

Effects of Microalgae on Nutrigenomics and Stress Biomarkers in Koi Carp, *Cyprinus Carpio*, (LINNAEUS, 1758) Raised in Aquaponic System

Aderotimi Tobi Mcheal¹, Gbadamosi O Kazeem^{2*} and Olanipekun Olamide Samuel¹

¹Department of Fisheries Technology, Federal Polytechnic, Ile Oluji, Ondo State, Nigeria

²Department of Fisheries and Aquaculture Technology, Federal University of Technology, PMB 704 Akure, Nigeria

ABSTRACT

This study evaluated the dietary effects of freshwater microalga, *Spirulina platensis* on the growth performance, nutrient utilisation and stress biomarkers in Koi carp, *Cyprinus carpio* fingerlings raised in aquaponic system with fluted pumpkins. Five feeds were formulated with different inclusion levels of spirulina at 0, 25, 50, 75 and 100% each representing treatment 1 (control), 2, 3, 4 and 5 respectively. The aquaponic system used is a pyramid Nutrient Film System (NFT) using a Poly Vinyl Chloride base with a small hole made for plant to float placed in a small disposable plastic filled with growth media and conventionally used Gravel (granite stones) which was used as control to support the plants for the plant to grow. The result showed that there were significant differences ($P < 0.05$) in the growth and nutrient utilization parameters with treatment 4 with 75% spirulina inclusion having the best protein efficiency ratio, feed conversion and feed efficiency ratios. The results from the stress biomarker analysis indicated that *Spirulina platensis* reduced liver stress and enhances liver function in *Cyprinus carpio* fingerlings, as evidenced by the decreasing ALT and AST levels in the liver of fish with spirulina diets.

*Corresponding author

Gbadamosi O Kazeem, Department of Fisheries Technology, Federal Polytechnic, Ile Oluji, Ondo State, Nigeria.

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Introduction

Ornamental fish are aquatic animals kept in an aquarium for display, shows, studies, and aesthetics. According to ornamental fish include fish, invertebrates, crustaceans, mollusk, and live rock [1]. They are a significant component of the global ornamental fish trade, which is a multi-billion-dollar industry. It contributes to the livelihoods of many people, particularly in developing countries where ornamental fish breeding is a source of income for local communities. The state of ornamental fish trade in Nigeria is far from satisfactory. Nigeria being a tropical country has a very high potential for ornamental fish production and exportation. Fish and fishery products remain some of the most traded food commodities in the world, 2020.

Koi carp, *Cyprinus carpio*, is a valuable ornamental fish due to its coloration, patterning and scalation. Koi carp, often simply referred to as "koi," are a species of ornamental fish that are highly prized for their vibrant colours and distinctive patterns. They come in a wide range of colours and patterns, including red, white, yellow, black, and blue. The most popular varieties include Kohaku (white body with red markings), Taisho Sanke (white body with red and black markings), Showa Sanshoku (black body with red and white markings), and Bekko (solid colour with black markings).

Microalgae are microscopic algae invisible to the naked eye. They are phytoplankton typically found in freshwater and marine

systems, living in both the water column and sediment. They are unicellular species which exist individually, or in chains or groups. *Spirulina* (*Arthrospira platensis*) is a protein-rich source because it contains approximately 60% protein. Microalgae are essential in aquaculture feeding, where they are used extensively as live feed, particularly for molluscs and marine fish larvae. However, these can also be used in dried form within production diets, for example *Arthrospira platensis* also known as *Spirulina* (blue-green algae) [2].

Understanding protein expression in fish provides valuable insights into physiological adaptations to dietary interventions. Proteins, especially those involved in stress responses, immune function, and metabolic pathways, are sensitive to dietary manipulation. Dietary inclusion of microalgae can modulate the expression of key proteins such as heat shock proteins, antioxidant enzymes, and cytokines, which serve as molecular indicators of health and performance. Therefore, evaluating protein expression offers a molecular perspective on how microalgae affect fish health.

Stress biomarkers in fish are crucial indicators of their physiological response to various stressors, aiding in the assessment of their health and welfare. The enzyme aspartate aminotransferase (AST) is widely distributed in erythrocytes and tissues, principally heart, liver, muscles, and kidneys. Alanine aminotransferase (ALT), also known as glutamate-pyruvate transaminase (GPT) and ALT, formerly known as serum glutamic pyruvic transaminase

(SGPT), is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas can serve as a stress biomarker in fish, particularly in response to physiological stressors. The general objective of this study is to assess the dietary effects of microalga supplementation on growth performance and stress biomarkers in Koi carp (*Cyprinus carpio*) cultivated in a recirculatory aquaculture system.

Materials and Methods

The study was carried out at the Teaching and Research farm of the Department of Fisheries and Aquaculture Technology, The Federal University of Technology Akure, Ondo State. One hundred and seventy healthy Koi Carp, *C. carpio* fingerlings were procured from Pristine farm in Ibadan, Nigeria. The fish were distributed randomly into the recirculatory aquaculture system at ten fish per tank in three replicates.

Ingredients	T1	T2	T3	T4	T5
Fish meal (65%)	17.21	17.21	17.21	17.21	17.21
Soyabean meal (45%)	17.21	12.91	8.61	4.30	0.00
Spirulina (54%)	0.00	4.30	8.61	12.91	17.21
GNC (43.5%)	17.21	17.21	17.21	17.21	17.21
Yellow maize	33.38	33.38	33.38	33.38	33.38
Fish oil	11.00	11.00	11.00	11.00	11.00
Vitamin	2.00	2.00	2.00	2.00	2.00
Starch	2.00	2.00	2.00	2.00	2.00
Total	100	100	100	100	100

The experimental design was conducted over 56 days in 15 recirculatory system tanks with 5 treatments in total (including the control), with each treatment containing 3 replicates (triplicates). Each treatment was represented by different supplementation levels of Spirulina-Additives. The graded levels of the Spirulina-Additives were 0%, 25%, 50%, 75%, and 100% per 100g for each

Table 1: Growth and Nutrient Utilization Parameters of *Cyprinus Carpio* fed with Spirulina Diet During the 56 Days Experimental Trial

TRTS	T1	T2	T3	T4	T5
IW	0.62 ± 0.01 ^a	0.69 ± 0.01 ^c	0.67 ± 0.02 ^b	0.63 ± 0.01 ^a	0.68 ± 0.02 ^{bc}
FW	1.08 ± 0.01 ^a	1.07 ± 0.01 ^a	1.12 ± 0.02 ^a	1.19 ± 0.02 ^b	1.08 ± 0.05 ^a
WG	0.47 ± 0.02 ^b	0.38 ± 0.01 ^a	0.45 ± 0.03 ^b	0.56 ± 0.02 ^c	0.39 ± 0.05 ^a
%WG	75.86 ± 3.97 ^b	54.82 ± 1.85 ^a	67.58 ± 5.35 ^b	89.44 ± 3.93 ^c	57.58 ± 7.14 ^a
FI	0.97 ± 0.36 ^a	0.74 ± 0.03 ^a	0.75 ± 0.06 ^a	0.79 ± 0.10 ^a	0.68 ± 0.16 ^a
FCR	2.10 ± 0.86 ^a	1.94 ± 0.09 ^a	1.67 ± 0.04 ^a	1.39 ± 0.13 ^a	1.72 ± 0.22 ^a
FER	0.52 ± 0.17 ^a	0.52 ± 0.03 ^a	0.60 ± 0.01 ^{ab}	0.72 ± 0.07 ^b	0.59 ± 0.08 ^{ab}
SGR	1.13 ± 0.36 ^a	0.72 ± 0.39 ^a	0.78 ± 0.33 ^a	0.88 ± 0.07 ^a	0.82 ± 0.16 ^a
PER	1.50 ± 0.50 ^a	1.48 ± 0.07 ^a	1.71 ± 0.04 ^{ab}	2.06 ± 0.21 ^b	1.68 ± 0.23 ^{ab}
%SURV	90.00±0.00 ^a	90.00± 0.00 ^a	90.00± 0.00 ^a	90.00 ± 0.00 ^a	60.00± 0.00 ^b

SDS PAGE Analysis for the Protein Expression of *Clarias Gariepinus* Liver Tissue

The protein expression profiles of the liver tissue from *Cyprinus carpio* specimens were analyzed using SDS-PAGE as represented in Figure 3 and quantified with GelAnalyzer software showed in Figure 4. The analysis revealed distinct, dose-dependent changes in protein expression corresponding to the varying inclusion levels of microalgae in the feed. The control group (T1) established the baseline hepatic protein profile.

diet, denoted as T1 (Control), T2, T3, T4, and T5 in diets 1, 2, 3, 4, and 5 for *C. carpio* as shown in Table 1.

All data were collected and recorded before and after the experimental trial. The blood and liver of the fish was collected for ALT (Alanine aminotransferase) and AST (Aspartate aminotransferase) analysis.

Statistical Analyses of Data

All data collected were checked for normality using one-way analysis of variance (ANOVA), and homogeneity of variance was tested using Levene's test to determine significant differences in the means. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 22.0 for Windows). Where significant differences were found, the means were separated using Duncan's multiple range test. A probability level of less than 0.05 was used to indicate significant differences among the treatments.

Results and Discussion

Water quality during the experimental trial were at a stable condition suitable for Koi carp aquaculture. The growth and nutrient utilization parameters for each treatment were measured and is shown in Table 1. Treatment 2 had the highest protein content, aligning with the observations of Robinson (2020) on the importance of protein-rich diets in supporting optimal animal performance. The result showed there were significant differences ($P < 0.05$) in the growth and nutrient utilization parameters with treatment 4 having the best protein efficiency ratio, feed conversion ratio and feed efficiency ratio [5-9].

The results from the stress biomarker analysis indicated that *Spirulina platensis* reduces liver stress and enhances liver function in *Cyprinus carpio* fingerlings, as evidenced by the decreasing ALT and AST levels in the liver. Ahmed (2016) observed that dietary *Spirulina* supplementation in Nile tilapia reduced ALT and AST levels, indicating lower stress levels and better liver function, which corresponds to the decreasing ALT and AST trends seen in the current study for *Cyprinus carpio*.

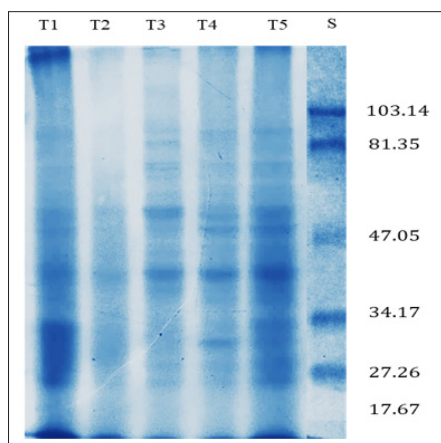


Figure 1: SDS Page Analysis of *Clarias Gariepinus* Juvenile fed with Experimental Diets

Stress Biomarkers

The results from the stress biomarker analysis indicated that *Spirulina platensis* reduces liver stress and enhances liver function in *Cyprinus carpio* fingerlings, as evidenced by the decreasing ALT and AST levels in the liver as shown in Table 2. However, the increase in blood AST at higher inclusion levels suggests other non-liver-related stress factors may be at play. These findings support the use of *Spirulina* as a dietary supplement for improving liver health in fish but also highlight the need for further research into the systemic effects of high *Spirulina* inclusion levels on overall fish health.

Ahmed et al., (2016) observed that dietary *Spirulina* supplementation in Nile tilapia reduced ALT and AST levels, indicating lower stress levels and better liver function, which corresponds to the decreasing ALT and AST trends seen in the current study for *Cyprinus carpio*.

The increase in blood AST with *Spirulina* inclusion may suggest that while liver stress is reduced, other factors like metabolic or muscular stress could be responsible for the elevated levels. This could be due to the higher metabolic demands of the fish as they convert *Spirulina* into biomass, a hypothesis that has been explored by researchers such as Borges et al., (2015).

Table 2: ALT and AST level in Blood and Liver of Koi Carp (*Cyprinus carpio*) Fingerlings Fed with *Spirulina Platensis* Diet

Treatment	T1	T2	T3	T4	T5
Blood (ALT)	24.87 ± 0.32 ^d	20.01 ± 0.27 ^c	18.66 ± 0.23 ^b	18.19 ± 0.29 ^b	14.67 ± 0.04 ^a
Blood (AST)	15.18 ± 0.39 ^a	24.35 ± 0.63 ^b	24.55 ± 1.21 ^b	25.22 ± 0.36 ^b	30.15 ± 0.35 ^c
Liver (ALT)	19.87 ± 0.32 ^d	15.69 ± 0.16 ^c	15.28 ± 0.11 ^c	14.25 ± 0.05 ^b	13.43 ± 0.01 ^a
Liver (AST)	22.01 ± 0.27 ^d	20.35 ± 0.07 ^c	18.03 ± 0.18 ^b	17.36 ± 0.70 ^b	15.58 ± 0.30 ^a

Data Presented are Means and Standard Deviation (Mean ± SD) for Ten Fish from Three Replicates (n=3) *Values with Different Superscripts on the Same Row Indicate Significant Difference at P>0.05nALT- Alanine Aminotransferase, AST- Aspartate Aminotransferase.

Conclusion

Treatment 4 with 75% inclusion of *Spirulina platensis* has the highest nutrient utilization performance in the diet of the fish. The study showed that spirulina is a viable ingredient in the culture of ornamental fish such as *Cyprinus carpio* and it is therefore recommended.

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