contains tryptophan (1.5% DM) and vitamin B6 (0.8% DM). Taking into account that the tryptophan requirement of *O. niloticus* is 1% of the diet, with the inclusion of 1% *A. platensis*, it was increased to 1.15%. The vitamin B_6 requirements in juvenile *Oreochromis* sp. are 15 to 16 ppm of the diet (Shiau & Hsieh, 1997), but in the current study that was increased to 80 ppm (500% more than estimated control levels) by adding 1% of *A. platensis*.

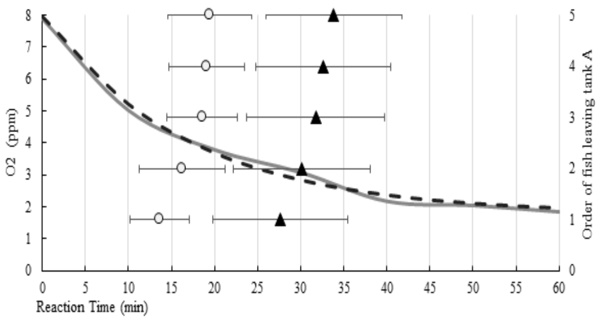

Figure 2. Mean (±SD) exit times of the first five fish per treatment (n=6), differentiating between controls (O) and *Arthrospira platensis* supplemented tilapia (▲). The continuous line is the O_2 concentration (ppm) in Tank A, and a regression curve (---) was used to calculate the O_2 concentration at which fish exited [y (O_2) = 1.78+6.19*0.94x (min), *r*=0.98].

In fish, body length is a strong predictor of individual positions in a hierarchy (Ward *et al.*, 2006). Large fish are presumably more proactive, dominating other individuals, taking prime feeding sites and aggressively excluding smaller subordinate competitors (Webster & Hixon, 2000). In our study, although it appeared that smaller fish left Tank A first, we did not find significant differences with regard to fish size. However, the control fish left Tank A in groups, as compared to the fish fed *A. platensis* who left individually, which supports the idea that when fish which feel threatened they seek the safety of others. Finally, although it was not our aim to measure resistance to hypoxia, the results also suggest that SPR fish were more tolerant to low oxygen levels. This is in accordance with other studies in humans, where *Arthrospira* sp. supplementation increases cardiovascular capacity (Kalafati *et al.*, 2010). Moreover, it has been found that *Arthrospira* sp. increase hematocrit levels of *O. niloticus* (Ibrahem *et al.*, 2013), which improves the efficiency of oxygen consumption. This finding is important for the aquaculture industry since oxygen levels are often a limiting factor for production (Abdel-Tawwab *et al.*, 2015).

In summary, supplementing *O. niloticus* fry with *A. platensis* strain PCC 9108 increased survival and the proportion of individuals that adopted a proactive coping style, based on the results from a two-choice test. The results also suggest that *A. platensis* increased resistance to hypoxia, which is important for intensive aquaculture production.

References

Abdel-Tawwab M, Ahmad MH, 2009. Spirulina (*Arthrospira platensis*) as a growth and immunity promoter for Nile tilapia, *Oreochromis niloticus* (L.), challenged with pathogenic *Aeromonas hydrophila*. Aquac Res 40 (9): 1037-1046. https://doi.org/10.1111/j.1365-2109.2009.02195.x

Abdel-Tawwab M, Hagras AE, Elbaghdady HAM, Monier MN, 2015. Effects of dissolved oxygen and fish size on Nile tilapia, *Oreochromis niloticus* (L.): growth performance, whole-body composition, and innate immunity. Aquacult Int 23 (5): 1261-1274. https://doi.org/10.1007/s10499-015-9882-y

Adel M, Yeganeh S, Dadar M, Sakai M, Dawood MA, 2016. Effects of dietary *Spirulina platensis* on growth performance, humoral and mucosal immune responses and disease resistance in juvenile great sturgeon (*Huso huso Linnaeus,* 1754). Fish Shellfish Immunol 56: 436-444. https://doi.org/10.1016/j.fsi.2016.08.003

Anzola B, 2013. The use of tryptophan in shelter dogs to treat stress-related anxiety disorders. Rev Cient 23 (1): 26-32.

Barreto RE, Volpato GL, 2011. Ventilation rates indicate stress-coping styles in Nile tilapia. J Biosci 36 (5): 851-855. https://doi.org/10.1007/s12038-011-9111-4

Berrios-Hernandez JM, Snow JR, 1983. Comparison of methods for reducing fry losses to cannibalism in tilapia production. Prog Fish Cult 45 (2): 116-118. https://doi.org/10.1577/1548-8659(1983)45[116:COMFRF]2.0.CO;2

Delarue JOCP, Matzinger O, Binnert C, Schneiter P, Chiolero R, Tappy L, 2003. Fish oil prevents the adrenal activation elicited by mental stress in healthy men. Diabetes Metab 29 (3): 289-295. https://doi.org/10.1016/S1262-3636(07)70039-3

EC, 2003. Regulation Nº 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrient. Official Journal of the European Union, 43. [Lex - 32003R183].

FAO, 2016. El estado mundial de la pesca y la acuicultura. 2016. Contribución a la seguridad alimentaria y la nutrición para todos. Roma. 224 pp.

Ferrari S, Horri K, Allal F, Vergnet A, Benhaim D, Vandeputte M, Chatain, B, Bégout ML, 2016. Heritability of boldness and hypoxia avoidance in European seabass, *Dicentrarchus labrax*. Plos One 11 (12): e0168506. https://doi.org/10.1371/journal.pone.0168506

Huntingford FA, Adams C, Braithwaite VA, Kadri S, Pottinger TG, Sandøe P, Turnbull JF, 2006. Current issues in fish welfare. J Fish Biol 68 (2): 332-372. https://doi.org/10.1111/j.0022-1112.2006.001046.x

Ibrahem MD, Mohamed MF, Ibrahim MA, 2013. The role of spirulina platensis (*Arthrospira platensis*) in growth and immunity of Nile tilapia (*Oreochromis niloticus*) and its resistance to bacterial infection. J Agr Sci 5 (6): 109-117. https://doi.org/10.5539/jas.v5n6p109

James R, Sampath K, Thangarathinam R, Vasudevan I, 2006. Effect of dietary spirulina level on growth, fertility, coloration and leucocyte count in red swordtail, *Xiphophorus helleri*. Isr J Aquac 58: 97-104.

Jourdan JP, 1999. Cultivez votre spiruline, manuel de culture artisanale de la spiruline. Antenna Technology, Paris, France.

Kalafati M, Jamurtas AZ, Nikolaidis MG, Paschalis V, Theodorou AA, Sakellariou GK, Kouretas D, 2010. Ergogenic and antioxidant effects of spirulina supplementation in humans. Med Sci Sports Exerc 42 (1): 142-151. https://doi.org/10.1249/MSS.0b013e3181ac7a45

Khanzadeh M, Fereidouni AE, Berenjestanaki SS, 2016. Effects of partial replacement of fish meal with spirulina platensis meal in practical diets on growth, survival, body composition, and reproductive performance of three-spot gourami (*Trichopodus trichopterus*) (Pallas, 1770). Aquacult Int 24 (1): 69-84. https://doi.org/10.1007/s10499-015-9909-4

Laursen DC, Olsén HL, de Lourdes Ruiz-Gómez M, Winberg S, Höglund E, 2011. Behavioural responses to hypoxia provide a non-invasive method for distinguishing between stress coping styles in fish. Appl Anim Behav Sci 132 (2): 211-216. https://doi.org/10.1016/j.applanim.2011.03.011

McCarty MF, 2007. Clinical potential of spirulina as a source of phycocyanobilin. J Med Food 10 (4): 566-570. https://doi.org/10.1089/jmf.2007.621

Phang SM, Miah MS, Yeoh BG, Hashim MA, 2000. Spirulina cultivation in digested sago starch factory wastewater. J Appl Phycol 12 (3): 395-400. https://doi.org/10.1023/A:1008157731731

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.r-project.org. [21 Sept, 2013].

Sharma S, Sharma S, Sharma KP, 2005. Protective role of spirulina feed in a freshwater fish (*Poecilia reticulata Peters*) exposed to an azo dye-methyl red. Ind J Expl Biol 43: 1165-1169.

Shiau SY, Hsieh HL, 1997. Vitamin B6 requirements of tilapia *Oreochromis niloticus* x *O. aureus* fed two dietary protein concentrations. Fish Sci 63 (6): 1002-1007. https://doi.org/10.2331/fishsci.63.1002

Shor-Posner G, Feaster D, Blaney NT, Rocca H, Mantero-Atienza E, Szapocznik J, Baum MK, 1994. Impact of vitamin B6 status on psychological distress in a longitudinal study of HIV-1 infection. Int J Psychiatry Med 24 (3): 209-222. https://doi.org/10.2190/7VD8-DA67-8T9L-UCHL

Sørensen C, Johansen IB, Øverli Ø, 2013. Neural plasticity and stress coping in teleost fishes. Gen Comp Endocr 181: 25-34. https://doi.org/10.1016/j.ygcen.2012.12.003

Takeuchi T, Lu J, Yoshizaki G, Satoh S, 2002. Effect on the growth and body composition of juvenile tilapia (*Oreochromis niloticus*) fed raw spirulina. Fish Sci 68 (1): 34-40. https://doi.org/10.1046/j.1444-2906.2002.00386.x

Teimouri M, Amirkolaie AK, Yeganeh S, 2013. The effects of spirulina platensis meal as a feed supplement on growth performance and pigmentation of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 396: 14-19. https://doi.org/10.1016/j.aquaculture.2013.02.009

Ungsethaphand T, Peerapornpisal Y, Whangchai N, Sardsud U, 2010. Effect of feeding spirulina platensis on growth and carcass composition of hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*). Maejo Int J Sci Technol 4 (2): 331-336.

Van Soest PV, Robertson JB, Lewis BA, 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Anim Sci 74 (10): 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Velasquez SF, Chan MA, Abisado RG, Traifalgar RFM, Tayamen MM, Maliwat GCF, Ragaza JA, 2016. Dietary spirulina (*Arthrospira platensis*) replacement enhances performance of juvenile Nile tilapia (*Oreochromis niloticus*). J Appl Phycol 28 (2): 1023-1030. https://doi.org/10.1007/s10811-015-0661-y

Ward AJ, Webster MM, Hart PJ, 2006. Intraspecific food competition in fishes. Fish Fish 7 (4): 231-261. https://doi.org/10.1111/j.1467-2979.2006.00224.x

Webster MS, Hixon MA, 2000. Mechanisms and individual consequences of intraspecific competition in a coral-reef fish. Mar Ecol Prog Ser 196: 187-194. https://doi.org/10.3354/meps196187

Yeganeh S, Teimouri M, Amirkolaie AK, 2015. Dietary effects of *Spirulina platensis* on hematological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Res Vet Sci 101: 84-88. https://doi.org/10.1016/j.rvsc.2015.06.002

Zarrouk C, 1966. Contribution a l'etude d'une cyanobacterie: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthese de *Spirulina maxima* (Setchell et Gardner) Geitler. PhD thesis, University of Paris, France.