Effect Of Dietary Supplementation Of*Chlorella vulgaris* On liver biomarkers and lipid profile on *ClariasGariepinus*

Abstract:

Clariasgariepinus is a commercially important species of fish in Nigeria and heavily cultured by many farmers.The study of effect of dietary supplementation of *Chlorella vulgaris* on some liver biomarkers and lipid profile of *Clariasgariepinus* was investigated for 12 weeks. Five groups of fish were used, one served as control and four were fed with different algal inclusion of 5%,10%,15% and 20%. At the end of the feeding trial, the fish were anesthetized with clove oil and blood samples were collected and analyzed. The results revealed that supplementation of *C. vulgaris*in diets of *C. gariepinus* resulted in a higher percentages of protein (43.0- 47.3) and lower AST(230.0) and ALT(54.0) in algae groups as compared with control protein(39.67), AST(497.3) and ALT(180.7).The lipid profile revealed that the treated groups had better outcomes than the control.The results showed significant variations in the serum biochemical parameters and lipid profile of the studied fishes, throughout the different treatments. Therefore, the current study highlighted the 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). *C. gariepinus* is an omnivore creature that eats anything, and has several distinct properties, including a high capacity to grow, higher feed conversion rate, high acclimatization to low water quality and high survival rate. It is one of the greatest widely recognized experimental species of fish in toxicity research(Abdelbaky *et al*., 2022).

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

Chlorella vulgaris (CV) is a freshwater-based single-celled algae, which contains the highest quantity of chlorophyll of all plants. It is a superfood with an abundant nutrient containing various vitamins and minerals, 18 amino acids and 60% protein. Chlorella Growth Factor (CGF), which is a phytonutrient is one of its unique properties. CGF is abundant in the nuclei of algae, made up of vitamins, nucleic acid- associated substances, amino acids, proteins, peptides and sugars (Raji *et al*. 2020).

Chlorella is a rich source of good-quality protein with amino acids, polysaccharides, lipids, vitamins, minerals, and nutrient-rich bioactive substances, presuming numerous physiological activities. High concentrations of photosynthetic pigments and many primary carotenoids, such as α-carotene, β-carotene, lutein, ascorbic acid, and α-tocopherol, have been reported in C. vulgaris. These carotenoids, β-1, 3 glucan, and phenolics are active immunostimulators and can scavenge free radicals and blood cholesterol. Vitamin B12 in Chlorella biomass is vital for blood cell formation and regeneration (Pradhan, *et al*.,2023). Hematological parameters are increasingly utilized as indicators of physiological stress responses to internal or external changes in fish. They serve as a vital tool for the effective and sensitive monitoring of the fish's physiological and pathological conditions(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 in fisheries laboratory of Biological Sciences 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

Thirty 30)were selected and divided into five groups, representing different treatments: a control group and four experimental groups. Each group consisted of three replicates, with 10 fish per tank, and 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, 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 means 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.

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%	$3.13\pm0.06C^{D}$	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 revealed a significant increase in total protein and albumin in fish- fed diets supplemented with *C. vulgaris* powder. These findings are consistent with those of Abbas *et al*. (2020) and Saberi *et al.* (2017), who observed higher protein and albumin levels in fish-fed *Chlorella*. They found that the inclusion of *C. vulgaris* in diets for 8 weeks significantly elevated total protein, albumin, and globulins in the serum of treated carp. The elevation of serum protein and albumin levels at various algal inclusion levels in this study may be attributed to the beneficial metabolites in *C. vulgaris,* which enhance liver function and improve blood parameters, resulting in lower values in the control group. The findings of this research 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 in all biochemical parameters for the control group compared to the algal-treated diets.This is similar to what the present study documented. Fish albumin is a water-soluble protein found in fish muscle tissue. It plays a vital role in transporting metabolites, such as fatty acids, hormones, and bilirubin, as well as regulating blood and osmoregulation processes. Additionally, albumin aids in filtering fluids within body tissues. In cultured fish species, the albumin content is influenced by factors such as species and size, feeding levels, feed availability and quality, and the digestible energy content of the feed(Nurfaidah *et al*., 2021)

Akbary and Raeisi, (2020) also reported similar positive results in their study on the effect of dietary supplementation of *Chlorella 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. These findings are consistent with the results of the current study, where the control group exhibited lower protein levels than the treated groups. This could be attributed to the essential metabolites in *C. vulgaris*, which provide the necessary amino acids to build up higher protein concentrations in the fish. Moreover, Abdelhamid *et al*. (2019) studied the factors affecting fish blood profiles and found that total protein and glucose levels improved when fish were fed diets containing clover seeds, thereby enhancing the health status of the fish. This is in line with the present study, where fish fed with *Chlorella* also exhibited improved protein levels, indicating a positive effect on their health. In another study by Rahdari *et al*., (2020) on immune responses and haematological variables of cultured great sturgeon (*Huso huso*) subjected to 11-ketotestosterone implantation, higher haematological and biochemical values were observed in the 11-ketotestosterone-treated group compared to the untreated group. This aligns with 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 in the present study, which likely contributed to the improvement in the biochemical and haematological profiles of the fish. Identical positive results were observed by Pradhan *et al.* (2020) in their study on the protective effects of *Chlorella 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. This aligns with the findings of the present study, where the algae-treated groups exhibited improved biochemical parameters. The observed improvements 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 for 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, with lower values observed in the *C. vulgaris* treated groups, and the lowest levels recorded at the 10% algal inclusion. This is not consistent with the findings of Sayed *et al*. (2022), who investigated the immunological and hemato-biochemical effects on catfish exposed to dexamethasone. Their study also reported higher biochemical parameter values, aligning with the trends observed in the current research, where algae supplementation appeared to 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 *C. vulgaris*- treated groups showed lower values. These findings align with the results of the present 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. On the other hand, Abdelhamid *et al*. (2019) observed higher ALT and AST values in fish fed with clover seed diets compared to the control, which does not align with the findings of the current research.

However, both transaminases (AST and ALT) in the treated groups of the present study were not significantly affected. This suggests that *C. gariepinus* may have improved the quality of the feed being fed. The inclusion of *C. vulgaris* in the diet might have contributed to better physiological regulation, allowing the fish to tolerate and adapt to the stress without a marked increase in liver enzyme levels. Furthermore, ADESINA, (2017) In the study on hematological and serum biochemical profiles of *Clariasgariepinus* juveniles fed diets containing different inclusion levels of mechanically extracted sunflower seed meal, it was observed that the treated groups exhibited higher PCV, HB, RBC, and WBC counts, 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 alterations in the 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 in line with the results obtained in the present study, where higher hematological values were established 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 contributed to improved blood health and liver function in *Clariasgariepinus*, similar to the effects seen in the sunflower seed meal study. Yarmohammadi *et al.*, (2021)also reported that there were decreases in ALT and AST levels in patients supplemented with *C. vulgaris*, while higher values were observed in patients who were not supplemented with the algae. This supports the findings of the present study, where *C. vulgaris* supplementation in fish diets led to lower ALT and AST levels, indicating potential liver protection and improved overall health. The present findings are also consistent with the study by Kim *et al.*, (2015)) on the effects of dietary supplementation of 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 (Okoye*et al.*, 2016). In their study on Reference Intervals for the Serum Biochemistry and Lipid Profile of Male Broodstock African Catfish at Varied Ages, they also reported higher lipid content, emphasizing the importance of lipid metabolism and transport in fish physiology.

The present study revealed a marked decrease in total cholesterol and LDL levels in the treated groups, along with a slight increase in HDL levels in the 5% *C. vulgaris*-treated group. Additionally, higher triglyceride levels were observed in the treated groups compared to the control. These findings align with the results of Abbas *et al*. (2020), who reported similar lipid outcomes. This suggests that *C. vulgaris* exhibits a lipid-lowering effect, potentially improving the lipid profile and overall health of the fish (Amira*et al.*, 2022), in their study on the effects of a microalgae-inoculated diet on the growth performance and blood parameters of Nile tilapia, established higher cholesterol and triglyceride values in the control group. This finding is consistent with the results of the present study. Similarly, Akbary and Raeisi (2020), in their investigation of the effect of dietary supplementation of *Chlorella vulgaris* on various physiological parameters of grey mullet, observed 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 and lower levels in algae-treated groups, further supporting the current study's observations. This may be attributed to the presence of algae, which has lipid-lowering properties. Conversely, Xu *et al*., (2014) and KHANI *et al.*, (2017) demonstrated that Chlorella powder could effectively reduce blood cholesterol, though it did not significantly affect glucose levels in Gibel carp. These findings align with the present study, emphasizing *C. vulgaris's*potential role in improving lipid profiles.

Conclusion:

Based on the findings of current study,it was concluded that fish fed with algae-based diet had the best biochemical characteristics as compared with those in the control group. Therefore, *C. vulgaris* inclusion in *C. gariepinus*feed can essentiallyimprove the physiology of these fish.

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