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Ensuring Safe Consumption-Comprehensive Health and Safety Analysis of Fish Grown in Algae-Based Wastewater Systems

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ABSTRACT

The global demand for fish is on the rise; consequently, there is a need for sustainable aquaculture practices to address environmental degradation, resource depletion and increased operational costs. In this study, the African catfish (*Clarias gariepinus*) fingerlings were used: one group received CO_2-enriched algae cultivated with diesel exhaust (experimental), while the other was provided with standard fish feed (control). Length and weight were systematically observed in a controlled environment (25°C, pH 7.0), post-experiment analyses of haematological, biochemical, and heavy metal parameters were conducted utilizing spectrophotometry, auto-analyzers, and histological evaluations. Fishes from the experimental group showed improved growth rates, decreased mortality, and enhanced immune responses to the algae-rich composition of nutrients. Proximate and phytochemical analyses showed nutritional advantages in favor of algae-fed fish, explaining higher protein and lower fat content. The potential benefits presented by algae were countered by histological signs of immune response and tissue damage seen in the experimental fish. This study concluded that algae-based wastewater systems offer a bright promise for sustainable aquaculture, yet their potential applications rely upon optimized cultivation procedures and strict operational rules to assure environmental integrity and safety of human health. Future research should optimize algae development by examining species-specific growth trends, nutrient enrichment strategies, and CO_2 absorption efficiency from diesel exhaust.

INTRODUCTION

Fish are in demand globally due to its nutritious benefits and growing popularity. Fish aquaculture faces environmental deterioration, resource depletion, and high operating expenses **(Bahadar & Khan, 2013)**. To assure food security and reduce environmental consequences, eco-aquaculture methods must be investigated and developed.

Algal wastewater treatment for aquaculture is being considered. Nutritionally dense algae may replace traditional fish feed and lessen dependence on environmentally and economically unsustainable feed sources. Wastewater algae bioremediate by sequestering

nutrients and pollutants, improving water quality for sustainable fish farming **(Sing *et al.*, 2013)**.

These benefits notwithstanding, wastewater-fish enterprises' safety and suitability are disputed. Fish bioaccumulation of heavy metals, pathogenic bacteria, and other contaminants from algal-wastewater systems must be thoroughly researched. Some algae can regulate waterborne contaminants, however the extent to which algae fish production systems affect fish health and edibility is unknown **(Dalrymple *et al.*, 2013)**. Intensive fish-feed production still puts strain on marine biodiversity since most feed firms use wild fish species as inputs. Overfishing disrupts the marine ecology, and fish-feed production uses a lot of land and energy **(Dalrymple *et al.*, 2013)**. Therefore, establishing cost-effective, sustainable, and nutritionally acceptable alternatives like algae-based diets from treated wastewater is crucial.

The effluents laden with nutrients from fish farms may cause eutrophication, which causes algal blooms, oxygen loss, and aquatic creature mortality **(Iancu *et al.*, 2012)**. Thus, treating wastewater with algae in aquaculture reduces pollution and creates a circular bioeconomy by turning waste into fish farm input **(Sing *et al.*, 2013)**.

The project aimed to determine the safety and acceptability of algal-based wastewater fish for human consumption. Assessing heavy metal deposition, microbial contamination, and fish nutrition will aid sustainable aquaculture techniques. The results will be used to manage wastewater-based fish farming models that are ecologically sustainable, commercially viable, and food safety-compliant

MATERIALS AND METHODS

1. Fish cultivation and farming experiment

1.1 Selection of fish

Sixteen fingerlings of the African catfish (*Clarias gariepinus*) of equal size (1.6 kg) and age (three weeks) were selected for the experiment.

The fish were randomly assigned into two groups:

1. Group A (Experimental Group): Eight fish grown in wastewater and fed algae being enriched with CO_2 from diesel exhaust.
2. Group B (Control Group): Eight fish grown in clean water and fed with regular fish feed.

Fig. 1. Catfish (*Clarias gariepinus*)

1.2 Algae cultivation and preparation for fish feeding

Prior to the start of the experiment, alga were grown under conditions enriched with CO_2 for three weeks for the experimental group (Group A). The enrichment of algal biomass and nutrients utilized captured diesel exhaust gases. Algae were fully cultured and harvested, washed and stored in a refrigerator set to 4°C until use. Control fish (Group B) were fed with a fish feed from Rumuosi that is commercially available.

1.3 Feeding protocol

The feeding protocols include:

i. Group A (Experimental): Fish were fed with CO_2-enriched algae three times a week on designated feeding days at 11 AM.
ii. Group B (Control): Fish were fed standard commercial fish feed daily and at a common feed rate.

1.4 Experimental conditions

Both experimental groups were maintained under controlled environmental conditions that were kept constant throughout six weeks of the entire experiment:

i. Water temperature was maintained at 25°C ± 1°C.
ii. pH value was maintained at 7.0 ± 0.2.

1.5 Growth monitoring and data collection

Every week, the growth of fish was looked into for their length and weight changes.

i. Initial measurements: Beginning of the experiment, both groups averaged 15cm.
ii. Measurements at the end of the six weeks:
    a. Group A; average length of 25cm and final weight of 2.4kg (average weight gain of 0.8kg).
    b. Group B: Average length of 22cm and weight of 2.1kg (average weight gain-0.5kg).

1.6 Mortality tracking

Recording mortality rates was another activity carried out in the course of the experiment:

i. Group A (Experimental): One fish mortality (12.5% mortality rate).
ii. Group B (Control): Two fish mortalities (25% mortality rate).

1.7 Post-experiment analysis

After the completion of the experiment, all the fish that survived were transported from the Experimental Station to Divic Specialist Laboratory, Rivers State, where a set of biochemical, hematological, heavy metal, and histological analyses were carried out to assess the influence of the different feeding regimes on fish health and suitability for humans.

2. Fish samples analysis method

2.1 Hematological analysis of fish samples

Sample collection

Blood samples were taken from fish below the gills through a surgical incision and were collected into K2-EDTA tubes to avoid clotting. The samples were then analyzed for various hematological parameters.

Estimation of packed cell volume (PCV)

The packed cell volume of fish blood was measured using the centrifuged method to quantify the proportion of red blood cells (RBCs) in whole blood.

Methodology

A 2mL blood sample was collected in an EDTA tube and mixed thoroughly. A capillary hematocrit tube was then filled to two-thirds of its length with the blood sample and sealed with plastisol. The tubes were centrifuged for 5 minutes at 3000 rpm. After centrifugation, the packed cell volume (PCV) was measured using a microhematocrit tube reader and expressed as a percentage of the total blood volume.

Hemoglobin (Hb) estimation

The hemoglobin levels were estimated based on a formula derived from PCV values. From the estimated PCV value we calculated the Hemoglobin count using the given formula:

$$\text{Hb Count} = PCV/3$$

Platelet count

The objective of this method was to estimate the number of platelets in the fish sample by using the Brecker-Cronkite method with ammonium oxalate. In this method, whole blood was diluted with 1% ammonium oxalate that hemolyzes the red blood cells but leaves the platelets. The platelets were then counted under a microscope with the aid of a phase-contrast hemocytometer.

Procedure

In a clean test tube, a solution of 0.38mL of 1% ammonium oxalate was prepared, to which 0.02mL of whole blood (K2 EDTA treated) was added. The tube was gently tapped to properly mix the contents. The counting chamber was prepared; it was cleaned, and the cover glass was assembled. The blood portion was then carefully introduced into the chamber with a Pasteur pipette. The chamber was left undisturbed for 20 minutes in a moist petri dish, and the platelets were counted using a 40× objective of the microscope within the four-square corners of the chamber.

$$\text{Platelet count} = (Cells\ counted \times Dilution\ Factor)/(Depth \times Area)$$

Total white blood cell (WBC) count

Total WBC counting utilized Turk's fluid which lyses red blood cells so that white blood cells may be stained for counting microscopically.

Procedure

A solution was made containing 0.38mL of Turk's fluid and to this, 0.02mL of whole blood was added to effect dilution. This was cautiously placed in a hemocytometer with white blood cells counted in four large squares under the microscope, followed by calculations of total WBC using the same formula as that for platelet count.

Differential white cell count

The white cell differential count was determined using the Leishman stain.

Procedure

A drop of blood was well mixed and applied to a clean slide, then spread at 45° to form a thin smear. After air drying, the smear was stained with Leishman stain for 2 minutes and treated with buffer water (pH 6.8) for 5 minutes. After rinsing and drying, the stained slide was examined under a 100× oil immersion microscope to identify and count different types of white blood cells.

2.2 Pathological analysis of fish samples

Blood collection and sample preparation

Blood samples were collected by jugular incision and put in plain red-capped containers. They were left to clot after which they were centrifuged at 3,000rpm for 5 minutes. The extracted serum was kept in properly labeled bijou bottles awaiting pathology analysis.

Estimation of sodium and potassium

Sodium and potassium were estimated by electrochemical analysis with an ISE autoanalyzer.

Methodology

The autoanalyzer was initially calibrated with standard solutions. The autoanalyzer aspirated 200µL of each sample, which were then measured/given as sodium and potassium concentrations on the screen.

Estimation of serum bicarbonate

The concentration of serum bicarbonate was estimated by back titration.

Methodology

Hydrochloric acid was added to the serum liberated CO_2. The mixture was then titrated with sodium hydroxide to get the original pH. Bicarbonate concentration was determined using the formula:

$$\text{Conc. of Bicarbonate} = (1 - X) \times 50$$

Where, X is titrated volume of NaOH.

Estimation of urea

The concentration of urea in fish serum was determined by the diacetyl monoxime (DAM) method, which is colorimetric in nature. The concentration of urea was determined using the formula:

$$\text{Conc. Of Urea} = (Absorbance\ of\ test)/(Absorbance\ of\ standard) \times Conc.of\ Standard$$

Estimation of creatinine

Creatinine concentration in fish samples was determined using the Jaffe-slot method, based on the orange color formation due to creatinine reaction with alkaline picrate. Concentration of creatinine calculated as:

$$\text{Conc. Of } \mathbf{Creatinine}\ (Absorbance\ of\ test)/(Absorbance\ of\ standard) \times Conc.of\ Standard$$

Estimation of total and conjugated bilirubin

Bilirubin determination was performed by the Jendrassik and Grof method, which determines total bilirubin and conjugated bilirubin based on the action of diazotized sulfanilic acid. Total bilirubin reacts in the presence of caffeine and tartrate to form a blue-colored complex, measured at 600nm. The conjugated bilirubin reacts with normal saline under alkaline condition and is recorded at the 540nm line.

Estimation of total protein

The concentration of total protein was determined according to the Biuret colorimetric method. The reagent Biuret interacts with the peptide bonds of proteins in an alkaline medium to give a purple-colored complex, which was recorded at 540nm. The protein concentration was calculated as:

$$\text{Conc. of Total Protein} = (Absorbance\ of\ test)/(Absorbance\ of\ standard) \times Conc.\ of\ Standard$$

Estimation of albumin

The concentration of the albumin was determined by Bromocresol Green (BCG) method. In buffered solutions, albumin was bound with Bromocresol green to give a green color. The absorbance read at 600nm, and the intensity of that color was directly proportional to the concentration of albumin present.

$$\text{Conc. Of Albumin} = (Absorbance\ of\ test)/(Absorbance\ of\ standard) \times Conc.\ of\ Standard$$

Estimation of liver enzymes (ALT, AST, ALP, GGT)

Important liver enzymes, namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT), were estimated on an automated biochemical analyzer of the type BS-120S.

Estimation of uric acid, magnesium, inorganic phosphate, and calcium

These biochemical parameters were assessed by Mindray BS-120S. Allantoin, a red-violet molecule at 520nm, may be oxidized from uric acid. In alkaline media, magnesium reactions with xylidyl blue generate color. A phosphomolybdate compound formed by inorganic phosphate may be detected at 340nm. Calcium and O-Cresolphthalein form a violet combination at 570nm.

Estimation of lipids (Total cholesterol, triglycerides, HDL-cholesterol)

Using a Mindray BS-120M automated lipid measurement machine, total, triglycerides, and HDL cholesterol were estimated. Triglycerides were measured by lipase and total cholesterol by enzymatic hydrolysis and oxidation. After precipitating LDL and VLDL, HDL was isolated.

Heavy metal analysis using atomic absorption spectrophotometry (AAS)

The concentration of heavy metals in fish tissue was determined using atomic absorption spectrophotometry (AAS) according to the American Public Health Association (APHA) Method.

Sample digestion for solid samples

About 2g of dried fish sample was digested using a mixture of nitric acid (HNO_3), perchloric acid ($HClO_4$), and sulfuric acid (H_2SO_4). The digest was then diluted up to 100mL with distilled water for AAS analysis.

Sample digestion for liquid samples

One liquid sample of 100mL was mixed with nitric acid and the mixture heated to reduce volume. The digest was filtered and diluted to 100mL for AAS analysis.

2.3 Proximate and phytochemical analysis of fish samples

Moisture content determination

Fish tissue were weighed before and after drying in an oven at 105°C. Moisture content was calculated as:

$$\%Moisture\ Content = (W1 - W2)/Weight\ of\ Sample\ \times 100$$

Where, W1 = weight of the petri dish and sample before drying and W2 = weight of the petri dish and sample after drying.

Ash content determination (AOAC, 1990)

In determining the ash content, the inorganic matter remaining from the complete combustion of organic material was quantitated and the ash content was calculated as:

$$\%Ash\ Content = (W3 - W1)/(W2 - W1)\ \times 100$$

Where, W1 is the weight of an empty platinum crucible, W2 is the weight of the crucible and sample before burning, and W3 is the weight of the crucible and ash after burning.

Crude fiber determination (AOAC, 1990)

The crude fiber content was determined after defatting the sample and subjecting it to acid treatment followed by alkali digestion. The crude fiber content was calculated as:

$$\%Crude\ Fibre = Weight\ of Fibre/Weight\ of\ Sample \times 100$$

Crude fat determination by Soxhlet extraction (AOAC, 1990)

Crude fat was determined by the Soxhlet extraction process using petroleum ether as solvent. The crude fat was calculated as follows:

$$\%Crude\ Fat = (Weight\ of\ Flask\ +\ Fat - Weight\ of\ Flask)/Weight\ of\ Sample \times 100.$$

Crude protein determination by Kjeldahl method (AOAC, 1990)

Crude protein was estimated according to the Kjeldahl method, which determines the nitrogen content in a sample. The nitrogen content was calculated using these formulas:

$$\%Nitrogen = Titre\ Value \times 0.01 \times 14 \times 4$$

$$\%Protein = \%Nitrogen \times 6.25$$

Carbohydrate determination (Differential method)

The carbohydrate content was estimated by difference using the following formula:

$$\%Carbohydrate = 100 - (\%Protein + \%Moisture + \%Ash + \%Fat + \%Fibre)$$

Estimation of total carotenoids and lycopene (Zakaria *et al.*, 1979)

The estimations of total carotenoids and lycopene concentration were carried out using extraction with petroleum ether followed by spectrophotometry. The concentrations were calculated using the following formulas:

$$Total\ Caroteenoids = A450 \times (Vol\ of\ Sample)/(Weight\ of\ Sample) \times 4$$
$$Lycopene = 3.12 \times A503 \times (Vol.\ of\ Sample)/(Weight\ of\ Sample)$$

2.4 Phytochemical analysis using GC-MS

Sample preparation and extraction

Bioactive compounds were identified and quantified using GC-MS. Air-dried fish samples were pulverized and extracted by Soxhlet extraction in 60°C ethanol for 4 hours. After washing and evaporating the extract, bioactive fractions were recovered. Pyridine reconstituted the dried extract before GC-MS analysis.

In this scenario, a GC-MS with an HP-5MS column employed helium as carrier gas and 70 eV electron ionisation for detection. The oven programming increased temperature from 50 (hold for 1 minute) to 300°C, at 5°C each minute (hold for 10 minutes). Quantification was done using chromatogram regions. Chemicals in the sample were identified by comparing their mass spectra and retention time to library standards (Replib and Mainlab).

2.5 Histological analysis

Sample collection and preparation

Fish samples were collected from both experimental and control groups. To avoid post-mortem alterations, samples were transported in ice-cooled containers for histological, haematological, and biochemical assessments.

Skin tissues were examined for illness or contamination. The tissues were fixed in 10% neutral-buffered formalin, dehydrated in ethanol, cleaned with xylene, and embedded in paraffin wax. The microtome was used to cut thin slices (4-5µm), which were then stained with H&E and mounted for microscopic inspection.

RESULTS AND DISCUSSION

1. Fish culture analysis

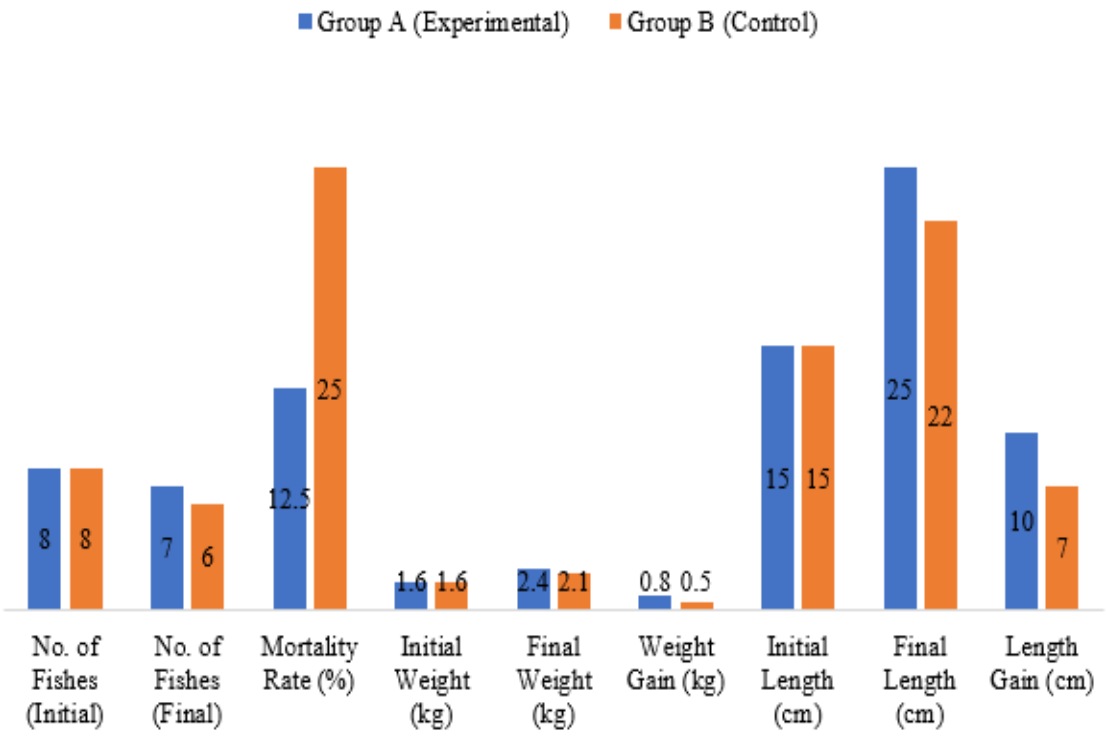

Fig. 2. Fish culture analysis

2. Analysis of fish samples

2.1 Hematological analysis

Table 1. Hematological results of fish sample

Parameter	Experimental 1	Experimental 2	Control 1	Control 2	Mean (Exp)	Mean (Control)
Neutrophil (%)	29	31	50	25	30	37.5
Lymphocytes (%)	60	55	40	58	57.5	49
Monocytes (%)	4	9	5	10	6.5	7.5
Eosinophil (%)	7	5	5	6	6	5.5
Basophil (%)	0	0	0	1	0	0.5
Packed Cell Volume (%)	15	25	25	22	20	23.5
Hemoglobin (g/dL)	3.33	6.67	8.33	7.33	5	7.83
Total WBC (10^9/L)	6	7	2	5	6.5	3.5
Platelet Count (×10^3/µL)	440	320	170	100	380	135

As seen in Table (1), the blood examination showed significant variations between experimental and control groups, emphasising the importance of food on fish health. Experiment 1 (29%) and experiment 2 (31%) had lower neutrophils than Control 1 (50%), indicating reduced inflammation, which is consistent with studies showing that algae-rich diets reduce inflammation due to their omega-3 content **(Khan & Abidi, 2010)**. However, lymphocytes were greater in experiment 1 (60%) and experiment 2 (55%) than Control 1 (40%), corroborating **Wu *et al.* (2013)** results that algae-based diets boost immunological responses. In Control 2 (10%) and experiment 2, monocytes were modestly higher, suggesting immunological activation (9%). Experiment 1 (15%) had a lower PCV than Control 1 (25%) and Control 2 (22%), suggesting anaemia.

The findings also showed that Experiment 1 and Experiment 2 had significantly lower haemoglobin levels (3.33 and 6.67g/ dL, respectively) than Control 1 (8.33g/ dL), indicating inadequate oxygen-carrying ability. This may be because traditional fish feed has more iron than algae-based diets, according to **Santhanam *et al.* (2023)**.

Higher total WBC counts in Experiment 1 (6.0 $\times 10^9$/L) and Experiment 2 (7.0 $\times 10^9$/L) indicate a greater immunological response, supporting **Sattanathan *et al.* (2022)** results that algal diets enhance immunity. In addition, platelet counts rose in both Experiment 1 (440 $\times 10^9$/L) and Experiment 2 (320 $\times 10^9$/L), suggesting effective clotting. Despite reduced haemoglobin and PCV for the experimental groups, immune indices improved, supporting **Lu *et al.* (2023)** claim that algae-based diets improve fish immunity.

2.2 Pathological analysis

Table 2. Pathological result of fish samples

Samples/Results	E1	E2	C1	C2
Sodium	158	156	143	139
Potassium	6.0	6.3	4.5	4.2
Bicarbonate	12	11	17	20
Urea	20.5	22.4	6.8	7.2
Creatinine	240	225	130	120
Total Bilirubin	10	14	25	31
Conjugated Bilirubin	35	8	6	Nil
Total Protein	10	14	25	31
Albumin	6	8	11	18
AST	486	476	138	104
ALT	54	21	14	23
ALP	110	89	37	44
GGT	48	53	6	9
Uric acid	0.02	0.02	0..01	0.007
Magnesium	2.0	1.5	0.5	0..4
Calcium	1.3	1.5	0.6	0.7
Inorganic phosphate	2.9	2.4	0.3	0.4
Total Cholesterol	0.1	0.07	0.2	0.3
Triglycerides	0.2	0.4	0.07	0.1
HDL Cholesterol	0.01	0.01	0.03	0.02

Table 3. Heavy metal result of fish samples

S/N	Parameter	Experiment (ppm)	Control (ppm)
1	Sodium, Na	8.89	7.922
2	Potassium, K	3.002	12.019
3	Magnesium, Mg	10.578	6.672
4	Iron, Fe	0.323	0.129
5	Chromium, Cr	0.092	0.039
6	Calcium, Ca	7.922	8.432
7	Zinc, Zn	0.722	0.919
8	Copper, Cu	0.996	0.484
10	Phosphorus, P mg/kg	8.057	9.223
11	Phytochemicals	12.56	78.58

Pathological examinations, as shown in Tables (2, 3), show differences between experimental (algae-fed) and control (regular diet) fish.

Electrolytes (Sodium, Potassium, Bicarbonate)

Experimental fish had greater sodium levels than controls (158, 156mmol/ L), suggesting improved electrolyte regulation. Compared to controls, experimental groups had higher potassium levels. Again, experimental fish had potassium levels of 6.0 and 6.3mmol/ L, whereas controls had 4.5 and 4.2. Bicarbonate levels were lower in experimental groups (12, 11mmol/ L) than control (17, 20 mmol/L), suggesting that the algae-based diet may affect fish acid-base regulation.

Kidney function (Urea, Creatinine)

The algae-based diet boosted protein metabolism or decreased renal excretion, since experimental groups had higher urea levels (20.5-22.4mmol/ L) than control groups (6.8-7.2). High protein meals from algae resulted in elevated creatinine levels (225-240µmol/ L) compared to control (120-130µmol/ L), suggesting greater muscle metabolism or renal stress.

Liver function

Experimental groups had lower total bilirubin levels (10-14µmol/ L) than control (25-31µmol/ L), indicating improved liver function. Conjugated bilirubin concentrations in experimental fish (8-35µmol/ L) were greater than control (6µmol/ L or undetectable), indicating enhanced detoxification. AST measurements were substantially higher in experimental meal treatments (476-486U/ L) than control (104-138U/ L), suggesting metabolic stress. Experimental fish had somewhat higher ALT concentrations (21-54U/ L) than control (14-23U/ L). ALP concentrations were much greater in experimental fish (89-110U/ L) than control (37-44U/ L), suggesting liver or bone stimulation.

Additionally, experimental fish had greater GGT (48-53U/ L) than control fish (6-9U/ L), showing that algal diets improve liver function.

Blood components (Total proteins, albumin)

Experimental groups had lower total protein levels (10 to 14g/ dL) than control groups (25 to 31g/ dL), indicating reduced protein production or storage. The experimental groups had lower albumin levels (6 to 8g/ dL) than the control groups (11 to 18g/ dL), suggesting reduced liver production of albumin, a protein needed for blood osmotic pressure and transport.

Calcium, magnesium, inorganic phosphate, cholesterol, triglycerides

Magnesium levels were greater in experimental groups (2.0mmol/ L Experiment 1, 1.5mmol/ L Experiment 2) than in control (0.5mmol/ L Control 1, 0.4mmol/ L Control 2). This indicates superior mineral absorption. Calcium levels were also higher in experimental groups (1.3mmol/ L in Experiment 1, 1.5mmol/ L in Experiment 2) than in control (0.6mmol/ L in Control 1, 0.7mmol/ L in Control 2), indicating enhanced bone health. Experimental groups had considerably greater inorganic phosphate levels (2.9mmol/ L in Experiment 1, 2.4 in Experiment 2) than in control (0.3 and 0.4mmol/ L, respectively), indicating a satisfactory mineral balance.

In lipid metabolism, experimental groups had lower total cholesterol (0.1mmol/ L in Experiment 1, 0.07 in Experiment 2) than in control (0.2, 0.3). This improved fat metabolism. However, triglycerides were higher in the two experimental groups (0.2mmol/ L in Experiment 1, 0.4 in Experiment 2) than in the control (0.07 in Control 1, 0.1 in Control 2), suggesting energy storage. HDL cholesterol decreased by 0.01mmol/ L in Experiment 1 and 2 compared in Control (0.03 and 0.02, respectively).

The algae enhanced fish mineral homeostasis, immunological activity, and energy storage, however protein utilisation was a possible metabolic stress indication in the experimental groups.

Heavy metal analysis

Heavy metal analysis demonstrated many experimental vs control group variations. The experimental group had higher levels of sodium (8.89 vs. 7.922ppm), magnesium (10.578 vs. 6.672ppm), iron (0.323 vs. 0.129ppm), and chromium (0.092 vs. 0.039ppm), indicating better metabolic support and nutrient absorption. In contrast, potassium mineral decreased in the experimental group (3.002 vs. 12.019ppm), indicating restricted potassium availability. The experimental group had decreased zinc and phosphorus levels, whereas the control had more antioxidant selenium (0.81 vs. 0.424ppm). Experimental group phytochemical levels were lower than control group levels (12.56 vs. 78.58ppm), suggesting fewer plant-derived nutrition consumption. Thus, whereas the experimental diet increased mineral absorption, it may have missed phytochemicals that affect antioxidant capacity and health.

2.3 Proximate analysis

Table 4. Proximate analysis result of fish samples

Parameter	Experimental skin (%)	Control skin (%)
Moisture	33.95	38.922
Fiber	7.336	6.885
Ash	5.917	15.085
Fat	0.637	4.068
Protein	23.8	18.9
Carbohydrate	28.36	16.14

Fish skin samples showed substantial changes between the experimental group given CO_2-rich algae and the control group fed regular fish feed by proximate analysis (Table 4). The experimental fish contained 23.8% protein compared to 18.9% in the control, indicating improved nutritional value. The experimental fish had 0.637% fat, whereas the control group had 4.068%, indicating health benefits and longer shelf life. Experimental fish had a tougher texture due to decreased moisture retention (33.95%) than control samples. The experimental fish contained more fiber (7.336%) and carbohydrate (28.36%), whereas the control had more ash (15.085%). This increases inorganic matter. These findings demonstrate CO_2-rich algae as a sustainable fish diet with enhanced nutritional quality, digestibility, and environmental benefits.

2.4 Histological analysis

The control fish had healthy epidermis and dermis and no inflammation, demonstrating that standard feed maintains healthy skin (Fig. 3) **(Joshi *et al.*, 2021)**.
The experimental group had thinning epidermis, lymphocyte infiltration, and necrosis, suggesting immunological responses. In extreme instances, epidermal ulceration and necrotic dermis eosinophilia reflect heightened immune activity, maybe related to allergies or pollutants. Research suggests that fish fed algae-based diets may cause tissue damage and immunological reactivity **(Garnica-Gómez *et al.*, 2023)**.

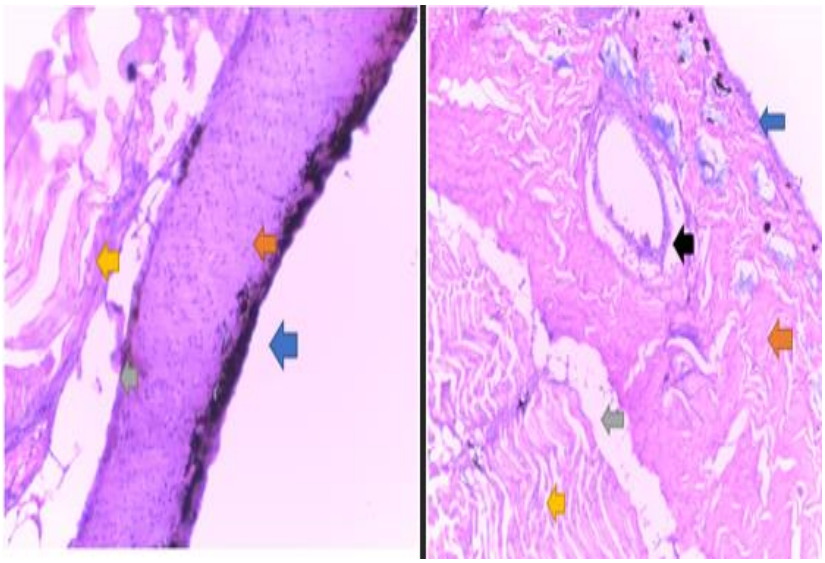

Fig. 3. Histological analysis of fish skin tissue from control culture

Blue arrow – Normal epidermal layer, Orange arrow – well structures dermis, Yellow arrow – collagen fibers in the connective tissue, Gray arrow – blood vessels within the dermis, and Black arrow – normal cellular organization

2.5 Phytochemical analysis

Table 5. Phytochemical analysis result of fish sample using GC-MS

Compound name	Amount (ppm)	Control (Fish feed)	Experimental (CO_2-enriched algae)
Daidzein	-	-	0.54
Daidzin	-	-	0.48
Daidzin	0.57	0.57	0.48
Cinnamic acid	1.30	1.30	0.48
Butein	0.39	0.39	0.29
Coumaric acid	1.52	1.52	-
Ferrulic acid	-	-	1.46
Ferrulic acid	0.13	0.13	1.46
Artemetin	18.27	18.27	-
Naringenin	0.72	0.72	0.44
Ellagic acid	-	-	0.72
Luteolin	0.77	0.77	0.23
Sinapinic acid	0.62	0.62	0.26
Kaempferol	0.72	0.72	0.54
Piperic acid	0.39	0.39	0.47
Flavone	0.43	0.43	-
Epicatechin	-	-	0.51
Epigallocatechi	0.30	0.30	0.30

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Quercetin	0.32	0.32	0.32
Flavon-3-ol	0.71	0.71	0.71
Gallocatechin 3 gallate	0.65	0.65	0.65
Robinetin	2.16	2.16	2.16
Myricetin	0.27	0.27	0.27
Nobiletin	0.15	0.15	0.15
Baicalin	-	-	-
Tangeretin	-	-	-
Artemetin	-	-	-
Naringin	-	-	-
Cinnamic acid	0.17	0.17	0.17
Coumaric acid	-	-	-
Syringic acid	-	-	-
Rosmarinic acid	-	-	-

Table (5) shows that bioactive substances including Daidzein (0.541ppm), Daidzin (0.475ppm), and Ferrulic acid (1.45951ppm) have greater concertation, suggesting better antioxidant capabilities.

Cinnamic acid was found in both groups, although its concentration was higher in the control group. CO_2-enriched algae increase phytochemical bioavailability in fish, enhancing nutritional and health advantages, supporting research on flavonoids in fish diets **(Khan & Abidi 2010)**.

CONCLUSION

Fish given algae-based feed had superior growth, nutrition, and mortality than fish fed standard fish meal. However, heavy-metal-rich untreated wastewater may harm fish tissue. Effective pre-treatment and safety evaluation are needed to properly utilize wastewater for fish.

Future research should optimize algae development by examining species-specific growth trends, nutrient enrichment strategies, and CO_2 absorption efficiency from diesel exhaust. According to food safety regulations, contamination control should also include sophisticated water treatment and heavy metal remediation. The economic feasibility, market acceptability, and nutritional benefits of algae-based feed compared to traditional algae-based feed must be assessed.

Governments, academic institutions, and private firms must collaborate to commercialize and expand algae-based aquaculture. Aquaculture companies may

embrace these green, cost-effective, and scalable options that ensure global food security and save the environment by pushing legislation and scientific advances.

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