Health Sciences Research
2017; 4(1): 1-5
http://www.aascit.org/journal/hsr
ISSN: 2375-379X

American Association for Science and Technology

Keywords
Nile catfish,
Spirulina platenesis,
Subfeeding, Energy Stores,
Electrolyte

Received: March 28, 2017
Accepted: April 18, 2017
Published: June 13, 2017

Effects of Dietary Supplementation of *Spirulina platensis* on the Physiological Parameters of *Clarias gariepinus* After Exposure to Food Shortage Stress

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Citation
Mustafa Ahmed Fawzy, Alaa El-Din Hamed Sayed, Nasser Sayed Abou Khalil. Effects of Dietary Supplementation of *Spirulina platensis* on the Physiological Parameters of *Clarias gariepinus* After Exposure to Food Shortage Stress. *Health Sciences Research*. Vol. 4, No. 1, 2017, pp. 1-5.

Abstract
Fishes are exposed naturally to fluctuation in the food availability along its life which adversely impact their physiological conditions. Dietary inclusionof *Spirulina platensis* in aquatic food formulation has a wide range of welfare and economic advantages. Hence, this study had been constructed to highlight the potential influences of food shortage on the serum metabolites and electrolyte variables of Nile catfish, and the possible modulatory impacts of graded *Spirulina* doses (1.25, 2.5, and 5g/Kg diet) applied for 2 months following this dietary stress. Limited dietary resource (2% of body weight) resulted in exhaustion of energy stores, impairment of renal function, and disturbance in electrolyte balance. *Spirulina* alleviated the depletion of energy reserves without any beneficial modulation regarding electrolyte equilibrium. Therefore, further studies are warranted to examine the most optimum dose of *Spirulina* and duration of exposure to achieve the most favorable impacts on both levels of metabolic and ionic balances.

1. Introduction

Nutritional status of the fishes faces many realistic challenges in the aquatic environment especially during migration, transportation, and disease outbreak, and fluctuations in prey resource availability, environmental temperature and water quality [1]. It heavily impacts performance and physiological condition of fishes [2]. Most of light is sheded on complete fasting and fasting-refeeding models, whereas studies on energy intake limitation in aqua-creatures are quite rare. Disrupting energy balance accompanied by feed restriction in fishes was exemplified by depletion of lipid and protein contents, increased retention of long-chain polyunsaturated fatty acids, alteration of phospholipid composition, downregulation of stearoyl-CoA desaturases, and upregulation of muscle-specific lipoproteinlipase [3]. Nowadays, one of the most attractive research topics in the field of aquafeed is supplementation of fishes with alternative sources of protein which satisfy many physiological and economic requests. In this fascinating area, algae have emerged as interesting unconventional protein additives in fish meals, particularly in tropical and subtropical regions where algae production is high [4]. In consequence of their richness in balanced amino acid profiles, vitamin B, pigments, nucleotides and carbohydrates, dietary algal inclusion was associated with improvements of growth, feed utilization, lipid metabolism, physiological activity, stress response, disease resistance, and carcass quality in fishes [5]. Of these algae, *Spirulina platensis* was in the vicinity of focus due to its nutritional constituents of protein, carotenoid, omega-3 and -6 polyunsaturated fatty acids, gamma linolenic acid, sulfolipids, glycolipids, polysaccharides, vitamins, and minerals. Owing to its high dietary profile, it was considered as a strong fighter against starvation and malnutrition in the world [6]. Actually, the literature is punctuated with several evidences indicating its contribution as a positive modulator of body weight, biochemical performance, food conversion, and cytoprotection in fishes [7].

It is worthy to note that this investigation is a continuation to a previously published study [8]. However, the mission of the current work is to elucidate the possible influences of dietary restriction on Nile catfish (*Clarias gariepinus*) on one side, and the promising utilization of *Spirulina platensis* as a gifted food supplement to combat the undernutrition on the another side, with special emphasis on the alternations in the serum metabolites and electrolyte outcome measures.

2. Materials and Methods

The culture of *Spirulina platenesis* was grown in Zarrouk's medium [9] at pH 9 and incubated at 30°C under continuous illumination fluorescent light of 48.4 µmole.$m^{-2}.s^{-1}$. The alga was cultivated in 50 L photobioreactors using a unialgal semi-continuous culture [10]. For diet preparation, aliquot of the algal suspension was filtered through Whatman glass fiber (0.45 µm), and the obtained pellet was weighed and added to the basal diet in different doses.

Ninety six healthy Nile catfish (490±17.8 g) were caught from the fish farm of Faculty of Agriculture, Assiut University, Egypt. They were reared in aerated glass tanks (100 L capacity) and acclimatized for two weeks. They were fed commercial pellets at a rate of 5% of wet weight twice daily.

Isolated *Spirulina* culture was added to the basal diet whose nutritional constituents were illustrated in table 1 to represent graded concentrations at 0.0 (control), 1.25, 2.5 or 5.0 g *Spirulina*/kg diet. Each concentration of *Spirulina* was suspended in 100 mL distilled water and added to the diet, and blended for 40 min to make a pasty mixture. The pastes were separately passed through a grinder, and pelleted (1 mm diameter) in a paste extruder. The diets were air-dried and stored in plastic bags in a refrigerator for further use. The mean values of water quality during the study were the following: temperature 29±0.4°C, dissolved oxygen 6.9±0.3 mg L^{-1}, pH 7.5±0.08, and electrical conductivity 269.25±13.65 µmho cm^{-1}.

Table 1. *Nutritional composition of the basal diet of Nile catfish (Clarias gariepinus).*

Ingredient	Percentage
Soybean meal (48%)[a]	30.1
Cotton meal (41%)	10.0
Menhaden meal (61%)	4.0
Corn grain	33.6
Wheat middlings	20.0
Dicalcium phosphate	0.6
Catfish vitamin and mineral mix[b]	0.2
Fat/oil	1.5

[a]Values in the parentheses represent percentage protein.

The adapted fishes were exposed to food shortage stress by feeding at 2% of body wet weight daily for two months. After that, fishes were weighed and classified randomly into 5 groups according to the concentrations of *Spirulina*. Each group contained three replicates (8 fishes/100 L tank). Then, fishes were exposed to different concentrations of *Spirulina*; 0.0 (control), 1.25, 2.5 or 5.0 g *Spirulina*/kg diet for two months.

At the end of experiment, six of eight fishes from each group were randomly selected and anesthetized using tricainemethanesulfonate (0.4 g L^{-1}). Blood was collected by cardiac puncture using sterilized syringes. Serum was isolated after centrifugation at 3000 rpm for 10 min, and stored at -80°C until assay of the studied parameters latter on. Creatinine (Cr), uric acid, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), glucose, cholesterol, and total protein (TP) were determined by colorimetric kits. The concentrations of HCO_3^-, Na^+, K^+, Cu^{+2}, Fe^{+2} and Ca^{+2} were measured in serum using atomic absorption spectrophotometer (GBC Model 300).

Statistical analyses were applied on the data obtained from completely randomized design with three replications. One-way ANOVA was used to test the effects of the dietary treatments. Duncan multiple range test was also applied to compare the means when a significant difference ($p<0.05$) was detected by ANOVA. All the statistical analyses were done using SPSS program version 10 (SPSS, Richmond, VA, USA).

3. Results

As illustrated in table 2, the feed-restricted fishes were characterized by significant hypoglycemia, hypocholesterolemia, and hypoproteinemia. All doses of *Spirulina* appeared to be equipotent in alleviating hypoglycemia. Serum TC increased to the same level of control group following supplementation with the first two doses of *Spirulina* with exception of the third one. Analysis of variance demonstrated a stepwise increase in serum TP by using the graded doses of *Spirulina*.

Liver function markers (AST, ALT, and ALP) did not significantly change in the feed-restricted fishes. On the contrary, the significant drops in serum uricacid and Cr levels of malnourished fish were obvious in comparison with the control ones, reflecting a decline in the metabolic utilization of protein. Serum uric acid levels were reduced in the algal supplemented groups. Most significant elevation in serum Cr levels was apparent after administration of 2.5 g of *Spirulina*/Kg diet, followed in potency by 5 g of *Spirulina*/Kg diet.

Table 2. *Serum levels of biochemical parameters in Nile catfish in the different experimental groups.*

Parameter \ Group	Group I	Group II	Group III	Group IV	Group V
Glucose (mg/dl)	82.03±0.35^{c}	63.07±2.25^{a}	67.73±0.97^{b}	70.40±1.2^{b}	68.77±0.03^{b}
TC (mg/dl)	215.0±1.00^{b}	206±2.89^{a}	211.33±0.33^{b}	213.33±1.45^{b}	203.67±1.45^{a}
TP (mg/dl)	3.67±0.12^{b}	2.97±0.12^{a}	3.63±0.22^{b}	3.97±0.12b^{c}	4.30±0.2^{c}
AST (μ/l)	36.9±0.49^{c}	36.18±1.09^{bc}	33.84±0.15^{a}	34.35±0.12^{a}	35.02±0.26^{ab}
ALT (μ/l)	18.77±0.27^{c}	17.37±0.88^{bc}	15.70±0.32^{ab}	16.63±0.38^{ab}	15.36±0.57^{a}
ALP (μ/l)	42.0±0.58^{a}	43.7±1.45^{ab}	45.0±0.58^{bc}	46.3±0.33^{c}	44.3±0.33^{abc}
Uric acid (mg/dl)	25.4±0.4^{b}	20.97±0.69^{a}	21.97±0.33^{a}	22.30±0.36^{a}	22.23±0.52^{a}
Cr (mg/dl)	0.46±0.01^{d}	0.32±0.01^{a}	0.33±0.01^{ab}	0.36±0.01^{c}	0.35±0.01^{bc}

Group I (control group); Group II (feed-restricted group); Groups III, IV, and V(feed-restricted group supplemented with *Spirulina* at graded doses of 1.25, 2.5, and 5g/Kg diet, respectively).
TP, total protein; TC, total cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Cr, creatinine.
Results are expressed as means±SEM of 6 fishes per group.
Different letters within the same raw indicate significance at $p<0.05$ (one-way ANOVA followed by Duncan multiple range test).

As shown in table 3, it was evident that the feed-restricted fishes suffered from electrolyte disturbances as indicated by hypernatriemia, hypokalemia, hypercupremia, hypoferremia, hypocalcaemia, and metabolic alkalosis. The undernutrition-induced hypernatremia and hypokalemia failed to be modulated by any one of the three doses of *Spirulina*.

SerumHCO_3 levels of either fishes supplemented with 2.5 g of *Spirulina*/Kg diet or those supplemented with 5 g of *Spirulina*/Kg diet were significantly higher than those of the feed-restricted groups. Supplementation of malnourished fishes with any level of algal doses did not result in significant changes in serum Cu^{+2} levels. However, the serum Cu^{+2} levels of the feed-restricted fishes, which received the higher two doses of *Spirulina*, were significantly higher than those of the adequately nourished ones. As compared with the feed-restricted fishes, each of the second and third doses of *Spirulina* caused the most potent increase in serum Fe^{+2} levels. In comparison with the feed-limited fishes, the first dose of *Spirulina* was efficient in overcoming the dietary limitation-induced hypocalcaemia. Whereas, the second and third doses of *Spirulina* were responsible for hypercalcaemia.

Table 3. *Serum levels of electrolyte in African catfish in the different experimental groups.*

Parameter \ Group	Group I	Group II	Group III	Group IV	Group V
Na^{+} (μg/ml)	116.3±2.19^{a}	123.3±0.88^{b}	124.7±0.33^{b}	125.3±0.33^{b}	125.3±0.33^{b}
K^{+} (μg/ml)	4.74±0.28^{b}	3.8±0.25^{a}	3.97±0.09^{a}	4.13±0.09^{a}	3.97±0.07^{a}
HCO_3^- (μg/ml)	13.00±0.58^{a}	15.33±0.88^{b}	16.10±0.46^{b}	19.33±0.33^{c}	18.67±0.88^{c}
Cu^{+2} (μg/ml)	98.8±0.62^{a}	105.8±3.17^{bc}	103.8±1.2^{ab}	108.5±0.32^{bc}	110.9±1.47^{c}
Fe^{+2} (μg/ml)	17.50±0.3^{c}	12.93±0.13^{a}	13.93±0.47^{ab}	14.90±0.45^{b}	15.07±0.27^{b}
Ca^{+2} (μg/ml)	48.42±0.84^{b}	42.13±1.16^{a}	48.79±0.34^{b}	51.59±0.58^{c}	52.14±0.58^{c}

Group I (control group); Group II (feed-restricted group); Groups III, IV, and V (feed-restricted group supplemented with *Spirulina* at graded doses of 1.25, 2.5, and 5g/Kg diet, respectively).
Results are expressed as means±SEM of 6 fishes per group.
Different letters within the same raw indicate significance at $p<0.05$ (one-way ANOVA followed by Duncan multiple range test).

4. Discussion

In the present work, the major hallmark in the malnourished fishes was the depletion of energy resources. This could be attributed to restricted feeding, as well as impaired digestive and absorptive intestinal functions following consumption of food in a quantity not enough to supply the energetic needs of fishes [11]. It is noteworthy that the broad nature of conflicting data which had been emerged from literature regarding the biochemical endpoint modulation during caloric deficiency, reflecting a high degree of specificity in specie adaptive responses, and variation in undernutrition periods, along with other confounding factors.

The hypoglycemia in the current experiment is compliant with that occurred in rainbow trout, but is contradictory with hyperglycemia in tilapia [12, 13]. Reduced activity of amylase [14], a well-known enzyme involved in the carbohydrate digestion, may be implicated in the existing negative glucose equilibrium. The observed hypoglycemia revealed the maintenance of constant rate of glucose utilization that was greater than the rate of endogenous glucose production under the state of inadequate nutritional supply. The reduction in serum TC level in the present study is similar to the finding of [15]. It may be attributed to mobilization of lipid as an energetic source or reduced number and activity of ribosomes [16].

The enhancement in the activity of hepato-pancreatic amylase, and the rate of gluconeogenesis and glycogenolysis may result in a significant increase in the serum glucose level of *Spirulina*-treated Nile catfish in this study [17, 18]. A dose-related increase in the serum TC level was observed in the malnourished fishes treated with *Spirulina*, and confirmed by increased fat digestibility due to enhanced absorption [17]. *Spirulina* inclusion in the diet of feed-restricted fishes led to a significant dose-dependent increase in serum TP level as that observed in deltamethrin-poisoned Nile tilapia [19]. Enhanced protein digestibility, absorption, assimilation and protein synthesis [17] could be the contributory factors in raising TP level.

The stress of food shortage induced obvious decreases in serum urea and Cr levels as corresponding with [20]. Administration of *Spirulina* to feed-limited catfish resulted in reduction of AST and ALT in the same line with that occurred in deltamethrin intoxicated Nile tilapia [19]. The second and third doses of *Spirulina* increased Cr levels similar to the influences of natural cyanobacterial water blooms in Nile tilapia [21].

The feed-limited fishes were characterized by a significant elevation in serum Na^+ level and a significant reduction in serum K^+ level. Matching with these results, [12] found a marked increase in plasma Na^+ level in the feed-restricted Nile tilapia.

The obvious rise in HCO_3^- level of the malnourished fishes may be consequence of reduction in the levels of ketone bodies and lactate with a concomitant conservation of HCO_3^- buffering action [22].

Malnutrition-induced hypercupremia in this study is in parallel with the low hepatic Cu elimination in fasted European eel [23]. Hypoferremia is in the same line with [24]. A significant elevation in serum Fe^{+2} level of the feed-restricted fishes supplemented with *Spirulina* is matched with [21].

The significant reduction in serum Ca^{+2} level of the feed-limited fishes is in a harmony with that observed in rainbow trout [25]. Therefore, the reduction in the energy stores by malnutrition is postulated to limit the Ca^{+2} pump activities leading to a significant decrease in serum Ca^{+2} level in the feed-restricted fishes. *Spirulina* restored Ca^{+2} ATPase to near normal levels by decreasing triglycerides and phospholipids back to control values, and protecting sulfhydryl group from oxidative damage through inhibition of membrane lipid peroxidation in the study of [26]. So, the increase in Ca^{+2} ATPase pump activity may explain the ability of higher doses of *Spirulina* to increase serum Ca^{+2} level in the present experiment.

In conclusion, the devastating impacts of feed limitation for 2 months on Nile catfish were manifested by broad disruption in the metabolic and electrolyte outcome indicators. On the other hand, the supplementation with graded doses of *Spirulina* succeeded in restoring the energetic resources and alleviating some electrolyte disturbances.

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