Effect of Dietary Supplementation of*Chlorella vulgaris* on liver biomarkers and lipid profile on *Clariasgariepinus*

Abstract:

Clariasgariepinus is an important commercial fish species in Nigeria and is widely cultivated by many farmers. This study investigated the effects of dietary supplementation with *Chlorella vulgaris* on liver biomarkers and lipid profiles in *Clariasgariepinus* over 12 weeks. Five groups of fish were used in the study; one group served as the control, while the other four groups were fed different concentrations of the algae at 5%, 10%, 15%, and 20%. At the end of the feeding trial, the fish were anesthetized using clove oil, and blood samples were collected for analysis. The results indicated that the supplementation of *C. vulgaris* in the diets of *C. gariepinus* led to higher protein percentages (ranging from 43.0% to 47.3%) and lower levels of AST (230.0) and ALT (54.0) in the algae-fed groups compared to the control group, which had a protein percentage of 39.67, AST levels of 497.3, and ALT levels of 180.7. Additionally, the lipid profiles showed that the treated groups had better outcomes than the control group. The findings revealed significant variations in the fish's serum biochemical parameters and lipid profiles across the different treatments. Therefore, this study highlights the positive impact of microalgae as a physiological booster in *C. gariepinus*.

Keywords: *Chlorella vulgaris, Clariasgariepinus,* biochemical parameters, lipid

Introduction:

Fish is one of the most valuable sources of protein food worldwide; people obtain about 25% of their animal protein from fish. It is the most easily affordable source of animal protein for the average Nigerian (Nsikak *et al*., 2021). *Clariasgariepinus* is an omnivorous species that consumes a wide variety of foods. It has several distinct characteristics, including a high growth rate, an impressive feed conversion rate, resilience to low water quality, and a high survival rate. Additionally, it is one of the most widely recognized species used in toxicity research involving fish(Abdelbaky *et al*., 2022).

Chlorella is a genus of green microalgae belonging to the phylum Chlorophyta and the class Chlorophyceae. It is a highly versatile and widely studied organism, known for its broad applications due to its adaptability, ease of cultivation, and rich nutrient profile (Xu *et al*.,2014)

Chlorella vulgaris (CV) is a freshwater, single-celled algae recognized for containing the highest amount of chlorophyll among all plants. This superfood is rich in nutrients, providing a variety of vitamins and minerals, 18 amino acids, and 60% protein content. One of its unique properties is the Chlorella Growth Factor (CGF), a phytonutrient abundant in the cells of the algae. CGF consists of vitamins, nucleic acid-related substances, amino acids, proteins, peptides, and sugars (Raji *et al*. 2020).

Chlorella is a rich source of high-quality protein, containing essential amino acids, polysaccharides, lipids, vitamins, minerals, and bioactive substances that support various physiological functions. *Chlorella vulgaris* is known to have high concentrations of photosynthetic pigments and primary carotenoids, including α-carotene, β-carotene, lutein, ascorbic acid, and α-tocopherol. These carotenoids, along with β-1,3-glucan and phenolics, act as active immunostimulants, capable of scavenging free radicals and lowering blood cholesterol levels. Additionally, vitamin B12 found in Chlorella biomass is crucial for blood cell formation and regeneration(Pradhan, *et al*.,2023).Hematological parameters are increasingly used as indicators of physiological stress responses in fish, helping to monitor their physiological and pathological conditions effectively and sensitively (Sharma & Shukla, 2021). Blood, being a sensitive connective tissue, is easily influenced by environmental factors (Fazio *et al*., 2021). This study aimed to investigate the effect of *Chlorella vulgaris* on biochemical characteristics (Protein, AST, ALT, Albumin and lipid profile) of *Clariasgariepinus*

MATERIALS AND METHODS

Study Area

The research was conducted inthe fisheries laboratory of The BiologicalSciences Department of Gombe State University, Gombe, Gombe State, Nigeria.

Preparation of experimental fish:

The pre-experiment activities included the procurement of fish from a fish farm under the State Ministry of Agriculture in Gombe. A total of 150*Clariasgariepinus* fingerlings were purchased and transferred to the fisheries laboratory for the experiment. The fish were acclimatized to the new conditions and fed a basal diet, twice the amount of 3% of their body weight.

Treatments design

A total of thirty fish were selected and divided into five groups, which included one control group and four experimental groups. Each group had three replicates, with ten fish per tank. The fish were fed a diet supplemented with varying concentrations of C. vulgaris.

Experimental diets and formulations:

Dried *Chlorella* powder was used in this study. Before the experiment began, a proximate analysis of both the algae and the reference diet was conducted. The diets were prepared by grinding the feed materials into a powder using a local grinder and then thoroughly mixing them with water to achieve a suitable dough texture. The dough was passed through a manual meat mincer and shaped into 2 mm diameter pellets. The pellets were then dried, packed into polypropylene bags that had been labeled beforehand, sealed, and stored for the experiment (Raji *et al*., 2020).

Feeding design

The control group was fed a commercial diet without *Chlorella vulgaris* (CV). The treatment groups were fed diets supplemented with 5% (CV 5), 10% (CV 10), 15% (CV 15), and 20% of dry powdered *Chlorella vulgaris*. The experiment lasted for 12 weeks, during which the fish were fed twice daily, at 8:30 AM and 5:00 PM

Blood sampling and analysis:

The experimental fish were starved for 24 hours before sampling to ensure accurate results. To minimize the potential effects of stress on the analyzed parameters, the fish were anesthetized using clove oil at a concentration of 40 mg/L (Saberi *et al*., 2017). Blood samples were collected from the caudal peduncle of the fish using 1 mL sterile disposable plastic syringes with 25-gauge needles. The same blood samples were collected into non-heparinized tubes for serum biochemistry analysis. The samples were immediately centrifuged at 4°C at 5000 rpm for 10 minutes. The serum was then carefully collected using a micropipette and stored at −80°C until further analysis of biochemical parameters.

The biochemical parameters measured included total protein (TP), albumin (ALB), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) (Esmaeili, 2021)were determined bymeans of standard enzymatic methods as described by Bush (1991). Lipid profiles, including serum triglycerides, cholesterol, HDL, and LDL, were measured using the Advia 2400 chemistry system (Raji *et al*., 2020).

Statistical analysis

The obtained data were analyzed using one-way ANOVA (Analysis of Variance) to assess the effect of microalgae supplements. A p-value of less than 0.05 was considered statistically significant. When the ANOVA test produced a significant F-value, post-hoc comparisons between groups were made using Tukey's multiple comparisons test. Statistical analysis was performed using SPSS version 16(Raji *et al*.,2020).

RESULTS:

Effect of *Chlorella vulgaris* on Biochemical parameters:

The results of the ANOVA show significant differences in the biochemical parameters between the dietary treatments.

Aspresentedin Table 1 the result showed that there are significant differences betweenAST **(Aspartate Aminotransferase)** and **ALT (Alanine Aminotransferase)**levels: ($F=4.93$, $p<0.019$ and $F=6.55$, $p<0.007$, respectively). The control group (CV 0%) had the highest values for both enzymes (497.3±74.0 for AST and 497.3±74.0 for ALT), while the lowest values were recorded in the CV 10% group (230.0±45.5 for ALT and 53.67±15.57 for AST).**PRT (Total Protein)**: No significant difference was observed in total protein levels ($F=1.25$, $p>0.352$). The highest total protein value was observed in the CV 5% group (47.33±5.03), and the lowest in the CV 0% group (39.67±1.53).**ALB (Albumin)**: Similarly, no significant difference was observed for albumin levels ($F=1.50$, $p>0.274$). The highest albumin value was recorded in the CV 0% group (16.67±0.58), while the lowest value was found in the CV 5% group (15.00±1.00) as described in Table 1.

Table 1: Effect of *Chlorella vulgaris* on the Biochemical parameters of *C. gariepinus*

Treatments	AST(IU/L)	ALT(IU/L)	PRTN(g/L)	ALB(g/L)
CV 0%	497.3±74.0^{A}	180.7±74.01^{A}	39.67±1.53	16.67±0.58
CV 5%	294.7±18.8^{B}c	63.67±13.58^{B}	47.33±5.03	15.00±1.00
CV 10%	230.0±45.5c	53.67±15.57^{B}	45.67±6.11	16.00±1.00
CV 15%	430.7±150.7^{AB}	69.33±6.35^{B}	46.67±7.09	16.00±1.00

CV 20%	268.3±95.2 c	54.00±13.75^{B}	43.00±2.00	16.67±1.16
P-VALUE	0.019	**0.007**	**0.352**	0.274

The results were expressed as mean ± standard error of the mean. Means with different letters in each column indicate a significant difference. The abbreviations used are as follows: **ALT** = alanine aminotransferase, **AST** = aspartate aminotransferase, **PRT** = total protein, and **ALB** = albumin

Lipid profile:

CHl (Cholesterol): A significant difference was observed in cholesterol levels between the dietary groups (F=22.58, p<0.00). The highest cholesterol value was recorded in the control group (CV 0%) (3.70±0.10), while the lowest was found in the CV 5% group (3.03±0.06) as shown in Table 2.As presented in table 2**, HDL (High-Density Lipoprotein)**: There was a significant difference in HDL levels (F=6.67, p<0.007). The highest HDL values were observed in the CV 0% group (0.43±0.06) and 5%, while the lowest levels were seen in the CV 15% and CV 20% groups (0.30±0.00).**LDL (Low-Density Lipoprotein)**: No significant difference was observed for LDL levels (F=0.80, p>0.55). The highest LDL values were recorded in the CV 0%, CV 10%, and CV 15% groups (0.10±0.00), while the lowest was observed in the CV 20% group (0.093±0.012).**TRGL (Triglycerides)**: A significant difference was observed for triglyceride levels (F=4.32, p<0.028). The highest triglyceride value was recorded in the CV 15% group (2.73±0.15), while the lowest was found in the control group (CV 0%) (2.27±0.06) as seen in table 2.

Table 2 : Effect of *C. vulgaris* on Lipid profile of *C. gariepinus*

Treatments	**CHl(mmol/l)**	**HDL(mmol/l)**	**LDL(mmol/l)**	**TRGL(mmol/l)**
CV 0%	3.70±0.10^{A}	0.43±0.06^{A}	0.10±0.00^{A}	2.27±0.06c
CV 5%	3.03±0.06^{D}	0.43±0.06^{A}	0.09±0.01^{A}	2.30±0.27^{B}c
CV10%	3.13±0.06C^{D}	0.37±0.06^{AB}	0.10±0.00^{A}	2.57±0.15^{AB}
CV15%	3.33±0.15^{B}	0.30±0.00^{B}	0.10±0.00^{A}	2.73±0.15^{A}
CV 20%	3.27±0.06^{B}c	0.30±0.00^{B}	0.09±0.01^{A}	2.50±0.10^{AB}c

P-value	0.000	0.007	0.552	0.028

CHL = total cholesterol, **TRG** = triglycerides, **HDL** = high-density lipoprotein, and **LDL** = low-density lipoprotein. Results were expressed as mean ± standard error of the mean. Means with different letters in each column indicate a significant difference.

Discussion:

In the present study, the results showed a significant increase in total protein and albumin levels in fish fed diets supplemented with *Chlorella vulgaris* powder. These findings are consistent with those of Abbas *et al.* (2020) and Saberi*et al*. (2017), who found higher protein and albumin levels in fish that were given *Chlorella*. They noted that including *C. vulgaris* in the diets for eight weeks significantly elevated total protein, albumin, and globulins in the serum of treated carp. The increase in serum protein and albumin levels at various algal inclusion levels in this study is likely due to the beneficial metabolites in *C. vulgaris*, which enhance liver function and improve blood parameters, leading to lower values in the control group.

These findings align with those of Amira *et al*. (2022), who studied the effects of microalgae-inoculated diets on growth performance and blood parameters in Nile tilapia. They reported significantly higher values for all biochemical parameters in the control group compared to the algal-treated groups, consistent with the results of the present study. Fish albumin is a water-soluble protein found in fish muscle tissue, playing a critical role in transporting metabolites such as fatty acids, hormones, and bilirubin, while also regulating blood and osmoregulation processes. Additionally, albumin helps filter fluids within body tissues. In cultured fish species, the albumin content is influenced by factors such as species, size, feeding levels, feed availability and quality, and the digestible energy content of the feed (Nurfaidah*et al*., 2021).

Akbary and Raeisi, (2020) reported similar positive results in their study on the impact of dietary supplementation with *C. vulgaris* on various physiological parameters of grey mullet (*Mugil cephalus*). They observed higher protein and glucose levels in the algae-treated groups compared to the control group. These findings are consistent with the current study, where the control group exhibited lower protein levels than the treated groups. This could be due to the essential metabolites in *C. vulgaris* that provide the necessary amino acids to achieve higher protein concentrations in the fish indicating a positive impact on their health.

In another study by Rahdari*et al.* (2020) on immune responses and hematological variables of cultured great sturgeon (*Huso huso*) subjected to 11-ketotestosterone implantation, higher hematological and biochemical values were observed in the 11-ketotestosterone-treated group compared to the untreated group. This supports the findings of the present study, where higher values in some biochemical parameters were seen in the algal-treated groups compared to the control. These changes could be attributed to the combined effects of the hormone and the inclusion of algae, which likely improved the biochemical and hematological profiles of the fish.

Identical positive results were also observed by Pradhan *et al*. (2020) in their investigation of the protective effects of *C. vulgaris*-supplemented diets on antibacterial activity and immune responses in Rohu fingerlings (*Labeorohita*) subjected to *Aeromonas hydrophila* infection. They reported higher biochemical parameters in the *C. vulgaris* groups compared to the control group. This aligns with the findings of the present study, where the algae-treated groups showed improved biochemical parameters. The observed enhancements in blood characteristics could be attributed to the inclusion of *C. vulgaris*in the diet, which likely enhanced the overall health and immune responses of the fish.

AST (aspartate aminotransferase) and ALT (alanine aminotransferase) are key enzymes used to assess liver function. Under normal conditions, the levels of these enzymes remain low. However, an increase in their levels can indicate impaired liver function or liver damage. These enzymes are released into the bloodstream when liver cells are damaged, and their elevated levels serve as markers of cell membrane damage, reflecting liver dysfunction or injury(Sayed *et al*., 2022).

The present study revealed significantly higher values of ALT and AST in the control group, while lower values were observed in the groups treated with *C. vulgaris*. The lowest levels were recorded at a 10% algal inclusion. This finding is not consistent with the results from Sayed *et al*. (2022), who examined the immunological and hemato-biochemical effects on catfish exposed to dexamethasone. Their study also reported higher biochemical parameter values, which aligns with the trends observed in the current research, suggesting that algae supplementation may support better liver function and overall health in the treated groups.

Abbas *et al.* (2020) reported significantly higher ALT, AST, uric acid, and creatinine values in the control group, while the groups treated with *C. vulgaris* showed lower values. These findings correlate with the current study, where the treated groups had lower ALT and AST levels, and the control group exhibited higher values. This could be attributed to the presence of anti-stress compounds in the algae, which may have reduced stress levels in the fish, consequently lowering the production of these enzymes.

However, both transaminases (AST and ALT) in the treated groups of the present study were not significantly affected. This suggests that *C. vulgaris* may have improved the quality of the feed being used. The inclusion of *C. vulgaris* in the diet might have contributed to better physiological regulation, allowing the fish to tolerate and adapt to stress without a marked increase in liver enzyme levels.

Furthermore, Adesina (2017) studied the hematological and serum biochemical profiles of *Clariasgariepinus*juveniles fed diets containing different inclusion levels of mechanically extracted sunflower seed meal. They observed higher packed cell volume (PCV), hemoglobin (HB), red blood cells (RBC), and white blood cells (WBC) counts in the treated groups, while the control group had lower values. The biochemical parameters, including protein, albumin, globulin, AST, and ALT, were also higher in the treated groups compared to the control. These changes in hematological and biochemical parameters could be attributed to the inclusion of sunflower seed meal in the diets, which likely contributed to improved overall health and physiological status in the fish. These findings are consistent with the results obtained in the present study, where higher hematological values were found in the treated groups compared to the control. Additionally, protein and albumin levels were observed to be higher, while ALT and AST levels were lower in the treated groups. This suggests that the inclusion of *C. vulgaris* in the diet may have improved blood health and liver function in *Clariasgariepinus*.

The present findings are also consistent with the study by Kim *et al.* (2015), which examined the effects of dietary supplementation with spirulina and quercetin on growth, innate immune responses, disease resistance against *Edwardsiellatarda*, and dietary antioxidant capacity in juvenile olive flounder. In their study, higher ALT and AST levels were observed in the control group, while lower levels were found in the algae-supplemented groups. This aligns with the current study, where *C. vulgaris* supplementation resulted in lower ALT and AST levels, suggesting a similar positive effect on liver function and overall health in the treated fish.

Effect of *C. vulgaris* on Lipid of *C. gariepinus*

Lipid stores serve as major energy reserves in fish and are mobilized during sexual maturation, with stored lipids being redirected from tissues to the gonads to support their development. Lipoproteins play a crucial role in lipid transport within fish, as highlighted by a study focused on reference intervals for serum biochemistry and lipid profiles in male broodstock African catfish at varied ages. This study reported higher lipid content, emphasizing the significance of lipid metabolism and transport in fish physiology (Okoye *et al*., 2016).

The present study revealed a marked decrease in total cholesterol and low-density lipoprotein (LDL) levels in the treated groups, along with a slight increase in high-density lipoprotein (HDL) levels in the group treated with 5% *Chlorella vulgaris*. Additionally, higher triglyceride levels were observed in the treated groups compared to the control group. These findings align with those of Abbas *et al.* (2020), who reported similar lipid outcomes. This suggests that *C. vulgaris* exhibits a lipid-lowering effect that may improve the lipid profile and overall health of fish.

Further, a study on the effects of a microalgae-inoculated diet on the growth performance and blood parameters of Nile tilapia established that the control group had higher cholesterol and triglyceride values(Amira *et al*., 2022). This finding is consistent with the results of the present study. Similarly, Akbary and Raeisi (2020) investigated the effect of dietary supplementation with Chlorella vulgaris on various physiological parameters of grey mullet, observing higher cholesterol and triglyceride levels in the untreated group and lower levels in the treated groups. These results further confirm the lipid-lowering effects of *C. vulgaris* and its potential to improve the lipid profile in fish.

Kim *et al.* (2015) also reported higher cholesterol and triglyceride levels in the control group, with lower levels in the algae-treated groups, supporting the observations of the current study. This may be attributed to the lipid-lowering properties of algae. Conversely, Xu *et al*. (2014) and Khani*et al.* (2017) demonstrated that Chlorella powder could effectively reduce blood cholesterol, although it did not significantly affect glucose levels in Gibel carp. These findings align with the present study, emphasizing the potential role of *C. vulgaris* in improving lipid profiles.

Conclusion:

The current study concluded that fish fed an algae-based diet exhibited superior biochemical characteristics compared to those in the control group. The protein level, ALT, AST and lipid profile were seen to have better outcome in all the treated groups.Thesrefore, including *C. vulgaris* in the feed for *C. gariepinus*can significantly enhance the physiology of these fish.

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