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Effects of *Chlorella vulgaris* on Hematological Parameters of *Clarias gariepinus*

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ABSTRACT

A 12-week feeding trial was conducted to investigate the effects of dietary *Chlorella vulgaris* supplementation on the haematological parameters of *Clarias gariepinus*. The fish were divided into five groups: one fed on a control diet without algae supplementation, and four experimental groups fed diets containing 5%, 10%, 15%, and 20% *C. vulgaris* powder at 3% of their body weight, twice daily. Treated groups exhibited higher RBC, WBC, and Hct levels, with the highest values observed in the 15% and 20% groups, while the control group showed higher RBC indices. No significant differences were noted in RBC and MCV, but significant variations were observed in WBC, Hct, Hb, MCH, and MCHC at $p<0.05$. Based on the findings, dietary supplementation with more than 5% *C. vulgaris* can enhance the blood quality of catfish.

Keywords: *Clarias gariepinus*, *Chlorella vulgaris*, Haematological Parameters.

INTRODUCTION

Algae, as a diverse group of autotrophic organisms, play critical roles in aquatic ecosystems and exhibit fascinating survival strategies. Their ability to produce secondary metabolites is a key adaptation for protection against predators, pathogens, and competition (Bule *et al*., 2018). Recently, microalgae have garnered interest in food and nutrition due to the presence of high amounts of proteins, vitamins, polyunsaturated fatty acids and antioxidants, respectively (Bule *et al*.,2018). As an essential part of aquatic ecosystems like oceans and lakes, micro algae are of great significance to the aquatic environment, the health of aquatic animals, and the balance of the ecosystem. The cells of micro algae are rich in active nutrients such as proteins, polyunsaturated fatty acids (PUFAs), polysaccharides, and essential amino acids, which can promote the growth of fish, shrimp, crab, and shellfish. Therefore, they can be used as a basic feed for fish and other economic aquatic animals directly or indirectly (Ma *et al*., 2020).

Chlorella vulgaris (CV) is a freshwater based single-celled algae, which contains the highest quantity of chlorophyll of all plants. It is a super food with an abundant nutrients containing various vitamins and minerals, 18 amino acids and 60% protein (Raji *et al*. 2020).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

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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 have the ability to scavenge free radicals and blood cholesterol. Vitamin B12 in Chlorella biomass is vital for blood cell formation and regeneration (Pradhan *et al*., 2023).

Clarias gariepinus is a member of *Claridae* family and is a popular commercial species in Africa due to its good quality meat and nutritive values. *C. gariepinus* is an omnivore creature that eats anything, and has a number of 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).

The hematological characteristics of fish play a crucial role in assessing their overall health. However, factors such as diet composition, metabolic adaptations, and variations in activity levels are key contributors to changes in their hematological parameters (Eyiwumi *et al*., 2018). The complete blood count is a valuable tool for aquaculturists to monitor water quality, related soil conditions, and assess fish health in response to feed, disease treatments, and maturity confirmation (Habib *et al*., 2021).The research aimed to determine the impacts of *C.vulgaris* on the blood characteristics of *C.gariepinus* species

MATERIALS AND METHODS

Study Area

The research was conducted in fisheries laboratory of Biological Science Department, 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 *Clarias gariepinus* fingerlings were purchased and transferred to the fisheries laboratory for the experiment. The fish were acclimatized to the new conditions and fed on a basal diet, twice the amount of 3% of their body weight. At the start and end of the experiment, each fish was individually weighed, and their total lengths were recorded.

Experimental 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 on a diet supplemented with varying concentrations of *C. vulgaris*.

Feed and Feeding Design

The control group was fed on a commercial diet without *Chlorella vulgaris* (CV). *Chlorella vulgaris* powder was purchased from Japan. Four treatment diets were prepared that included the supplementation of 5% (CV 5), 10% (CV 10), 15% (CV 15), and 20% of dry powdered *Chlorella vulgaris*. Upon combining with the grounded feed, water was added and mixed well to obtained dough. The dough was pelleted with meat micter into considerably 2 mm particle size. It was dried and kept at a temperature of -4°C until use. The experiment lasted for 12 weeks, during which the fish were fed twice daily, at 8:30 AM and 5:00 Pm.

Water Quality Measurements

The experimental tanks used for the studies had a capacity of 150 L and were equipped with a closed re-circulation system. Each tank was fitted with top filter pumps and aeration diffusers (Sonic Air Pump P85, China) to

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ensure proper dissolved oxygen flow. The tanks were cleaned, disinfected, dried, and then filled with de-chlorinated water up to two-thirds of their total volume. To prevent the fish from jumping out, the tanks were covered with mesh nets of 2.0–3.0 mm size. To maintain optimal water quality for the fish, 30–50% of the water was replaced every 2 to 3 days. Water quality was monitored regularly according to APHA (1992). Using an Extech DO700 meter, dissolved oxygen levels were maintained above 4.0 mg/L, with water temperatures between 26-27°C.

Table 1: Nutritional information of basal diet

Nutrients	minimum	Maximum%
Crude protein	45	-----
fat	8.00	12.00
ash	----	8.00
Crude fiber	2.00	4.50
moisture	------	8.00
calcium	1.50	2.00
phosphorous	0.10	-----
sodium	0.30	----

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 samples were transferred into 1.5 ml heparinized tubes (Trittau, Germany) for hematological analysis. The heparinized blood samples were then stored in a refrigerator at 4°C until further analysis.

The complete blood count (CBC) included the measurement of the following parameters: red blood cells (RBC), white blood cells (WBC), hematocrit (Hct, %), and hemoglobin (Hb, g/L), which were analyzed using an automated hematology analyzer (Sysmex XN, Germany). Other related parameters such as MCV, MCHC, and MCH were calculated using equations according to Fazio *et al*., 2016

MCV = Hct x10/RBC,

MCH= Hgb x 10/RBC,

MCHC(gd/l) = (Hgb/Hct) x 100

Where MCV= Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin and MCHC = Mean Corpuscular Haemoglobin Concentrations.

Statistical Analysis

The obtained data were analyzed using one-way ANOVA (Analysis of Variance) to assess the effect of *C. vulgaris* on *C. gariepinus* supplements. A p-value < 0.05 was considered statistically significant.

RESULTS

The impacts of *Chlorella vulgaris* (algae) inclusion levels and their interactions on the hematological parameters of *Clarias gariepinus* were investigated. The inclusion of *Chlorella vulgaris* (algae) in the diet of *Clarias gariepinus* resulted in notable changes in hematological parameters. For red blood cells (RBC), no significant differences were found across the diets ($F = 1.48$, $p > 0.279$). However, the mean values for the 20%, 15%, 5%, and 10% Chlorella diets were significantly higher than the control (0%). A significant difference in white blood cell (WBC) count was observed between dietary levels ($F = 7.29$, $p=0.005$), with the highest value recorded in the CV 15% group (6.27 ± 0.321), followed by CV 5% (5.87± 0.87), and the lowest in the control group (4.17± 0.25).

For hematocrit (Hct), a significant difference was observed between the dietary levels ($F = 4.54$, $p<0.024$), with the 15% (38.67 ± 1.53) and 20% (38.00 ± 1.00) diets showing significantly higher values than the control (33.67 ± 3.21). Regarding hemoglobin (Hb),

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significant differences were found between diets (F = 193, p<0.000), with the highest mean value in the control (CV 0%) at 10.47 ± 0.153, while the lowest was in CV 20% (8.100 ± 0.100), at p = 0.000. There were no significant differences or interactions observed for Mean Corpuscular Volume (MCV) (F = 0.47, p = 0.758), but significant differences were recorded for mean corpuscular hemoglobin (MCH) (F = 13.50, p = 0.000) and mean corpuscular hemoglobin concentration (MCHC) (F = 97.80, p = 0.000). The highest MCV value was recorded in CV 15% (101.67 ± 9.11), and the lowest in CV 20% (93.00 ± 0.00). The control group showed the highest values for both MCH (24.667±0.577) and MCHC (28.667 ± 0.577), while the lowest values were found in CV 20% (MCH: 21.667 ± 0.577, MCHC: 21.33 ± 0.577) Table 2.

Table 2: Effects of *Chlorella vulgaris* (algae) on the Hematological parameters of *Clarias gariepinus.*

Treatments	RCB(10^6cells/mm³)	WBC(10^3cell/mm)	HCT%	Hb(g/dl)	MCV(fl)	MCH	MCHC
CV 0%	3.33±0.404^{B}	4.17±0.25^{B}	33.67±3.21^{B}	10.47±0.15^{A}	99.3±6.34	24.67±0.58^{A}	28.66±0.58^{A}
CV 5%	3.80±0.61^{AB}	5.87±0.87^{A}	34.00±1.00^{B}	9.90±0.10^{B}	99.33±14.22	22.67±0.58^{B}	27.33±0.58
CV 10%	3.60±0.17^{AB}	5.53±0.32^{A}	36.38±1.53^{AB}	9.03±0.058c	101.0±6.6	22.67±0.58^{B}	22.67±0.58 c
CV 15%	3.83±0.50^{AB}	6.27±0.32^{A}	38.67±1.53^{A}	8.100±0.15^{D}	101.67±9.11	21.67±0.58^{B}	22.33±0.58 c^{D}
CV 20%	4.10±0.10^{A}	5.36±0.50^{AB}	38.00±1.0^{A}	8.10±0.100^{E}	93.0±0.0	21.67±0.58^{B}	21.3±0.58^{D}
P-value	0.279	0.005	0.024	0.00	0.758	0.00	0.00

RBC- Red blood cells, WBC- White blood cells, HB- Haemoglobin, HCT- Haematocrit, MCV- Mean corpuscular, MCHC- Mean cell haemoglobin concentration, MCH- Mean cell haemoglobin.Values are expressed as mean ± standard error. Different letters (a, b, c, d, e) in the columns represent significant differences (Tukey's test, p≤0.01).

DISCUSSION

Hematological parameters are increasingly utilized as indicators of physiological stress responses to environmental or dietary changes in fish. These parameters serve as a crucial tool for effectively and sensitively monitoring the physiological and pathological conditions of fish (Sharma and Shukla, 2021). In vertebrates, metabolic processes occur within the blood vascular system, which regulates vital life functions. As the body's communication tissue, it serves as the medium through which all cells interact and exchange necessary substances (Kandeepan, 2014). In the present study, the results showed a significant increase in red blood cells, white blood cells, and hematocrit (HCT) percentage in all the algal-supplemented diets, with the 15% and 20% inclusion levels showing the highest values. The control group exhibited significantly lower values of these parameters compared to the treated groups. These findings are consistent with the study by Abbas *et al.* (2020), which examined the pharmacological effects of *Chlorella vulgaris* in tilapia fish and reported higher values in all hematological parameters. This improvement in hematological parameters could be attributed to the inclusion of algae in the fish diets, which likely enhanced these values. Saberi *et al.*, (2017) also reported higher values in RBC, WBC, HCT, and MCV in the algae-treated

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groups, while the control group showed lower values but higher Hb, MCH, and MCHC. These findings are in complete agreement with the present study, where the control group had significantly higher MCH, MCHC, and Hb, while the treated groups displayed lower values in these parameters but higher values in other blood characteristics. This could be attributed to the inclusion of algae in the diets, which likely improved the blood parameters.

In the present study, the results showed a significant increase in red blood cells, white blood cells, and hematocrit (HCT) percentage in all the algal-supplemented diets, with the 15% and 20% inclusion levels showing the highest values. This in accordance to the study conducted by Amira *et al*. (2022) on the effects of microalgae-inoculated diets on growth performance and blood parameters of Nile tilapia, higher values were observed in the hematological parameters of the algae-treated groups compared to the control. This aligns with the findings of the present study, where higher values were seen in the algae treated groups, particularly at the 15% and 20% inclusion levels, while the control group showed lower values. This could be attributed to the inclusion of algae in the diets, which likely improved the blood parameters. Furthermore, Abdulrahman *et al*., (2018), in his study on the physiological effects of micro algae. *Chlorella sp.* on common carp (*Cyprinus carpio*), reported similar positive results, with higher hematological parameters observed in the algae-treated groups compared to the control. This finding is consistent with the results of the present study, which showed higher values in the supplemented groups compared to the control. Based on these results, the inclusion of *C. vulgaris* (CV) in the feed of *C. gariepinus* led to an increase in all measured hematological parameters. An enhancement in WBC, RBC, HCT, Hb, and MCV levels was observed in fish fed with CV at varying concentrations. This improvement can be attributed to the positive effects of the bioactive components in CV, such as vitamins and alkaloids, which likely support immune function and overall fish physiology. Moreover, Habib *et al.*, (2021) reported higher values of RBC, Hb, and PCV in farmed fish, with lower values in wild fish, as well as lower WBC and MCH. These findings are similar to the results observed in the present study. The results of the present study demonstrated that supplementing *C. gariepinus* diets with *Chlorella* led to higher RBC, WBC counts, HCT, and MCV values, while the control group exhibited lower values. A study was conducted to evaluate the toxic effects of chlorpyrifos on Nile tilapia (*Oreochromis niloticus*). Hematological assessments revealed significant differences in hemoglobin concentration, white blood cell (WBC) counts, and red blood cell (RBC) numbers between the chlorpyrifos-treated group and the control group (Hossain *et al*., 2022). Furthermore, in another study on the effects of *Chlorella vulgaris* and germinated barley powder on blood as prebiotic sources in the diets of common carp (*Cyprinus carpio* L.). The findings showed that dietary supplementation with *Chlorella* and germinated barley significantly increased hemoglobin levels, red blood cell (RBC) count, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Additionally, white blood cell (WBC) counts were notably affected (Sleman *et al*., 2021). All these findings were in total agreement with what the present study recorded with *C. vulgaris* groups exhibited significant changes than the ones fed with untreated diet (Sleman *et al*., 2021).

CONCLUSION

It can be concluded that inclusion of micro alga such as *Chlorella vulgaris* in the diet of *C. gariepinus* generally improved the blood

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characteristics. The changes in red blood cells, white blood cells, hematocrit, MCH, MCHC resulted in the betterment of the blood of these fish than the untreated ones. Therefore, incorporating CV into fish feed may play a stimulatory role in boosting the blood qualities and its physiological health.

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