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Differential quantities of carbon quantum dots incorporated feed on the growth and biochemical traits of Zebrafish (*Danio rerio*)

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Abstract Carbon quantum dots possess excellent hydrophilicity and cell permeability, making them very suitable for water-based and bio-related applications. However, there is a lack of research on the utilization of carbon quantum dots in fish feed. The results of this investigation showed that carbon quantum dots have high adsorption peaks at 355 nm in the UV-visible absorption spectra. SEM image was observed at the wavelength range from 13.77 to 13.69. EDAX spectrum showed three peaks and was located on thespectrum between 0.1 KeV and 3 KeV. FT-IR spectral observation was at a wavelength range from 4000 to 500cm^{-1}. The condition factor of Zebrafish exhibited an increase in all the experimental feeds as compared to the original condition. The consumption of feed, the efficiency of converting feed into growth, growth rate, percentage of growth, rate of growth relative to body size, assimilation of nutrients, metabolism, and efficiency of gross and net growth were all higher in F4 when it contained 1.5 ml of synthesized carbon quantum dots (1.39±0.28, 0.53±0.16, 0.05±0.005, 20.96±4.98, 1.36±0.28, 1.32±0.26, 5.85±2.14, and 6.08±1.30). The results conclude that 1.5 ml of carbon quantum dots incorporated in feed enhanced Zebrafish's growth and biochemical traits.

Keywords: Carbon quantum dots, Growth, Biochemical traits, Zebrafish

Introduction

Carbon Quantum Dots (CQDs), often referred to as Carbon Dots (CD), are nanomaterials that have a zero-dimensional structure and a size of less than 10 nm. They are composed of almost spherical nanoparticles that are also smaller than 10 nm (Baker and Baker, 2010). Possessing special qualities such as being easy to use, soluble in a large amount of water, penetrating cells, and illuminating at different wavelengths when excited (Roy *et al*., 2015; Havrdova *et al.,* 2016). The compounds of silver, cadmium, zinc, mercury, selenium, lead etc. can form QD, and are widely used in drug delivery, biomedical research and various therapy applications. QD are unique from other nanoparticles because of their properties such as long-term fluorescence resistance against photobleaching, excellent signal brightness,

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high adsorption coefficients, light emission, simultaneous excitation of different fluorescence colours etc (Singh *et al*., 2018). CQDs may be able to replace traditional metal-based quantum dots in the disciplines of chemical sensing, biosensing, bioimaging, photovoltaics, photocatalysis, and drug targeting in the future (Wang *et al*., 2016). Owing to its extensive applications, high hydrophilicity, and cell penetration, the biosafety of CQDs warrants careful attention. (Sun *et al*., 2015). However, few studies haven't been done on adding CQDs to fish feed. According to certain preliminary research, CQDs were extremely safe and biocompatible in their surroundings. Zebrafish is one of the most widely used model organisms due to their fast embryonic development and short generation time. Transparent Zebrafish embryos are most favourable to fluorescence bioimaging of nanoparticles (Kang *et al*., 2015). Zebrafish had more than 10,000 genes which are similar to that of humans. Hence, in the present study, the incorporation of different quantities of carbon quantum dots in the feed on growth and biochemical traits of zebrafish *Danio rerio* was assessed for the first time. The Carbon Quantum Dots were synthesized chemically and characterized using UV-visible spectroscopy, SEM, EDAX, and FT-IR. Six feeds were prepared with differential quantities of chemically synthesized carbon quantum dots (F1-Control, F2 - 0.5ml, F3 – 1ml, F4-1.5ml, F5- 2 ml and F6-2.5ml) and feed ingredients are fish meal, groundnut oilcake, wheat flour, and tapioca flour.

Materials and methods

Synthesis of CQDs

Citric acid ($C_6H_8O_7$), L-Histidine ($C_6H_9N_3O_2$) and distilled water were collected from the Department of Biology, The Gandhigram Rural Institute - Deemed to be University, Gandhigram, India. Analytical grade chemicals were all utilized in the synthesis of Carbon Quantum Dots; no additional purification was required. Before being used, every glass wares was dried and cleaned three times using deionized water. Carbon Quantum Dots (CQDs) were chemically synthesized by the hydrothermal method for this study. Citric acid (pellets) 2 gm and 0.7 gm L-Histidine (pellets) become a solution in 50 ml of distilledwater in a conical flask. The colourless solution is stirred well using a stirrer for 5 minutes under 300 rpm. Then the stirred sample solution is poured into Teflon and placed in a muffle furnace for 8 hrs at 150°C. The colourless solution turned into brown colour which represents the presence of CQDs. Then it is centrifuged for 20 minutes at 10000 rpm at normal room temperature. Using a syringe collect the sample into Eppendorf tubes.

Characterization

Using a spectrophotometer UV-Vis Double Beam DUV 3500, UV-Vis spectroscopy studied the physical properties and primary characterization of carbon quantum dots that were chemically synthesized. An energy-dispersive X-ray spectrometer (EDAX) was used to analyze the chemical composition of CQDs, while Tesco SEM-VEGA III lmu, a type of scanning electron microscopy working at 20 kv in the vacuum, was used to study the morphology of the CQDs. Using Thermo-Scientific NICOLETTI IS5, the functional group of CQDs was recorded over a 4000–400 cm^{-1} spectral range.

Experimental study and feeds

For the growth study, Zebrafish fingerlings (0.120 ± 0.020g) were purchased from Reo Pets, Dindigul, Tamil Nadu, India, for growth experiments. Fish were subsequently brought to the laboratory in polythene bags that were filled with oxygenated water. Fish were allowed to acclimatize for 10 days at 28 ± 2^0C in glass aquariums measuring 60L ×45B×45 H centimetres. Fish meal, groundnut oil cake, wheat flour, and rice bran were utilized in a dry pellet feed during acclimation.

The selection of necessary components for feed preparation is dependent on the fish's ability to receive nutrients. The feed's protein content was estimated using the Micro-Kjeldhal method, and it was then produced. The sources of protein and carbohydrates used to prepare feed are fish meal and ground nut oil cake, tapioca powder, and wheat flour. All of the items were dried before being pulverised and sieved using a 425-micron sifter to prepare the experimental feed. After measuring and properly mixing all the materials, 130–150 ml of sterile distilled water was used. After being autoclaved for 30 minutes at 1000C, the mixed feed tough was allowed to cool to ambient temperature. Fish and sunflower oils are employed as lipid sources in the feed after cooling, along with supplevite mix, sodium chloride, sodium benzoate, and varying amounts of carbon quantum dots (F1-Control, F2 (0.5ml CQD), F3 (1 ml CQD), F4 (1.5 ml CQD), F5 (2 ml CQD), and F6 (2.5 ml CQD) were incorporated with the feed and extruded. The formulated feed was kept in an airtight jar at -20°C until used to avoid contamination (Table 1).

For the present investigation, a uniform size of Danio rerio zebrafish (0.120±0.020g) was selected. The fish were then put in an 18-litre rectangular glass tank (45cmL × 22cmB × 22cmH). Each aquarium had ten fish added to it. Every feeding trace was kept in triplicate. The prepared experimental meal was provided to the fish twice a day for an hour each, from 8 to 9 am and 4-5 pm, as they were raised on an ad libitum diet. The fish were fed for an hour without being disturbed, and then the unfed portions were gathered and dried until they reached a constant weight. The fish excrement was gathered and dried at 95°C in the hot air oven each day prior to the water being changed in an effort to disturb the fish as little as possible. The experimental tank's water content was replenished with approximately 70% fresh tap water. The trial ran for a total of 21 days. The zebrafish's final body length and weight were calculated while they were still alive on the twenty-first day. After the muscles, liver, and gills were removed from several tanks, biochemical analyses were carried out.

Table 1. Composition of different ingredients in the experimental feed (g\100 gm) of Zebrafish

S No.	Composition	Feed 1 (Control)	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6
1	Fish meal	33.75	33.75	33.75	33.75	33.75	33.75
2	GNOC*	33.75	33.75	33.75	33.75	33.75	33.75
3	Wheat flour	11.25	11.25	11.25	11.25	11.25	11.25
4	Tapioca	11.25	11.25	11.25	11.25	11.25	11.25
5	Fish Oil	2	2	2	2	2	2
6	Sunflower Oil	2	2	2	2	2	2
7	Supplevite - mix	4	4	4	4	4	4
8	Sodium chloride	1	1	1	1	1	1
9	Sodium benzoate	1	1	1	1	1	1
10	Carbon Quantum Dots	-	0.5 ml	1 ml	1.5 ml	2 ml	2.5 ml

*GNOC – Ground Nut Oil Cake

Results

Using the UV visible absorption spectra, carbon quantum dots are capable of characterized. The wavelength range in which the absorbance spectra of CQDs were measured was 300–400 nm. The observed sharp bands are confined to 355 nm. As seen in Figure 1, Scanning electron microscopy revealed that the nanoparticles formed quasi-spherical particles less than 10 nm in size (Figure 2).

In EDAX, three peaks between 0.1 KeV and 3 KeV are displayed that were recorded on the CQD (Figure 3). The carbon quantum dots are directly connected to those maxima. Carbon was suggested by the highest peak, which was found at 0.1 KeV. It is evident that nitrogen is the source of the 0.2 KeV spectrum. The oxygen is linked to the third peak, which is situated at 0.3 KeV. The IR range of 4000-400 cm^{-1} was examined in the CQD FT-IR spectra. Using the peak value in the infrared radiation band as a guide, the FT-IR analysis was performed to determine the different functional groups of active components. The bands with 3327.57, 1636.3, and 1098.26 linked to N-H Secondary Amine, C = C Alkene, and C-O

Aliphatic Ether, respectively, were utilized to confirm the development of CQDs (Figure 4). Transillumination is a technique of sample illumination by transmission of light through the sample. The chemically synthesized CQDs showed green luminescence under a single ultraviolet light excitation (Figure 5).

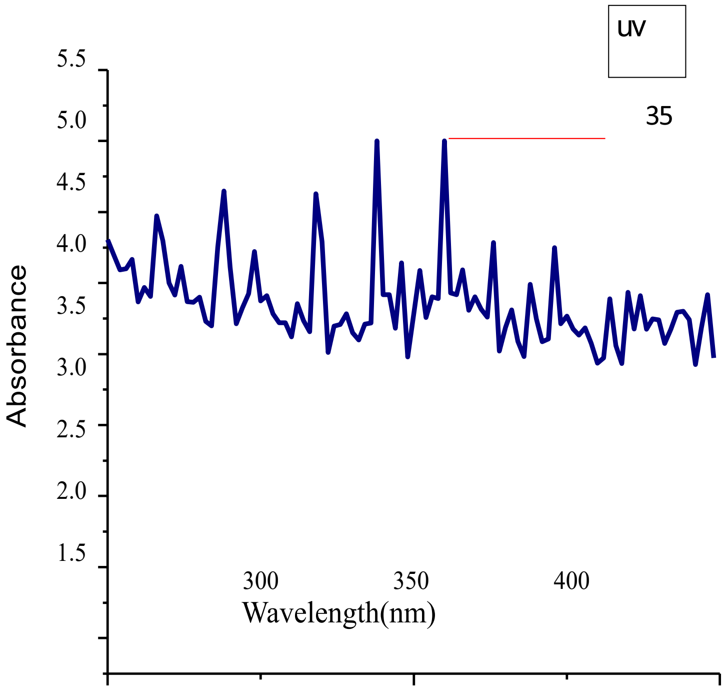


Figure 1. UV Vis Analysis image of CQD

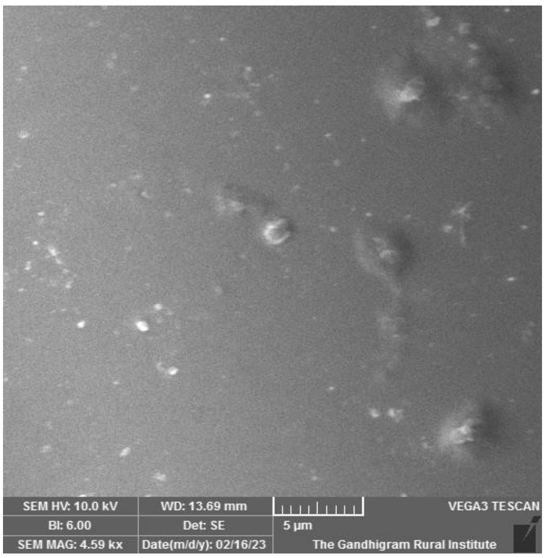


Figure 2. SEM Analysis image of CQD

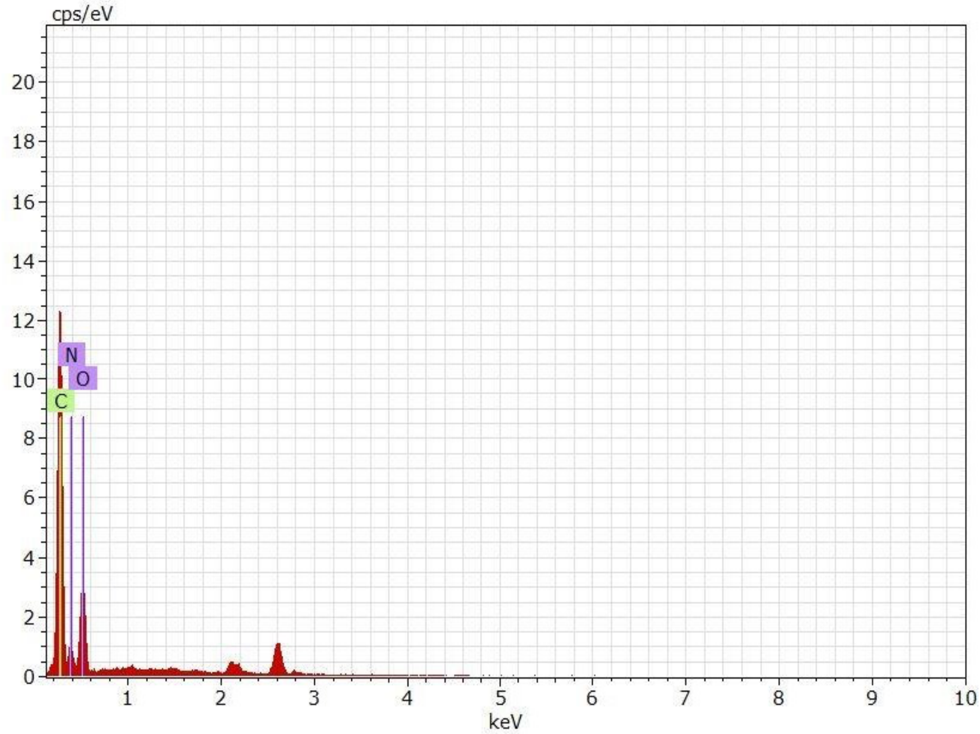


Figure 3. EDAX Spectrum image of CQD

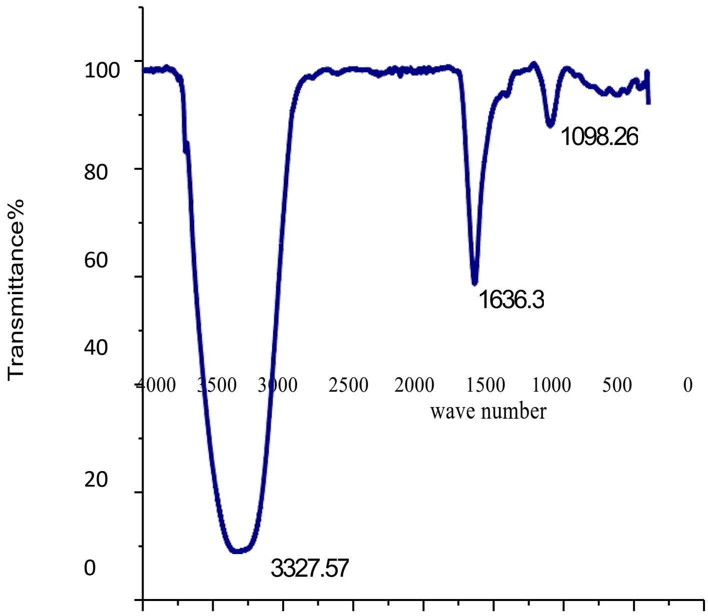


Figure 4. FT-IR spectrum graph of Carbon Quantum Dots

The condition factor (K) of Zebrafish, which were given varying amounts of experimental meals, is displayed in Table 2. The final condition factor is elevated in all of the experimental feeds. The different characteristics related to feed utilization and growth performance of Zebrafish are presented in Table 3. The analysis of variance (ANOVA) was conducted on growth parameters, including feed consumption, gross growth efficiency, and net growth efficiency (Table 4). The fish grown in feed I, II, III, IV, V, and VI had feed consumption rates of 7.27, 39.10, 4.58, 5.85, 3.6, and 2.5, respectively. Their feed conversion efficiency, growth, percentage growth, assimilation, metabolism, gross, and net growth efficiency were also measured. The concentration of protein, carbohydrate, and lipid (mg/g) in the muscle, gill, and liver of Zebrafish is greater in feed II, which contains 0.5 ml of carbon quantum dots, compared to other feeds (Figure 6).

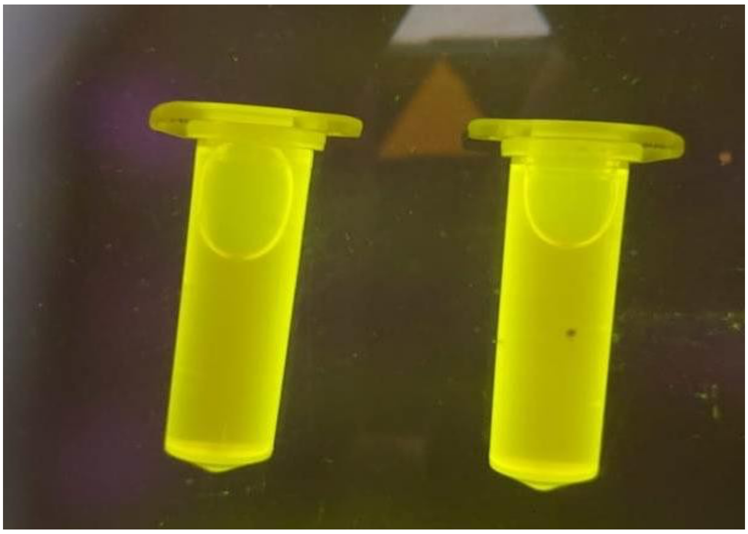


Figure 5. Green luminescence of carbon quantum dots under transilluminator

Table 2. Condition factor of zebrafish

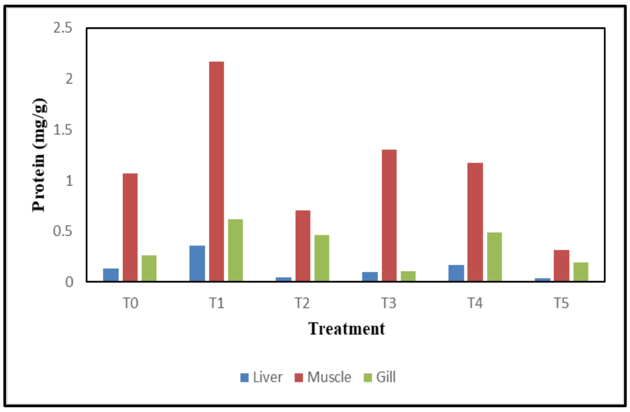
Feeds	Initial	Final
1 (Control)	1.202 ± 0.264	2.225 ± 1.036
2	2.155 ± 1.317	5.702 ± 3.124
3	1.390 ± 0.197	3.221± 0.611
4	1.074 ± 0.191	2.239 ± 0.549
5	0.947 ± 0.111	4.418 ± 0.480
6	1.067 ± 0.197	3.108 ± 1.002

Table 3. Feed utilization and Growth parameters of zebrafish in relation to the different quantities of Carbon Quantum Dot

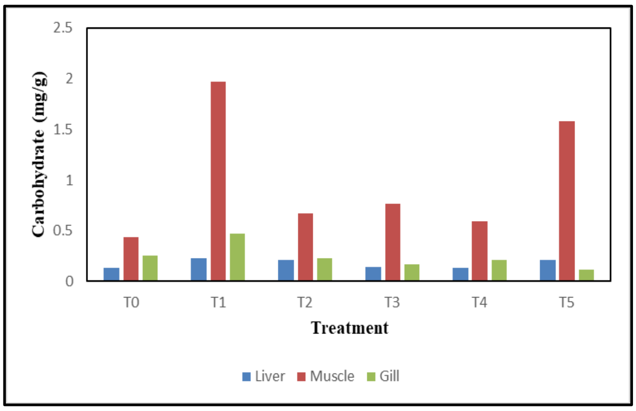
Parameters	Experimental Feeds					
	Feed I (Control)	**Feed II (0.5ml)**	**Feed III (1ml)**	**Feed IV (1.5 ml)**	**Feed V (2 ml)**	**Feed VI (2.5ml)**
Feed consumption (g/g live wt./21days)	1.13±0.29	0.86± 0.11	0.99±0.17	1.39±0.28	1.29±0.06	1.03±0.27
Feed Conversion Efficiency	0.53±0.43	3.02± 2.15	0.39± 0.23	0.53±0.16	0.16±0.14	0.22±0.13
Feed Conversion Ratio	7.27±6.51	2.69± 39.06	5.85±2.14	2.50±1.47	1.86±1.66	4.58± 2.32
Growth	0.07±0.05	0.18± 0.28	0.04± 0.02	0.05±0.005	0.023± 0.02	0.03±0.25
Percentage Growth	21.16± 13.02	16.30± 31.89	18.89± 12.54	20.96±4.98	10.75±6.38	7.86±6.84
Assimilation	1.09±0.29	0.83± 0.11	0.95± 0.17	1.36±0.28	1.25±0.06	0.99±0.27
Metabolism	1.02±0.32	0.65± 0.39	0.91± 0.16	1.32±0.26	1.23±0.08	0.94±0.27
Gross Growth Efficiency (%)	7.27±6.51	3.91± 1.71	4.58± 2.32	5.85±2.14	3.6±2.2	2.5±1.47
Net Growth Efficiency(%)	7.54±6.81	2.57± 0.72	4.77± 1.40	6.08±1.30	3.8±1.0	2.56±0.49

Table 4. Analysis of variance of growth parameters (feed consumption, growth, gross growth efficiency, net growth efficiency) of Zebrafish

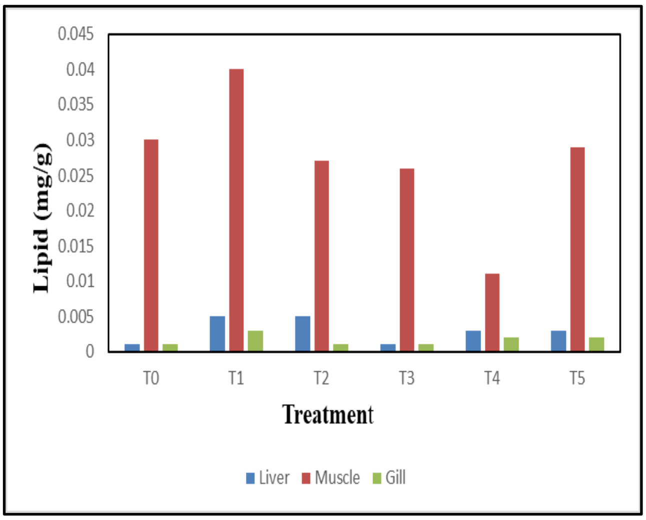
Parameters	Sources	Sum of squares	df	Mean Square	F	Sig
Feed consumption	Between Groups	0.268	5	0.054	2.859	0.063
	Within Groups	0.225	12	0.019		NS
	Total	0.492	17			
Growth	Between Groups	0.035	5	0.007	16.695	0.000
	Within Groups	0.005	12	0.000		S
	Total	0.040	17			
Gross Growth Efficiency	Between Groups	428.028	5	85.606	7.775	0.002
	Within Groups	132.125	12	11.010		S
	Total	560.153	17			
Net Growth Efficiency	Between Groups	31.529	5	6.306	6.955	0.003
	Within Groups	10.879	12	0.907		S
	Total	42.408	17			



6 (a)



(6b)



6(c)

Figure 6. Effect of CQD incorporated feed on protein (6a), carbohydrate (6b) and lipid (6c) contents of Muscle and gill of Zebrafish

Discussion

The study of chemically synthesized CQDs offers a valuable contribution to the field of nanotechnology. In this study, CQDs are synthesized by hydrothermal bottom-up method using citric acid as precursor and histidine as reducing agent. Plino Innocenzi *et al.* (2019) reported that crystalline powdered citric acid monohydrate (CAM) is employed as a precursor to fluorescent carbon dots. Bing Zhang *et al*. (2010) synthesized CQDs using the one-step method by hydrothermal treatment of ascorbic acid. Iwinska and Pietrusiewicz (2020) reported that using the hydrothermal carbonization process, CQD was created from gelatin and baby milk that is hypoallergenic.

The primary characterization of CQDs was analyzed using a UV-Vis spectrophotometer and the peak absorbance was observed at a wavelength of 355nm. According to Seyedeh Masoumeh Ghoreishi *et al.* (2020), the CQD exhibited a peak at 260 nm and at roughly 380 nm associated with the C=C and C=O transportation-related π-π∗ and π-n∗ transitions. According to Guo *et al.* (2016), the excitation spectrum of the CQDs revealed two peaks that were roughly centred at 240 and 330 nm. According to Wang *et al*. (2017), the UV region (260–320 nm) has a tail that extends into the visible spectrum. Rani *et al*. (2020) reported two distinct peaks in the UV-Vis spectrum of CQDs, located at 224 and 280 nm.

By utilizing a scanning electron microscope (SEM) to examine the morphology and the structure of the chemically synthesized Carbon Quantum Dots, it was discovered that the quasi-spherical particles were less than 10 nm. Jlassi *et al.* (2020) reported that the carbon dots have a 4.5 nm size and are spherical in shape. According to Abinaya *et al.* (2021), the carbon dots' SEM image revealed that the particles are uniformly distributed and spherical. Three peaks between 0.1 KeV and 3 KeV may be seen in the EDAX spectrum that was recorded on the CQD. The greatest peak on the spectrum is associated with carbon at 0.1 KeV, 0.2 KeV, and 0.3 KeV, respectively, and is the highest peak for carbon. According to Dager *et al.* (2019), carbon quantum dots are made up only of carbon and oxygen peaks, meaning that there is no elemental signature of any other foreign contaminants present in the manufactured C-QDs.

The verification of carbon quantum dots (CQDs) synthesis was accomplished by utilising Fourier Transform Infrared Spectroscopy (FT-IR) to analyse the functional group of the CQDs. The bands with frequencies of 3327.57, 1636.3, and 1098.26 were associated with N-H Secondary Amine, C = C Alkene, and C-O Aliphatic Ether, respectively. Ghoreishi *et al.* (2020) utilised FT-IR to characterise the surface functional groups. The peaks observed between 3200 and 3500 cm^{-1} are attributed to the hydroxyl group. The stretching band of H-N is observed at around 3000–3300 cm^{-1}.The H-C and carbonyl groups are shown by the peaks observed at 1400 and 1600 cm−1, respectively. The presence of C-N and C-O groups can be identified by the bands observed at 1120 cm-1 and 1342 cm−1, respectively. Sutanto *et al.* (2020) found that FTIR measurements showed NH and OH stretching bonds in the CDQs at specific wavenumbers: 3186 cm−1, 3182 cm−1, 3184 cm−1, 3181 cm−1, and 3178 cm−1.

The wavenumbers demonstrate the abundance of amino and hydroxyl groups on the CDs' surface, indicating their strong hydrophilic qualities, which may contribute to their increased stability in aqueous solution. According to Guo *et al.* (2016), the stretching vibration of N-H and O-H was linked to the broad absorption band at around 3300 cm^{-1} in the Fourier transform -infrared (FT-IR) spectra of CQDs. At about 1690 cm^{-1}, a prominent peak was identified as the stretching vibration band of C=O. The existence of carboxylic acid and other functional groups containing oxygen was verified by these studies. The peak, located approximately at 1515 cm^{-1}, was found to coincide with the N-H stretching and bending vibration bands, indicating the existence of functional groups comprising amino acids. Additionally, the stretch of vibrations C-H, C=C, and C-C was linked to the absorption bands at 2930, 1410, and 1330 cm^{-1}, confirming the existence of aryl and alkyl groups. According to Rani *et al.* (2020), the CQD spectra displayed peaks at 3378 cm^{-1}, which were related to the stretching vibration of O-H groups, 1608 cm^{-1}, which was assigned to C=O stretching, and 1130 cm^{-1}, which was attributed to CO stretching. Transillumination is a method of illuminating a sample by passing light through it. Under a single UV light stimulation, the chemically produced CQDs exhibit green luminescence.

The zebrafish condition factor (K) was assessed to compare food quality. All meals with different levels of Carbon Quantum Dots improved Zebrafish's final condition factor. All feed utilisation indicators were higher for feed IV. Feed utilisation, growth, gross growth efficiency, and net growth efficiency varied greatly. Feed II had more protein, carbohydrate, and fat in Zebrafish muscle, gill, and liver than the other feeds.

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