



**COMBINATORIAL EFFECT OF DIETARY  
CONJUGATED LINOLEIC ACID AND QUERCETIN  
ON FEED INTAKE, GROWTH AND SURVIVAL OF  
*PANGASIONODON HYPOPTHALAMUS*  
(SAUVAGE, 1878) FINGERLING REARED AT  
LOW TEMPERATURE**

Dissertation submitted in partial fulfilment  
of the requirements  
for the degree of

**M.F.Sc. (Fish Nutrition and Feed Technology)**

by

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**SEPTEMBER, 2024**

**Pankaj Kumar Singh**, 2022. Combinatorial effect of dietary conjugated linoleic acid and quercetin on feed intake, growth and survival of *Pangasionodon hypophthalmus* (sauvage, 1878) fingerling reared at low temperature, M.F.Sc. Dissertation, ICAR-Central Institute of Fisheries Education (University Established Under Section 3 of UGC Act 1956), Panch Marg, Off Yari Road, Versova, Andheri (W), Mumbai – 400 061

**ISBN: 978-93-48499-98-1**



**Dedicated to...**

**My Family, Friends and  
Teachers**





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Dated: 27 September 2024

## CERTIFICATE

Certified that the dissertation entitled "COMBINATORIAL EFFECT OF DIETARY CONJUGATED LINOLEIC ACID AND QUERCETIN ON FEED INTAKE, GROWTH AND SURVIVAL OF PANGASIANODON HYPOPHthalmus (SAUVAGE, 1878) FINGERLING REARED AT LOW TEMPERATURE" is a bonafide record of independent research work carried out by Mr. Pankaj Kumar Singh during the period of study from December, 2023 to September, 2024 under our supervision and guidance for the degree of Master of Fisheries Science(Fish Nutrition and Feed Technology) and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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# DECLARATION

I hereby declare that the dissertation entitled **“COMBINATORIAL EFFECT OF DIETARY CONJUGATED LINOLEIC ACID AND QUERCETIN ON FEED INTAKE, GROWTH AND SURVIVAL OF *PANGASIONODON HYPOPTHALAMUS* (SAUVAGE, 1878) FINGERLING REARED AT LOW TEMPERATURE”** is an authentic record of work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.



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## ACKNOWLEDGEMENTS

*First and foremost, I am sincerely grateful to almighty GOD for unfailing care, mercy and blessing in all the works of my life.*

*I wish to express my heartfelt gratitude to my lifelines PAPA, MUMMY, BROTHERS, SISTERS and my whole family for immensely contributing in my success, supporting me in each and every step of my life and without whom this was an impossible task.*

*I would like to express my gratitude to Dr. Ravishankar C. N., Director and Vice Chancellor, Central Institute of Fisheries Education, Mumbai, and National Agricultural Higher Education Project (NAHEP) for giving me the chance to do research and providing me all facilities required throughout this research.*

*I express my sincere gratitude to Dr. N. P. Sahu, Joint Director, ICAR-CIFE, Mumbai for his valuable suggestions and corrections.*

*I am deeply indebted to my most respected guide Dr. Ashutosh D. Deo, Principal Scientist Fish Nutrition, Biochemistry and Physiology Division, ICAR- Central Institute of Fisheries Education, Mumbai for his continuous guidance, patience, encouragement and enthusiastic support throughout my entire research work and successful completion of this dissertation. I feel overwhelmed for his constant concern and his support is unforgettable. I am so lucky and proud to have him as my Guide.*

*I am grateful to Dr. Manish Jayant, Scientist, Fish Nutrition, Biochemistry and Physiology Division who as a member of the advisory committee always offered wholehearted support and*

guidance for this research work and guide me at each and every stage of my research work.

*I gratefully acknowledge **Dr. Subodh Gupta**, Scientist, Fish Nutrition, Biochemistry and Physiology Division, for his valuable suggestions coupled with amazing patience in guiding me at every stage of this study.*

*I gratefully acknowledge **Dr. Md Akhlakur**, Scientist, Fish Nutrition, Biochemistry and Physiology Division, for his valuable suggestions coupled with amazing patience in guiding me at every stage of this study.*

*I express my deep sense of gratitude to all of my teachers of FNBP Division namely, **Dr. Kedar Nath Mohanta** (Principal Scientist and HoD), **Dr. Parimal Sardar** (Principal Scientist), **Dr. Prem Kumar** (Scientist), **Dr. Sikendra Kumar** (Scientist), **Dr. Tincy Varghese** (Scientist) and **Dr. Shamna N** (Scientist) for their support, assistance and encouragement throughout my research period.*

*I thank my lovable senior **Dr. Ashraf**, for his overwhelming support in conducting the experiment and being there for me whenever I face any problem during the research. A special thanks to **Ms. Tejaswini** for her valuable suggestions, continued support, guidance, motivation, love and care. Her support helped me to write thesis in a better manner. I would like to thank **Mr. Shivkumar** for his continued support, sharing knowledge and kind help. I thank **Yash sir** for his valuable suggestions, continued support, guidance, motivation, and care. His support helped me to write thesis in a better manner. I would like to thank **Megha** for her valuable suggestions, continued support, guidance, motivation, and care. His support helped me to write thesis in a better manner.*

*This work would have not been completed without the valuable help and understanding of my friends Megha, Jenisha, Avvari Venkata Sai, Simon, Suraj, Avantika, Samrat, Anu, Bhanu, Dhivakar Anushree, Purva, Udit, Pallavi to whom I owe a lot. They have always been with me during difficult times of my research without any hesitation and supported me at every point of this work.*

*I whole heartedly thank my beloved seniors, Ashraf sir, Nisha mam, Chandan sir, Saiprasad sir, Praksh sir, Akhila ma'am, Veeramani sir, Shiv sir, Tejaswini ma'am, Revathi ma'am, Paul Nathaniel sir, Hussain sir, Madhulika mam, Bhaswati mam, Prashanth sir, Bhuvanesh sir for their selfless help, encouragement and support in my work and teaching me many things during my experiment.*

*I extend my special affectionate gratitude to my dear juniors, Chandrasekhar Pradhan, Suhas, Saurav, Hariharan, Maheswaran and Anvita for being part of my work and helping me in all situations.*

*I whole heartedly thank my beloved friends, Vineet Anand, Atul Kumar Singh, Debashis Mohanta, Bishu Das, Rajeev, Suraj and Mahesh for their selfless help, encouragement and support.*

*I am very much grateful to Ambrish, Sushma and Roshan for their kind gesture, encouragement and support during my entire research.*

*I am very much grateful to Mrs. Aruna Devi (Technical officer), Akshay Bhaiya, Prashant Bhaiya, Rohan Bhaiya, Suresh ji from FNB division for their sincere help with smiling face.*

*I am extremely grateful to one person who has a significant influence in my life, Dr. Shivendra Kumar (Professor, Dr. RPCAU, Pusa) who paved the path for me in the world of Fisheries science.*

*I shall be grateful to all the staff members of CIFE library and Librarian, for their substantial assistance in compiling the manuscript.*

*Last but not the least, I would like to thank to all who has helped me directly or indirectly in this research work and helped to make it success.*

*Date: 27-09-2024*

*Place: Mumbai*

A handwritten signature in blue ink that reads "Pankaj". The signature is written in a cursive style with a horizontal line underneath the name.

*PANKAJ KUMAR SINGH*

## सारांश

कम तापमान ( $18.0 \pm 1.0$  °C) पर पाले गए पैंगसियानोडोन हाइपोफ्थाल्मस (धारीदार कैटफ़िश) फ़िंगरलिंग्स में फ़ीड सेवन और विकास पर संयुग्मित लिनोलिक एसिड (सीएलए) और केरसेटिन (केर) के संयोजन प्रभाव को चित्रित करने के लिए 60-दिवसीय भोजन परीक्षण आयोजित किया गया था। संयुग्मित लिनोलिक एसिड और केरसेटिन के विभिन्न स्तरों के साथ सात आइसोनाइट्रोजेनस (37.0%) और आइसोकैलोरिक (412 किलो कैलोरी आहार ऊर्जा/100 ग्राम) व्यावहारिक आहार। नियंत्रण (0.0% सीएलए + 0.0% केर) T1 (0.5% सीएलए + 0.0% केर) T2 (1.0% सीएलए + 0.0% केर) T3 (0.0% सीएलए + 0.5% केर) T4 (0.0% सीएलए + 1.0% केर) T5 (0.5% सीएलए + 0.5% केर) और T6 (1.0% सीएलए + 1.0% केर) तैयार किए गए थे। यह प्रयोग 1.6 एल/मिनट की प्रवाह दर के साथ कम तापमान ( $18.0 \pm 1.0$  °C) पुनः परिसंचारी जलीय कृषि प्रणाली (आरएएस) में आयोजित किया गया था। आहार का सेवन और विकास पैरामीटर (वजन बढ़ना, वजन बढ़ना%, विशिष्ट विकास दर, फ़ीड रूपांतरण अनुपात, तापीय विकास गुणांक, फ़ीड दक्षता अनुपात, प्रोटीन दक्षता अनुपात और लिपिड दक्षता अनुपात) उत्तरजीविता (%) पाचन एंजाइम गतिविधियाँ (प्रोटीज, एमाइलेज और लाइपेज) चयापचय एंजाइम (एमिनोट्रांसफ़ेरेज, लैक्टेट डिहाइड्रोजेनेज, मैलेट डिहाइड्रोजेनेज, और ग्लूकोज-6-फॉस्फेट डिहाइड्रोजेनेज) सीरम पैरामीटर (सीरम कुल प्रोटीन, एल्बुमिन, ग्लोबुलिन, ए: जी अनुपात और ग्लूकोज) प्रतिक्रिया पैरामीटर के रूप में उपयोग किए गए थे। परिणाम से पता चला कि आहार में सीएलए और केर के आहार पूरक नियंत्रण (पी < 0.05) की तुलना में उपचार समूहों में महत्वपूर्ण उच्च फ़ीड सेवन के लिए नेतृत्व किया 1.0% सीएलए (पी < 0.05) के साथ खिलाया गया टी 2 समूह में उच्चतम फ़ीड का सेवन देखा गया उच्चतम वृद्धि दर और बेहतर फ़ीड रूपांतरण अनुपात टी2 (1.0% सीएलए) समूह में दर्ज किया गया, इसके बाद टी5 (0.5% सीएलए + 0.5% केर) था प्रयोगात्मक अवधि के दौरान, उत्तरजीविता दर 100% बताई गई थी। पाचन एंजाइम गतिविधियाँ जैसे; प्रोटीज, एमाइलेज और लाइपेज गतिविधियाँ टी 2 (1.0% सीएलए) में अधिक पाई गईं, इसके बाद टी 5 (0.5% सीएलए + 0.5% केर) टी2 (1.0% सीएलए) समूह में एस्पार्टेट एमिनोट्रांसफ़ेरेज और एलेनिन ट्रांसामिनेज गतिविधियों जैसे मेटाबोलिक एंजाइम कम हो गए, जबकि लैक्टेट डिहाइड्रोजेनेज, मैलेट डिहाइड्रोजेनेज और ग्लूकोज-6-फॉस्फेट डिहाइड्रोजेनेज गतिविधि में वृद्धि टी2 समूह में 1.0% सीएलए के बाद टी5 (0.5% सीएलए + 0.5% केर) में देखी गई। टी 2 (1.0% सीएलए) में सीरम कुल प्रोटीन और एल्ब्यूमिन में भी वृद्धि हुई, इसके बाद टी 5 (0.5% सीएलए + 0.5% केर) उपरोक्त परिणामों से प्राप्त निष्कर्ष में कहा गया है कि 1.0% सीएलए के आहार पूरक के बाद संयोजन पूरक (0.5% सीएलए + 0.5% केर) विकास, पोषक तत्वों के उपयोग, पाचन एंजाइम गतिविधियों को बढ़ा सकता है और कम तापमान पर पाले गए पैंगसियानोडॉन हाइपोफ्थाल्मस फ़िंगरलिंग में चयापचय गतिविधियों को प्रभावित कर सकता है।

# ABSTRACT

A 60-day feeding trial was conducted to delineate the combinatorial effect of Conjugated linoleic acid (cLA) and Quercetin (que<sup>r</sup>) on feed intake and growth in *Pangasianodon hypophthalmus* (striped catfish) fingerlings reared at low temperature ( $18.0 \pm 1.0$  °C). Seven isonitrogenous (37.0%) and isocaloric (412 kcal dietary energy/100 g) practical diets with varying levels of conjugated linoleic acid and quercetin *viz.* Control (0.0% cLA + 0.0% que<sup>r</sup>), T1 (0.5% cLA + 0.0% que<sup>r</sup>), T2 (1.0% cLA + 0.0% que<sup>r</sup>), T3 (0.0% cLA + 0.5% que<sup>r</sup>), T4 (0.0% cLA + 1.0% que<sup>r</sup>), T5 (0.5% cLA + 0.5% que<sup>r</sup>) and T6 (1.0% cLA + 1.0% que<sup>r</sup>) were prepared. The experiment was conducted in a low temperature ( $18.0 \pm 1.0$  °C) re-circulatory aquaculture system (RAS) with a flow rate of 1.6 L/min. Feed intake and growth parameters (weight gain, weight gain%, specific growth rate, feed conversion ratio, thermal growth coefficient, feed efficiency ratio, protein efficiency ratio and lipid efficiency ratio), survival (%); digestive enzymes activities (protease, amylase and lipase); metabolic enzymes (aminotransferases, lactate dehydrogenase, malate dehydrogenase, and glucose-6-phosphate dehydrogenase); serum parameters (serum total protein, albumin, globulin, A:G ratio and glucose) were used as response parameters. The result showed that dietary supplementation of cLA and que<sup>r</sup> in diet led to significant higher feed intake in treatment groups than control ( $p < 0.05$ ). Highest feed intake was observed in T2 group fed with 1.0% cLA ( $p < 0.05$ ). Highest growth rate and better feed conversion ratio was reported in T2 (1.0% cLA) group followed by T5 (0.5% cLA + 0.5% que<sup>r</sup>). Throughout the experimental period, survival rate was reported to be 100%. Digestive enzyme activities *viz.*; protease, amylase and lipase activities were found to be higher in T2 (1.0% cLA) followed by T5 (0.5% cLA + 0.5% que<sup>r</sup>). Metabolic enzymes like aspartate aminotransferase and alanine transaminase activities reduced in T2 (1.0% cLA) group, whereas increased lactate dehydrogenase, malate dehydrogenase, and glucose-6-phosphate dehydrogenase activity was observed in T2 group fed 1.0% cLA followed by T5 (0.5% cLA + 0.5% que<sup>r</sup>). Serum total protein and albumin were also increased in T2 (1.0% cLA) followed by T5 (0.5% cLA + 0.5% que<sup>r</sup>). The conclusion derived from above results state that dietary supplementation of 1.0% cLA followed by combinatorial supplementation (0.5% cLA + 0.5% que<sup>r</sup>) can increase growth, nutrient utilization, digestive enzyme activities and can affect metabolic activities in *Pangasianodon hypophthalmus* fingerlings reared at low temperature.

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# *INTRODUCTION*

# 1. INTRODUCTION

The world's population will increase by two billion people over the course of the next thirty years, from the present 7.7 billion to roughly 9.7 billion by 2050 (United Nations, Department of Economic and Social Affairs, Population Division (2019)). The key food production businesses are under tremendous strain to meet the rising demand resulting from this fast population growth. Taking care of issues related to appropriate nutrition provision is also necessary in order to guarantee a sufficient food supply. Fish is a great source of high-quality protein, and the aquaculture industry, which is increasing quickly, is essential to supplying the growing demand for farmed fish among the population (Subasinghe, 2009). Currently, 49% of the world's fish production comes from aquaculture (FAO, 2022).

The total amount of fish produced worldwide is estimated at 178 million metric tons (mmt). Approximately 49% of this production comes from aquaculture. *Pangasionodon hypophthalmus* makes up 5.1% of the production in this aquaculture industry (FAO, 2022). This plant is extensively grown in India, Myanmar, Thailand, Vietnam, and Cambodia. In Indian ponds and cages, pangasius farming has grown in popularity lately. India has reported producing about 0.7 mmt of pangasius, based on feed consumption data (Kaippilly, 2022).

Although aquaculture has a lot of promise, there are a lot of obstacles that need to be overcome in order to increase production in this industry. Global warming is a significant hazard that affects fisheries and aquaculture alike. Since fish cannot produce enough heat through metabolism, they are ectothermic species, meaning that the temperature of their surroundings controls their body temperature (Prosser and Nelson, 1981). According to Brander (2007), environmental changes-particularly those related to temperature - are ultimately to blame for effects like acidification, sea level rise, and other changes that alter the ecosystem as a whole and have an impact on the fisheries. Cheung *et al.*, (2008) provided a helpful prediction regarding the global redistribution of catch potential as a result of rising temperatures. Fish behaviour is significantly impacted by temperature. Temperature is widely recognized as the primary abiotic factor influencing various physiological processes in fish (Brett, 1971).

Many fish and marine animal deaths as a result of abnormally low winter temperatures have been reported; these deaths are known as "winterkills" (Storey & Gudger, 1936; Horwood & Millner, 1998; Hoag, 2003). Temperature variations in water can have a substantial effect on a number of physiological functions, including growth, metabolism, and reproduction (Portner and Peck, 2010; Cheng *et al.*, 2013; Shahjahan *et al.*, 2013, 2017). For example, during low water temperatures, the growth rate of cultured freshwater carps such as *Labeo rohita*, *Labeo catla*, *Cirrhinus mrigala*, *Ctenopharyngodon Idella*, and *Hypophthalmichthys molitrix* decreases, resulting in a shortened growing season of approximately four months (Afzal Khan *et al.*, 2004). Because of slower digestion rates, longer gut transit times, and slower gastrointestinal evacuation rates, cooler water temperatures may also have an adverse effect on nutritional digestibility (Miegel *et al.*, 2010). (Tsurkan, 2022) observed that low temperatures have a negative impact on the physiological state of carp, causing levels of ash and moisture to rise and body weight, fatness, and protein to drop. Because of its adaptability to a variety of environmental circumstances, pangasius does especially well in ponds with large stocking densities (Islam *et al.*, 2019). However, low water temperatures in the winter severely impede *Pangasius* growth, causing a decrease in feed intake and growth (Razzaque *et al.*, 2003). Farmers in northern India have suffered significant losses as a result of mass deaths that occurred in *Pangasius* ponds over the winter (Kumar *et al.*, 2022). As a result, it is imperative to protect and maintain fish growth when exposed to low temperatures. Several tactics are employed to increase production when temperatures drop. Since fish will typically consume less feed in colder weather and floating feed may drift to the shore before being consumed, many farmers choose to use slow-sinking feed during cooler months. These feeds are made by extrusion, similar to floating feeds, and have a higher density, which causes them to sink slowly. Moreover, feeding fish a high-energy diet and encouraging its utilization is a tactic during low-temperature periods. Lastly, feeding fish a diet that will increase feed intake in colder water is accomplished by adding metabolic modifiers to diets. These metabolic modifiers include L-carnitine and propylene glycol, among others. Given the effects of the many modifiers indicated above, conjugated linoleic acid and quercetin are the main subjects of this investigation. Conjugated linoleic acid, or CLA for short, is a class of linoleic acid (18:2n-6) variants distinguished by two conjugated cis/trans double bonds. These variations are frequently found in a wide range of foods, including dairy

products like cheese, butter, milk, and vegetable oils, as well as meat, poultry, and seafood. According to Pariza *et al.*, (2001), the main naturally occurring active forms of CLA are the trans-10, cis-12, and trans-9, trans-11 isomers. Many biological effects of CLA in terrestrial animals have been shown by research; these include a notable suppression of atherosclerosis (Lee *et al.*, 1994) and anti-cancer capabilities (Ha *et al.*, 1990).

Quercetin; fruits, vegetables, and nuts are prominent sources of this polyphenolic and lipophilic flavonoid (Bigliardi and Galati, 2013). It is usually found in nature in a glycosylated state, which is a complex including sugar molecules (Guo and Bruno, 2015). According to Yamamoto and Oue (2006), structurally, it is a plant pigment of the catechol type, containing two benzene rings that include phenolic hydroxyl groups and three central carbon atoms. Its remarkable antioxidant qualities originate from its capacity to engage with diverse enzymes and efficiently scavenge free radicals (Di Carlo *et al.*, 1999). By chelating reactive oxygen species (ROS), transforming them into energy, and providing H<sup>+</sup> ions to reduce lipid peroxidation, it reduces oxidative stress in fish. Additionally, it protects cell membranes by controlling the concentration of metals in fish tissues (Wei *et al.*, 2018).

Therefore, the current study aims to assess the combinatorial impact of conjugated linoleic acid and quercetin on feed intake, growth and physio-metabolic responses of *Pangasianodon hypophthalmus* in low-temperature conditions, with the following specific objectives:

## **OBJECTIVES**

To study the feed intake and growth of *Pangasianodon hypophthalmus* fingerlings fed with conjugated linoleic acid and quercetin at low temperature

To study the physio-metabolic responses of *Pangasianodon hypophthalmus* fingerlings fed with conjugated linoleic acid and quercetin at low temperature

*REVIEW  
OF  
LITERATURE*

## 2. REVIEW OF LITERATURE

### 2.1 Status of Indian Aquaculture and Aquafeed

In 2022-23, fish production in India increased to 175.45 lakh tonnes, with an average annual fish production rate of 7.98%. India's contribution to world fish production reached 8.92% in 2022-23 (Handbook on Fisheries Statistics, 2023). India boasts abundant aquatic resources, signalling considerable potential for further expansion in aquaculture shortly. There is currently a noticeable uptick in catfish farming, particularly in the production of *Pangasianodon hypophthalmus*, which contributes significantly to overall catfish aquaculture output. The estimated production of *P. hypophthalmus* is approximately 0.7 mmt, determined through feed consumption and interstate transportation of this fish (Kaippilly, 2022). According to Islam *et al.*, (2019), *P. hypophthalmus* possesses rapid growth, desirable size, strong market demand, and a favourable feed conversion ratio (FCR). This species is recognized for its resilience, ability to thrive in diverse environmental conditions, and ability to sustain high stocking densities. The aquafeed market reached 1.8 mmt in 2023 (IMARC, 2024).

### 2.2 Effect of Temperature on Fish

Fish are classified as ectotherms, which means that they depend on the temperatures of their external environment for warmth instead of producing enough heat through metabolism or other mechanisms (Prosser and Nelson, 1981). Their body temperatures are mainly determined by the surrounding environment, which is why they are categorized as obligatory poikilotherms and rigorous temperature conformers. Temperature variations have a significant impact on fish growth, food consumption, conversion rates, and other physiological processes since they are poikilotherms (Britz *et al.*, 1997; Azevedo *et al.*, 1998). According to Howerton (2001), fish thrive in tropical settings with water temperatures between 25 and 35 °C. The standard metabolic rate (SMR), which is the metabolic rate required for maintaining life and daily activities, rises with rising environmental temperatures in ectothermic fish due to the temperature dependence of biochemical reactions Volkoff and Rønnestad (2020). According to Willmer *et al.*, (2009), metabolic processes can

speed up by two to three times for every 10 °C increase in the surrounding temperature. Tsurkan (2022) found that while mean corpuscular volume (MCV) decreases, indicating an increase in haemoglobin content and concentration, the total content of red blood cells is reduced, along with an elevation in haemoglobin level, mean corpuscular haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC). Islam *et al.*, (2019) reported that *P. hypophthalmus* exhibits superior growth performance indicators, including weight gain, specific growth rate (SGR), and feed conversion ratio (FCR), at temperatures of 28 °C and 32 °C. When temperatures fall below 21°C, channel catfish exhibit irregular feeding behaviour and much lower feed intake than during the warmer months (Crawshaw, 1984; Robinette *et al.*, 1985; Robison, 1982). According to Kumar *et al.*, (2018), *P. hypophthalmus* has critical temperature maxima (CT<sub>max</sub>) of 41.77 °C and critical temperature minima (CT<sub>min</sub>) of 15.98 °C, respectively. These values mean that the species cannot withstand temperatures that are higher or lower than these.

### **2.3 Effect of Winter on Fish Culture**

India experiences four different seasons, according to the Indian Meteorological Department: summer (March to May), winter (January to February), monsoon (June to September), and post-monsoon (October to December). Winter used to last two months, but because to climate change, it now lasts almost four months. Wintertime temperatures in northern India usually vary from 10 to 18 °C. The methods used in aquaculture do not alter during this time, however fish consume little or no food. As a result, in an effort to save money, farmers frequently refuse to feed their fish or just give them very little.

The main factor affecting a number of factors, including fish physiology and pond conditions, is temperature. Research shows that the ideal winter water temperature is lowered by around two months, which affects the physiological condition of carp and causes them to lose body fat and weight (Tsurkan, 2022). Temperatures much below the maximum critical temperature for the species cause fish to lose appetite and cease feeding (Shafland and Pestrak, 1982). Because of slower digestion rates, longer gut transit times, and slower gastrointestinal evacuation rates, cooler water temperatures can limit the digestibility of nutrients (Miegel *et al.*, 2010).

Fish growth, metabolism, feed intake, and production are all impacted by their tendency to stay at the bottom of the pond rather than near the surface in colder weather. This behaviour increases the risk of toxic gas buildup, impairs the immune system, and increases fish susceptibility to diseases (Afzal Khan *et al.*, 2004). Low water temperatures were blamed for mortality rates that were seen in a number of pangas farms in Uttar Pradesh, North India, between December 2020 and January 2021 (Kumar *et al.*, 2022).

Adding metabolic modifiers to fish diet is a workable way to address the issues that low winter temperatures bring about. Animals are given metabolic modifiers, usually by injection, implantation, or feeding, to enhance a variety of characteristics including weight increase, feed efficiency, carcass production, meat quality, and nutritional composition in addition to extending shelf life (Dikeman, 2007). These modifiers are classified into many groups, such as somatotropin, ethanolamines or beta-agonists, vitamins or chemicals that resemble vitamins at supra-nutritional levels, 'designed' lipids, and anabolic steroids. It has been suggested that diets rich in phospholipids, cholesterol, L-carnitine, and polyunsaturated fatty acids (PUFA) can improve tolerance to low temperatures (Nobrega *et al.*, 2020).

## **2.4 Stress Response and Survival of Fish during Winter**

Stress is a typical physiological adaptation meant to help one cope with challenging external stimuli. In order to reestablish homeostasis and adjust to the new changes in the environment, stress triggers primary, secondary, and tertiary reactions. Neuroendocrine reactions and the production of catecholamine and corticosteroid hormones are examples of primary responses. Hematological, osmoregulatory, and metabolic alterations are examples of secondary reactions. According to Barton (2002), tertiary changes encompass alterations in growth and development, behavioral shifts, resistance to disease, and overall stress. Chronic cold stress is linked to altered liver function enzymes, as well as histopathological and oxidative stress biomarkers in the liver and gills (Refaey *et al.*, 2023).

The invasive environmental disruptions affect a variety of physiological and biochemical processes essential to survival. While short-term or acute

environmental changes have an inhibiting influence on fish's ability to operate normally and engage in their typical activities, long-term environmental changes can be fatal and are reliant on the length of exposure. While most of these depend on prior thermal history, these modifications are what allow acclimation or thermal tolerance to flourish in the new environment. Because the metabolic processes regulating these changes are likewise temperature-dependent, it has also been established from numerous studies that acclimatization to high temperatures occurs faster than acclimation to low temperatures. Fish use a variety of adaptation techniques to withstand cold temperatures and endure up to six months under a thin layer of ice.

Fish use their lowered metabolic rates and lethargic behaviour as a survival tactic when their food and oxygen supply are scarce. The only species of goldfish known to exhibit full temperature adjustment is *Carassius carassius* (Kanungo and Prosser, 1959). The liver is where most acclimation-related changes are likely to occur, and one can anticipate variations in these changes in terms of behaviour in both dormant species (e.g., eels; Walsh and Moon, 1983) and remaining active species (e.g., striped bass, *Morone saxatilis*; Stone and Sidell, 1981). According to Stone and Sidell (1981), after cold acclimation (5 °C), actively feeding striped bass showed an increase in hepatic lipid reserves and the Hepatosomatic index. In contrast, the American eel stopped feeding at 8 °C and showed a drop in its lipid reserves, enzyme activity, and hepatosomatic index (Walsh and Moon *et al.*, 1983). According to research by Kent *et al.*, (1988), catfish (*Ictalurus punctatus*) that were acclimated to lower temperatures (15 °C) displayed alterations in the liver's morphological, physiological, and biochemical characteristics in addition to cardiac hypertrophy. Schwarzbaum *et al.*, (1991) found that cold-acclimated *Rutilus rutilus* roaches had increased Na-K ATPase activity. According to studies, nototheniids' power outputs and muscle contraction velocities were about ten times greater than those of warm-water species accustomed to low temperatures.

Most organisms undergo metabolic reorganization of the cell membrane and cell to withstand the low temperatures. Research conducted by Campbell and Davies (1978) on blennies (*Blennius pholis*) has shown a 70% increase

in the number of mitochondria per cell in the liver. An increase in ATP production is the cause of this alteration.

## **2.5 Effect of Low Temperature on Immunity of Fish**

Fish's innate immune responses and immunological competence are greatly impacted by temperature, which makes it possible for opportunistic infections to infiltrate the fish. Additionally, parasites may have a stronger impact on host survival at lower temperatures (Doménech *et al.*, 1997). Low temperatures cause metabolism to slow down and gill excretion of ammonia to decrease, which impairs immunity and causes auto-intoxication (Hodkovicova *et al.*, 2020). Innate immune responses of fish at 19°C were significantly reduced due to a decrease in total leucocyte count, phagocytic activity, and respiratory burst activity. Orange spotted grouper held at 19°C and challenged with *Vibrio alginolyticus* showed higher mortality when compared to those held at 27°C (Cheng *et al.*, 2009).

Pathogenic microorganisms that are psychrophilic grow more readily at low temperatures. Aquaculture is negatively impacted by cold-adapted parasites, such as *Flavobacterium psychrophilum*, which kills a variety of freshwater fish species below 10 °C (Ferguson *et al.*, 2018).

## **2.6 Effect of Low Temperature on Growth and Feed Intake in Fish**

Fish feeding behaviour is significantly impacted by the water's temperature in the pond (Crawshaw, 1984). Lowering the temperature has an adverse effect on digestibility, growth, activity, feed intake, and the activity of digestive enzymes. It was discovered that magur grew more readily at 32 °C, and that 9.4 °C was the fatal temperature (Shafland and Pestrak, 1982). When temperatures dropped below 21 °C, channel catfish eating behavior changed and their feed intake significantly decreased as opposed to feeding throughout the summer (Crawshaw, 1984). Auburn University research also showed that catfish (0.45 kg) kept for the winter in ponds without food lost 9% of their body weight, while fish fed 1% of their body weight on days when the water temperature was above 12 °C gained 18% of their body weight (Lovell, 1989; Dunham *et al.*, 1987). The amount of weight growth

or loss depends on how severe the winter is; it has been seen that mild winters result in greater weight gain or loss than frigid winters.

Weight loss in the tissue and liver of yellow drum under cold stress was documented by Zhu *et al.*, (2020). Reduced feed intake at low temperatures resulted in poorer weight increase values in December and January in an experiment including three carp species: mrigal, rohu, and silver carp (Nazish and Mateen, 2011).

## **2.7 Effect of Low Temperature on Physiology of Fish**

Fish that are kept in cold water exhibit drastically reduced physiological responses. Temperature affects both passive and active ion transport pathways, which in turn affects the hydro-mineral state of bodily fluid. As an adaptive reaction to cold, the Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> plasma mineral composition seemed to reset in accordance with the acclimated temperature (Metz *et al.*, 2003). In carp acclimated to 15°C, it was shown that Na<sup>+</sup>/K<sup>+</sup>- ATPase expression and the number of chloride cells increased to make up for the lower reaction rate and the decreased activity of the Na<sup>+</sup> pump at low temperatures (Metz *et al.*, 2003). In order to compensate for the decreased enzymatic rates at low temperatures, serum glucose levels rose (Atwood *et al.*, 2003). Fish exposed to cold temperatures also showed a decrease in opercular activity. It is possible to hypothesise that an increase in membrane fluidity serves as a survival mechanism for low temperature adaptation. The increase in the amount of polyunsaturated fatty acids causes alterations in membrane fluidity (Farkas *et al.*, 2001; Tocher, 2003). Ion pump proliferation and decreased gill permeability are two more adaptive survival methods (Davenport and Sayer, 1993). Winter performance may be improved by high-conditioning factors (Prchal *et al.*, 2018).

Fish and other animals frequently exhibit homeoviscous adaptation, which increases fatty acid unsaturation to preserve membrane permeability and fluidity in order to maintain physiological balance amid extreme temperatures (Atwood *et al.*, 2003). A vital part of adjusting to cold is muscle fat. It has been suggested that reduced body weight or muscle fat throughout the winter, as well as reduced body fat before and after, may improve growth and survival in the growing seasons that follow. According to Prchal *et al.*, (2018), the reduced fat content would favour an increase in polyunsaturated fatty acids and an omega-3 fatty acid profile.

Phospholipids in the plasma and microsomal membranes re-distribute to maintain lipid miscibility and boost cryo-stability, which causes low-temperature acclimatization (Thieringer *et al.*, 1998).

## **2.8 Effect of Low Temperature on Metabolic Changes in Fish**

For cold-blooded creatures like fish, the primary factor affecting metabolic rate is water temperature (Brett, 1971). Standard metabolic rate (SMR) is highly dependent on water temperature, according to Fry (1971). Low temperatures reduce the activity of digestive and metabolic enzymes, which affects the metabolic rate through associated hormonal and gene expressions (Sala-Rabanal *et al.*, 2003). Suski and Ridgway (2009) found that during periods of decreased feed intake, fish regulated their metabolism by activating fewer enzymes, ion pumps, and ion channels in an effort to save maintenance expenses. According to Reid *et al.* (2021), cold shock and stress are frequently linked to oxidative stress (such as elevated lipid peroxidation), which causes an increase in the activity of antioxidant enzymes specific to the affected tissue and changes in metabolic processes.

Low threshold temperatures cause anaerobic metabolism to take over, gradually lowering energy levels (Kyprianou *et al.*, 2010). Protein misfolding and the creation of foreign protein structures are brought on by cold stress. Lipid metabolism changes as a result of cold acclimatization. Swimming is maintained even in extremely cold temperatures thanks to the oxidative metabolism of lipids (Kyprianou *et al.*, 2010). As a means of adapting to low temperatures and enhancing the functional ability of tissues, an increase in mitochondria has been seen (St-Pierre *et al.*, 1998).

Inadequate digestion and absorption of proteins is linked to cold-induced fasting. Due to the mobilization of amino acids from muscle during starvation, it was discovered that fish exposed to low temperatures had lower plasma concentrations of essential and non-essential amino acids (Ibarz *et al.*, 2010).

Within 8 days of fish being exposed to cold, there was a rapid decrease in perivisceral fat and a rapid buildup of lipids in the liver, resulting in fatty liver degeneration and major metabolic alterations (Ibarz *et al.*, 2010). Dietary intervention plays a critical role in alleviating temperature-related stresses, as nutrition has a

tremendous effect on this. The application of metabolic modifiers in feed can help address the issues during cold winter temperatures.

According to Dikeman (2007), metabolic modifiers are substances that are given to animals by feeding, injection, or implanting them in order to enhance many aspects of their nutritional profile, including visual meat quality, carcass meat yield percentage, feed efficiency, dressing percentage, and rate of weight gain. Anabolic steroids, somatotropin, phenethanolamines or beta agonists, vitamins or vitamin-like chemicals administered at supra-nutritional amounts, 'designed' lipids, and other modifiers are the six categories of metabolic modifiers. According to Nobrega *et al.*, (2020), a diet rich in PUFA, phospholipids, cholesterol, and L-Carnitine may increase tolerance to cold.

## **2.9 Studies on Effect of Various Metabolic Modifiers on Fish**

According to a number of publications, metabolic modifiers can increase fish feed intake (Steinberg *et al.*, 1994). According to (Tort *et al.*, 2004), supplementing sea bream with several metabolic modifiers such as vitamin E, C, and choline improves their growth and palatability. Luc *et al.*, (2023) found that the combination of fish and maize oil, *Aurantiochytrium sp.*, and sunflower oil were the best lipid sources for stimulating growth in cold-stressed Nile tilapia. Nitric oxide (NO), which is produced by L-arginine, raises plasma insulin, growth hormone, glucagon, catecholamine, and prolactin levels. Additionally, it changes the metabolism of skeletal muscles and increases metabolism (McConell *et al.*, 2006).

It has been reported that iodine can alter enzyme activity in channel catfish diet at a level of 9.6–60 mg I/Kg DM (Gatlin and Wilson, 1986). On Channel catfish, selenium at a level of 0.1–0.5 mg Se/kg increased growth and enzyme activity (Gatlin and Wilson, 1984). The impact of dimethyl- $\beta$ -propiothetin (DMPT), a dietary supplemented sulfonium molecule, on the growth of marine fish, including flounder, yellowtail, and red sea bream (Nakajima *et al.*, 1991). L-carnitine is an organic substance with multiple uses that is utilized as a carrier for  $\beta$ -oxidation, which generates energy (Li *et al.*, 2019). Maruthi (2022) investigated how L-carnitine affected *Labeo rohita* growth and feed intake at cold temperatures.

The outcomes showed that L-carnitine is essential for reducing the effects of cold stress. The effects of ashwagandha root extract and powder on *L. rohita* fingerlings were investigated by Naveen (2022). The results of the study demonstrated the beneficial effects of ashwagandha root powder and extract on rohu growth performance at low temperatures and feed intake. When fish are supplemented with pyrroloquinoline quinone, their mitochondrial number increases and their energy consumption rises accordingly (Bauerly *et al.*, 2011). The feed intake and growth of common carp fingerlings raised at low temperatures were improved by adding pyrroloquinoline quinone at a rate of 0.75 mg/kg to their diet (Gopika *et al.*, 2022). According to Revathi (2021), rohu fed with varying amounts of onion peel extract at low temperature showed enhanced development and feed intake.

Tejaswini (2021) investigated how *L. rohita* development and feed intake were impacted by a lemon peel extract high in flavanones. The results showed that fish growth performance, antioxidant capacity, and digestive enzyme activity are all enhanced by flavanone chemicals. According to Wang *et al.*, (2014), citrus flavonoids have a significant role in preventing serious chronic diseases. Dehydrated lemon peel added to the diet improved the immunological response, antioxidant status, and reduced stress in rainbow trout without affecting growth (Chekani *et al.*, 2021).

## **2.10 Conjugated Linoleic Acid**

Conjugated linoleic acid (cLA) is an important nutrient that is found in many different diets. Conjugated linoleic acid is most abundant in ruminant products, whereas vegetable products and some shellfish have very little levels (Fritche *et al.*, 1999). Any positional and geometric isomers of linoleic acid with alternating double bonds due to chemical or microbiological processes are referred to be cLA. Throughout the 18-carbon chain, these double bonds can appear at several points, including 7,9, 8,10, 9,11, 10,12, and 11,13. Furthermore, the three-dimensional structure of cLA can have different cis and/or trans configurations (Belury 2002; Rainer and Heiss 2004). Thanks to its many health effects (anti-lipogenic, anti-carcinogenic, anti-atherosclerotic, antioxidative, immunomodulatory, and antibacterial) cLA is a popular supplement in nutrition research. Some fish species have a greater ability to store cLA in their muscular tissues, which presents a potential

way to increase the amount of cLA that humans consume. Temperature, diet composition, and stage of reproduction are among the variables that can affect the fatty acid composition and, in turn, the amount of cLA in muscle lipids (Bandarra *et al.*, 2006; Kennedy *et al.*, 2005). Octadecadienoic acid's positional and geometric isomers combine to form conjugated linoleic acid (cLA) [linoleic acid (LA), 18:2n-6]. There are various isomers of conjugated linoleic acid; the most prevalent isomer in nature is cis-9, trans-11 (c9, t11). Fish began to deposit conjugated linoleic acid when a diet high in the fatty acid was introduced, and other fatty acids also began to deposit as a result. It may change fatty acid metabolism and bone mineralization because it reduces D-9 desaturase activity (Berge *et al.*, 2004). It is unlikely that dietary cLA will enhance channel catfish growth responses. Fish showed a considerable improvement in feed efficiency, carcass fat concentration, protein retention, and energy retention (Twibell *et al.*, 2003). CLA improves fish quality and decreases tissue fat deposition, and Chu's croaker can successfully integrate it in tissues with up to 2% dietary lipid without growth impairment (Huang *et al.*, 2017).

## 2.11 Quercetin

Common flavonol-type flavonoids include quercetin, which can be found in berries, onions, tea, apples, and other fruits and vegetables. It has been proposed as a possible anti-cancer chemical due to its antioxidative, anti-inflammatory, and vasodilating qualities (Erlund, 2004). In nature, quercetin is mainly found in glycosylated form, where it forms compounds with sugar molecules (Guo and Bruno, 2015). According to Yamamoto and Oue (2006), structurally speaking, it is a plant pigment of the catechol type with two benzene rings that contain phenolic hydroxyl groups and three central carbon atoms. Because of its remarkable antioxidant qualities, it can scavenge free radicals and interact with enzymes (Di Carlo *et al.*, 1999). This reduces oxidative stress in fish by chelating reactive oxygen species (ROS) and turning them into energy. Moreover, it preserves cell membranes by controlling metal concentrations in fish bodies and reduces lipid peroxidation by supplying H<sup>+</sup> ions (Wei *et al.*, 2018).

People usually get between 4 and 68 mg of quercetin through their diets each day, according to a number of studies done in the United States, Europe, and Asia (Hertog *et al.*, 1993, Hertog *et al.*, 1995, Knekt *et al.*, 1997, Rimm *et al.*, 1996).

Recent research indicates that the primary way quercetin is now available in the US is as a dietary supplement (Theoharides and Bielory 2004). The relationship between dietary quercetin and resveratrol may enhance antioxidant capacity, immune system performance, and blunt-nose bream lipid metabolism. High doses of quercetin supplementation may encourage the growth of certain fish species (Jia *et al.*, 2019). and improve the antioxidation and meat quality of grass carp, especially when supplementing with the suggested dose of quercetin (0.37 g/kg) (Xu *et al.*, 2019). Additionally, it has been noted that adding quercetin to the diet of tilapia may enhance growth performance, hepatopancreatic antioxidant potential, and digestive enzyme activity (Zhai *et al.*, 2014). According to Shi *et al.*, (2021), quercetin can improve mammalian immunity by influencing the expression of the NF- $\kappa$ B signalling pathway and antimicrobial peptides genes in mice. This was supported by Bhaskar *et al.* (2011), who showed quercetin's capacity to reduce prostaglandin-induced inflammation by activating the Toll-NF- $\kappa$ B signalling pathway.

Wang *et al.*, (2020) found that in aquatic species, optimal quercetin levels were associated with increased myeloperoxidase and lysozyme (Lys) activity in *Brachydanio rerio* var. Furthermore, Quercetin significantly increased *Oncorhynchus mykiss* serum's antibacterial potential and Lys activity (Awad *et al.*, 2013). Despite these results, studies on the effects of quercetin on aquatic animals mostly concentrate on plasma biochemical markers; its mechanisms for immune regulation are yet unknown. Through direct modulation of host immune responses, recent in vivo and in vitro investigations have demonstrated the powerful antiviral effects of Quercetin (Ganesan *et al.*, 2012; Rojas *et al.*, 2016). By preventing viral particles from attaching to host cells and replicating there, Quercetin demonstrated anti-Singaporean grouper iridovirus properties (Liu *et al.*, 2020). After *Procambarus clarkii* was challenged with the white spot syndrome virus (WSSV), Zhang *et al.* (2021) showed that Quercetin was able to reduce mortality and viral replication; however, the specific anti-WSSV mechanism is yet unknown. The strong antioxidant capacity of quercetin seems to be associated with its immunomodulatory effects (Li *et al.*, 2016). Pês *et al.*, (2016) provided evidence that Quercetin can strengthen the antioxidant defence system by increasing glutathione (GSH) levels and upregulating the gene expression of antioxidant enzymes. In a similar vein, Wang *et al.*, (2020)

reported that Quercetin increased Nrf2 expression in *Brachydanio rerio* var. to promote antioxidant enzyme activity.

Previous studies (Shin *et al.*, 2010a; Zhai *et al.*, 2015) demonstrated the effectiveness of Que in reducing oxidative stress caused by toxins or heavy metals by boosting fish antioxidant capacities. Furthermore, Quercetin is well known for being a strong free radical scavenger (Ulusoy and Sanlier, 2019); using free radical scavenging, (Eftekhari *et al.*, 2018) they were able to lessen the damage that aflatoxin B1 causes to the liver. According to Heijnen *et al.* (2002), the catechol group in the  $\beta$  ring and the -OH group of the AC ring are responsible for Quercetin's antioxidant qualities. However, there has been little research on Quercetin's antioxidant properties in crustaceans and much on fish. Animal health depends on the intestinal microbiota, which Quercetin may control to improve infection resistance and enhance good bacteria (Saccon *et al.*, 2021). Nevertheless, investigations into Quercetin's impact on intestinal microbiota in aquatic animals are scarce.

*MATERIALS*  
&  
*METHODS*

## **3. MATERIALS AND METHODS**

### **3.1. Site of the Experiment**

The experiment was conducted at wet laboratory of ICAR-Central Institute of Fisheries Education (ICAR-CIFE), Seven Bungalows Campus, Andheri (W) Mumbai, India-400061. While, digestive, metabolic & stress enzyme activities and serum parameters were accomplished in the laboratories of Fish Nutrition Biochemistry and Physiology division at ICAR–CIFE, Mumbai-400061.

### **3.2. Experimental Animal**

Five hundred number of striped catfish, *Pangasianodon hypophthalmus* fingerlings, with an average weight of  $10.0 \pm 0.5$  g, are striped catfish. The fish were purchased from a Powerkheda hatchery. The fish were shipped to the wet laboratory at ICAR-CIFE, Mumbai, sealed in airtight bags. Fish were carefully moved to circular tanks with a 1000 L capacity and a high air exchange rate. To reduce the stress of handling, the fish were kept in an undisturbed state for the entire night. In order to reduce handling stress, vitamin C and 0.2% salt treatment was administered the next morning. For 20 days, the stock was acclimated in well-aerated conditions and fish were fed with a diet containing 35.0% crude protein.

### **3.3. Chemicals and Glassware**

The glassware used throughout the experiment were neutral glass of Borosil and Qualichem. Chemicals (analytical grade) of various companies viz. SIGMA, SISCO research laboratory (SRL), Hi-media, Qualigens, Erba®, Merck etc. were used for analytical purposes.

### **3.4. Experimental Design and Setup**

The study was carried out in a low-temperature re-circulatory aquaculture system (RAS) for eight weeks connected with proper inlets and outputs with mechanical and/or biological filtering systems. An online chiller (6.0 tons) was utilized to maintain the low temperature of  $18 \pm 1$  °C. The flow rate of 1.6 L/min was maintained. Eighteen 100 L plastic circular tubs with plastic covers were used for the

experiment. Tubs were cleaned with water, disinfected with potassium permanganate solution (5 mg/L), and cleaned thoroughly with water. The tanks were filled with water and air stones were used to provide the adequate aeration.

Two hundred fifty-two fingerlings (mean weight,  $10.12 \pm 0.02$  g) were randomly assigned to seven treatment groups in triplicate. Chlorine-free water that was equipped with an appropriate aeration system. The temperature was lowered progressively until it reached the necessary temperature for the experiment—roughly a 1 °C drop in 2 days until the fish accepted the diet. To reduce heat loss from condensation, a Hilton sheet (5 mm thick) was wrapped around the tubs and the inlet-outlet pipes. Fish were fed with experimental diets twice daily. The water volume (90 L) was maintained at constant volume throughout the 8 weeks of culture and the siphoning of tanks were done once in 2 days to remove uneaten feed and faecal matter. Plate 1 provides an illustration of the experimental configuration. The fish's growth and overall health were evaluated by taking its body weight at intervals of 15 days. Before calculating body weight, the fish were starved for an entire night.



**Plate 1: Experimental setup**



**Plate 2: Chiller**



**Plate 3: Overhead storage tank**



**Plate 4: Canister filter**



**Plate 5: Fish in experimental tub**

**Table 1: Experimental design of 60 days feeding trial**

<sup>1</sup> Treatments	Particulars	Replications		
		R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>
C	Basal diet (0.0% <sup>2</sup> cLA + 0.0% quercetin)	CR1	CR2	CR3
T1	Basal diet + (0.5% cLA + 0.0% quercetin)	T1R1	T1R2	T1R3
T2	Basal diet + (1.0% cLA + 0.0% quercetin)	T2R1	T2R2	T2R3
T3	Basal diet + (0.0% cLA + 0.5% quercetin)	T3R1	T3R2	T3R3
T4	Basal diet + (0.0% cLA + 1.0% quercetin)	T4R1	T4R2	T4R3
T5	Basal diet + (0.5% cLA + 0.5% quercetin)	T5R1	T5R2	T5R3
T6	Basal diet + (1.0% cLA + 1.0% quercetin)	T6R1	T6R2	T6R3

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin  
<sup>2</sup>cLA – conjugated linoleic acid

### 3.5. Preparation of Experimental Diets

The experimental meals were prepared using readily available products from the local market, including fish meal, soybean meal, groundnut oil cake (GNOC), wheat flour, de-oiled rice bran, maize flour, sunflower oil, and fish oil. Seven diets that are isonitrogenous (37.0%), iso-lipidic (10.0%), and iso-caloric (412 kcal DE/100 g) but have different amounts of quercetin and conjugated linoleic acid (0.0%, 0.5%, and 1.0%). The diets were identified by the following codes: C, T1, T2, T3, T4, T5, and T6. There was no conjugated linoleic acid or quercetin in the control diet (C).

**Table 2. Composition of the experimental diets**

Ingredients composition (%)	<sup>1</sup> Treatments						
	C	T1	T2	T3	T4	T5	T6
<sup>2</sup> FM	7.00	7.00	7.00	7.00	7.00	7.00	7.00
<sup>3</sup> SBM	33.00	33.00	33.00	33.00	33.00	33.00	33.00
<sup>4</sup> GNOC	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Wheat flour	3.50	3.50	3.50	3.50	3.50	3.50	3.50
<sup>5</sup> DORB	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Corn flour	4.08	4.08	4.08	4.08	4.08	4.08	4.08
Sunflower oil	3.15	3.15	3.15	3.15	3.15	3.15	3.15
Fish oil	3.15	3.15	3.15	3.15	3.15	3.15	3.15
*Vit-Min mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Cellulose	2.00	1.50	1.00	1.50	1.00	1.00	0.00
Guar gum	1.00	1.00	1.00	1.00	1.00	1.00	1.00
<sup>6</sup> BHT	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Choline chloride	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vitamin C	0.08	0.08	0.08	0.08	0.08	0.08	0.08
cLA	0.00	0.50	1.00	0.00	0.00	0.50	1.00
Quercetin	0.00	0.00	0.00	0.50	1.00	0.50	1.00
<b>Total</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin  
<sup>2</sup>cLA – conjugated linoleic acid.

<sup>2</sup>FM, fish meal; <sup>3</sup>SBM, soybean meal; <sup>4</sup>GNOC, groundnut oil cake; <sup>5</sup>DORB, de-oiled rice bran; <sup>6</sup>BHT, butylated hydroxytoluene; \*Composition of vitamin mineral mix (Chelated Growmin Forte; quantity/kg): Vitamin A: 8,00,000 IU, vitamin D<sub>3</sub>: 80,000 IU, vitamin E: 600 mg, Nicotinamide: 1,200 mg, Cobalt: 2,200 mg, Copper: 4,700 mg, Iodine: 600 mg, Iron: 2,200 mg, Magnesium: 6,500 mg, Manganese: 3,300 mg, Potassium: 200 mg, Sodium: 40 mg, Sulphur: 0.95%, Zinc: 10,000 mg, Calcium: 30%, Phosphorous: 15%.

According to the feed formulation, each item was weighed (Table 2). After the ingredients were well combined, water was added to make the dough. The dough was prepared and then put in the autoclave to steam. Steaming took place for 20 minutes at 15 pressure and 121 °C. The dough was taken out and allowed to cool. cLA, quercetin, cellulose, guar gum, BHT, choline chloride, vitamin C, and vitamin-mineral premix were added together with the oils and thoroughly mixed. The automatic pelletizer (Uniextruder single screw extruder, S. B. Panchal & Co, Mumbai, India) was then used to pelletize the feed. The pellets, which had a diameter of 2 mm, were then allowed to air dry for a day before being placed in a hot air oven set at 60°C to dry them to a moisture content of less than 10%. Packed into labelled plastic containers that were airtight (Plate 6), the dried pellets were kept at room temperature until they were needed for feeding.



**Plate 6: Experimental diets**

### **3.6. Physico-Chemical Parameters of Water**

Temperature, pH, dissolved oxygen, carbon dioxide, and ammonia were among the water quality metrics that were measured during the experiment.

### **3.6.1. Temperature**

Using a dissolved oxygen meter (MERCK, Germany), which also recorded temperature, the water temperature in each of the experimental tubs was determined.

### **3.6.2. pH**

The pH was measured of all experimental tubs by a digital pH meter (LABINDIA).

### **3.6.3. Dissolved oxygen**

The dissolved oxygen was measured by membrane electrode method using dissolved oxygen meter (MERCK, Germany) for all the experimental tubs.

### **3.6.4. Free carbon dioxide**

The dissolved free carbon dioxide was measured by titrimetric method (APHA, 2005) and calculated using the following formula

$$\text{Carbon dioxide (mg/L)} = \frac{A \times N \times 44 \times 1000}{\text{Volume of sample (ml)}}$$

Where;

A = Volume of the titrant (NaOH)

N = Normality of the titrant (N/44)

### **3.6.5. Ammonia**

Un-ionized ammonia concentration was estimated using UV-Vis spectrophotometer at 635 nm wavelength by phenate method (APHA, 2005) and compared with the standard graph. The concentration was expressed as mg/L.

## **3.7. Proximate Composition of Experimental Diets and Fish**

Proximate analysis of the experimental diets and fish was done following standard methods (AOAC, 1995).

### 3.7.1. Moisture

By taking a known weight of the sample in the petri dish and drying it in a hot air oven at 105 °C until a constant weight was reached, the moisture content of the sample (i.e., experimental diets and fish) was ascertained. The following formula was used to compute the moisture content, which was used to reflect the variation in sample weight.

$$\text{Moisture (\%)} = \frac{\text{Wet weight of sample (g)} - \text{Dry weight of sample (g)}}{\text{Wet weight of sample (g)}} \times 100$$

### 3.7.2. Dry matter (DM)

Dry matter of the experimental diets was obtained by subtracting the moisture (%) from 100.

$$\text{Dry matter (\%)} = 100 - \text{Moisture (\%)}$$

### 3.7.3. Crude protein (CP)

Using the micro Kjeldahl method, the sample's nitrogen concentration was quantitatively measured through the processes of digestion, distillation, and titration. Utilizing Pelican (Kelplus–KES 06 INL R, India), the digestion was completed. After adding the concentrated H<sub>2</sub>SO<sub>4</sub> and the digestion mixture (K<sub>2</sub>SO<sub>4</sub> and CuSO<sub>4</sub> in a 9:1 ratio), the sample was heated for two to three hours at 400 to 500 degrees Celsius. A semi-automated distillation equipment (Kelplus Classic-DX vats (P), India) was used to distill the digested sample. For distillation, NaOH (40%) and boric acid (4%) were utilized. To determine the nitrogen percentage, the distilled sample was titrated with 0.1 N HCl after being distilled using Toshiro's indicator. The nitrogen percentage was multiplied by a factor of 6.25 to determine the crude protein percentage.

$$\text{Crude protein (\%)} = \text{N}_2 (\%) \times 6.25$$

#### 3.7.4. Ether extract (EE)

Petroleum ether (boiling point 40–60 °C) was used as the solvent in Soxhlet's equipment Pelican (Socsplus-SCS 08 AS, India) to estimate the ether extract. The following computation was done:

$$\text{Ether extract} = \frac{\text{Weight of flask with lipid extract (g)} - \text{Weight of flask (g)}}{\text{Weight of sample (g)}} \times 100$$

#### 3.7.5. Crude fibre (CF)

Using an FT 122 Fibertec™ (FOSS, Denmark) for acid (1.25% hydrochloric acid) and alkali (1.25% sodium hydroxide) digestion, oven drying (100 ± 2 °C), and then incinerated in a muffle furnace (at 550 °C for 6 h), the crude fiber content of the fat-free dry diet samples was determined. The following formula was used to perform the computation.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of dry sample before ashing (g)} - \text{Weight of ash (g)}}{\text{Weight of fat free sample (g)}} \times 100$$

#### 3.7.6. Total ash

Ash content was estimated by taking a known weight of the sample in a silica crucible and placing it in a muffle furnace at 550 °C for 6 h. The calculation was done as follows:

$$\text{Total ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

#### 3.7.7. Nitrogen-free extract (NFE) of feed

The nitrogen-free extract (NFE) of the tissue was calculated by subtracting the percentage of other nutrients from 100 (Hasting, 1969).

$$\text{NFE} = 100 - (\text{CP\%} + \text{EE \%} + \text{Ash\%} + \text{CF\%})$$

### 3.7.8 Gross energy (GE)

The gross energy (GE) of the diets was calculated by using mean GE values of protein, carbohydrates and lipids (Blaxter, 1989).

$$\text{Gross energy (kcal/100g)} = \{\text{CP (\%)} \times 5.65\} + \{\text{EE (\%)} \times 9.45\} + \{\text{NFE (\%)} \times 4.2\}$$

### 3.7.9 Digestible energy (DE)

The digestible energy value of experimental diets was calculated on the basis of standard physiological values (Halver, 1976) as per the following formula:

$$\text{DE (kcal/100g)} = \{\text{Protein (\%)} \times 4\} + \{\text{Lipid (\%)} \times 9\} + \{\text{Carbohydrate (\%)} \times 4\}$$

## 3.8. Feed Intake

Feed intake study was done on a daily basis after feeding the fish to ad-libitum and collecting the left-over feed by siphoning after 2 h of feeding. The collected feed was oven dried at 60 °C and weighed on an electronic weighing balance. This was carried out for whole experimental period.

$$\text{Total feed intake (g/fish)} = \frac{\text{Feed given (g)} - \text{left over feed collected (g)}}{\text{Number of fishes}}$$

$$\text{Feed intake (g/100g fish)} = \frac{\text{Feed given (g)} - \text{left over feed collected (g)}}{\text{Initial weight of fish (g)}} \times 100$$

$$\text{Voluntary feed intake (\%/day)} = \frac{\text{Feed given (g)} - \text{left over feed collected (g)}}{\text{Initial weight of fish (g)} \times \text{Total no. of days}} \times 100$$

## 3.9. Growth and Nutrient Utilization Parameters

Samplings were done at intervals of 15 day to assess the body weight of the fish. Fish were starved overnight before taking the weight.

### 3.9.1. Weight gain (WG)

The weight gain was estimated using the following formula

$$\text{WG (g)} = \text{Final wet weight (g)} - \text{initial wet weight (g)}$$

### 3.9.2. Percentage weight gain (WG %)

The percentage weight gain was calculated using the following formula

$$\text{Weight gain (\%)} = \frac{\text{Final weight (g)} - \text{initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

### 3.9.3. Specific growth rate (SGR)

The specific growth rate was calculated by the following formula

$$\text{SGR (\%/day)} = \frac{\text{Ln}(\text{final weight}) - \text{Ln}(\text{initial weight})}{\text{Experimental period (days)}} \times 100$$

### 3.9.4. Thermal growth coefficient (TGC)

Thermal growth coefficient was calculated by the following formula

$$\text{TGC} = \frac{\{\text{Final weight (g)}\}^{1/3} - \{\text{Initial weight (g)}\}^{1/3}}{\text{Duration of feeding (days)} \times \text{temperature (}^{\circ}\text{C)}} \times 1000$$

### 3.9.5. Feed conversion ratio (FCR)

The feed conversion ratio was calculated by the following formula

$$\text{FCR} = \frac{\text{Feed given (g dry weight)}}{\text{Body weight gain (g wet weight)}}$$

### 3.9.6. Protein efficiency ratio (PER)

The protein efficiency ratio was calculated by the following formula

$$\text{PER} = \frac{\text{Net weight gain (g)}}{\text{Protein fed (g)}}$$

### 3.9.7. Lipid efficiency ratio (LER)

The lipid efficiency ratio was calculated by the following formula

$$\text{LER} = \frac{\text{Net weight gain (g)}}{\text{Lipid fed (g)}}$$

### **3.9.8. Survival (%)**

At the end of the experiment, all the experimental tubs were dewatered and the number of the experimental animals in each tub was counted and the survival (%) was calculated by the following formula

$$\text{Survival (\%)} = \frac{\text{Total number of fish harvested}}{\text{Total number stocked}} \times 100$$

## **3.10. Enzyme Assays**

### **3.10.1. Tissue homogenate preparation**

Following the conclusion of the experiment, the fishes from each treatment group were gathered, anesthetized with clove oil, and the tissue—liver, gut, gills, and muscle—was promptly removed. Next, using a Teflon-coated mechanical homogenizer (REMI Equipment, Mumbai, India), a 5% tissue homogenate was made in a cold 0.25 M sucrose solution. The entire process was carried out in an ice-cold environment in order to maintain the enzymatic activity. The tissue homogenates were then placed in a chilled centrifuge machine (Heraeus Megafuge 8R Centrifuge, Thermofisher Scientific, Germany) and centrifuged for 10 minutes at 4 °C at 5000 rpm. After that, the supernatant was gathered into 2 ml eppendorf tubes and kept at -20° C in a deep freezer until the enzyme assays were completed.

### **3.10.2. Tissue protein estimation**

The Bradford method was used to quantify the total protein in each tissue sample used for enzyme tests (Bradford, 1976). The Bradford assay depends on the protein's ability to bind to the dye Coomassie blue G250. On flat bottom ELISA plates, the tissue homogenates (5 ul) were added to 250 ul of Bradford reagent and incubated for 10 minutes. Readings were made using a Biotek microplate reader at 595 nm. Wet tissue protein content was reported in mg/g.

### **3.10.3 Digestive enzymes**

#### **3.10.3.1. Protease activity**

Drapeau's (1974) casein digestion method was used to measure the amount of protease activity in the homogenate of intestinal tissue. A set of tubes containing an enzyme reaction mixture were incubated for five minutes at 37°C. The mixture contained 1% casein in 0.05 M Tris phosphate buffer (pH 7.8). A blank for reagent was also provided in the meantime. The tissues homogenate was then added to the mixture of enzymes. Ten minutes into the incubation period, 10% TCA was added to stop the reaction. Ten minutes was all that was needed to filter the entire content. The absorbance was measured using the filtrate that was produced. The number of enzymes required to release acid-soluble fragments equal to  $\Delta 0.001A_{280}$  per minute at 37 °C and pH 7.8 was used to express one unit of enzyme activity. Finally, the protease activity was expressed as a micromole of tyrosine released/min/mg protein.

### **3.10.3.2. Amylase activity**

Utilizing the DNS (3,5-di-nitrosalicylic acid) technique, as outlined by Rick and Stegbauer (1974), the amylase activity of the homogenate of intestinal tissue was determined. The amount of reducing sugars generated on carbs by  $\alpha$ -amylase and glucoamylase was used to determine the activity. Tissue homogenate, phosphate buffer (pH 6.9), and 1% (w/v) starch solution made up the reaction mixture. After that, the test tubes were incubated for 30 minutes at 37 °C. DNS was added after the incubation, and the mixture was heated to a boiling point for five minutes. Following cooling, distilled water was added to the reaction mixtures to dilute them, and the absorbance at 540 nm was measured. The benchmark used was maltose. The expression of amylase activity was micromoles of maltose released/min/mg protein.

### **3.10.3.3. Lipase activity**

Lipase activity was measured by spectrophotometry using the method outlined by Akhila *et al.*, (2024). The estimate of lipase activity in this assay is based on the release of p-nitrophenyl butyrate (p-NPB). For fifteen minutes, reaction mixtures with lipase and substrates (2.64 mM p-NPB in sodium acetate buffer, 0.05 M and pH 5.6, with 4% (v/v) Triton X-100) are incubated at 37 °C. Following this, the

reaction is stopped by the addition of 2 ml of acetone, and the matching absorbances are measured in a microplate reader at 405 nm.

### **3.10.4. Activities of protein metabolic enzymes**

#### **3.10.4.1. Aspartate aminotransferase (AST) activity**

The technique outlined by Wooten (1964) was used to quantify the AST activity in the liver tissue homogenate. The substrate is made up of 2 mM  $\alpha$ -ketoglutarate and 0.2 M DL-aspartic acid in 0.05 M phosphate buffer (pH 7.4). The substrate was added to the experimental and control tubes in an amount of 0.5 ml. Tissue homogenate (0.1 ml) was added, and the process was then initiated. After that, the assay mixture was incubated for 60 minutes at 37 °C. Subsequently, 0.5 ml of a 1 mM solution of 2,4 dinitrophenyl hydrazine (DNPH) was added to stop the reaction. The tubes were then left for 20 minutes at room temperature with sporadic shaking. After that, 5 ml of 0.4 N NaOH solution was added, and everything was well combined. The optical density (OD) of the reaction solution was measured at 540 nm in comparison to the blank after 10 minutes. In millimoles of oxaloacetate released/min/mg protein at 37 °C, the AST activity was measured.

#### **3.10.4.2. Alanine aminotransferase (ALT) activity**

The same methodology as for AST activity was applied to measure the ALT activity of liver tissue homogenates; however, the substrate employed was 0.2 M DL-alanine rather than DL-aspartic acid. The amount of pyruvate released per minute per milligram of protein at 37 °C was measured as the ALT activity.

### **3.10.5. Enzymes of carbohydrate metabolism**

#### **3.10.5.1. Lactate dehydrogenase (LDH)**

The LDH activity in liver tissue was measured using Wróblewski and Ladue's technique (1955). 2.7 ml of 0.1 M phosphate buffer (pH 7.5), 0.1 ml of NADH solution (2 mg NADH mixed in 1 ml of phosphate buffer solution), 0.1 ml of tissue homogenate, and 0.1 ml of sodium pyruvate made up the entire 3 ml of the reaction mixture. Following the addition of sodium pyruvate as a substrate, the reaction was initiated. For two minutes, the OD was measured at 340 nm at 30-second intervals.

Units/ mg protein/min at 37 °C were used to express the enzyme activity, with 1 unit being equivalent to 0.01 OD/min at 37 °C.

### **3.10.5.2. Malate dehydrogenase (MDH)**

The Ochoa (1955) approach was used to measure the MDH activity in liver tissue. 3 ml of the reaction mixture was made up of 0.1 ml of freshly prepared oxaloacetate solution (2 mg oxaloacetate dissolved in 2 ml chilled distilled water), 0.1 ml of tissue homogenate, 2.7 ml of 0.1 M phosphate buffer (pH 7.5), and 0.1 ml of NADH solution. The addition of the oxaloacetate solution as a substrate caused the reaction to begin. For two minutes, the OD was measured at 340 nm at 30-second intervals. At 37 °C, the enzyme activity was expressed as units/mg protein/min, with 1 unit being equivalent to 0.01 OD/min.

### **3.10.6. Enzyme related to lipogenesis**

#### **3.10.6.1. Glucose-6-phosphate dehydrogenase (G6PDH)**

The DeMoss (1953) method was used to measure the liver's glucose-6-phosphate dehydrogenase (G6PDH). 1.5 ml of 0.1 M tris buffer (pH 7.8), 0.2 ml of 2.7 mM NADP, 0.1 ml of tissue homogenate, 1.05 ml of distilled water, and 0.1 ml of 0.02 M glucose-6-phosphate (G6P) made up the 3 ml total reaction volume. Over the course of three minutes, the OD was measured at 340 nm at 15-second intervals against distilled water. The enzyme activity was represented in units/mg protein/min.

### **3.10.7. Enzymes of oxidative stress**

#### **3.10.7.1. Superoxide dismutase (SOD)**

Superoxide dismutase was measured in liver tissue using the technique outlined by Misra and Fridovich (1972), which was predicated on the enzyme's oxidation of the epinephrine-adrenochrome transition. After taking 50µl of the sample in the cuvette, 1.5 ml of 0.1 M carbonate-bicarbonate buffer (which contains 0.5 ml of 3 mM epinephrine and 57 mg/dl of EDTA; pH 10.2) was added and thoroughly mixed. An instantaneous 3-minute recording of the optical density change was made in a UV-visual spectrophotometer operating at 480 nm wavelength. The quantity of

protein needed to provide 50% inhibition of adrenaline auto-oxidation was defined as one unit of SOD activity.

### **3.10.7.2. Catalase (CAT)**

In liver tissue, catalase was measured using the Takahara *et al.*, (1960) method. 50µl of the enzyme source and 2.5 ml of 50 mM phosphate buffer (pH 7.0) were added to the test tube. A volume of one ml of H<sub>2</sub>O<sub>2</sub> solution was added to initiate the reaction. For three minutes, the absorbance drop was recorded at 240 nm at intervals of 15 seconds. In place of the H<sub>2</sub>O<sub>2</sub> solution, 1.0 ml of pure water was used to run the enzyme blank concurrently. At 37 °C, the enzyme activity was measured in nanomoles of H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein.

## **3.11. Serum Parameters**

### **3.11.1. Collection of blood and serum**

Fish were taken at random from every experimental tank and given 50 µl/L of clove oil to induce anaesthesia. After that, blood was drawn from the caudal vein into vials coated with EDTA. The fish were handled with extreme caution to prevent stress throughout the sampling process. The haematological parameters (haemoglobin, haematocrit, and RBC) were examined using blood samples. Blood was drawn and immediately placed into a dried Eppendorf tube for serum. For the purpose of clotting the blood, the tubes were left in a tilted posture at room temperature for one hour. Following the coagulation of the blood, the serum, or yellow straw-coloured supernatant, was carefully collected and transferred to another tube after centrifugation was performed for five minutes at 4000 rpm.

### **3.11.2. Serum total protein**

Using the kit, the serum total protein was calculated using the Biuret method (Reinhold, 1953). The concentration of proteins in the plasma is directly correlated with the strength of the purple colour complex that results from the binding of proteins with copper ions in the biuret reagent's alkaline medium.

Three test tubes were obtained, with the labels Blank (B), Standard (S), and Test (T). Each tube received two ml of distilled water and one ml of Biuret

reagent. A volume of 0.05 ml of protein standard was transferred to the test tube designated as standard, and an additional 0.05 ml of serum was added to the test tube. After thorough mixing, it was incubated for ten minutes at 37 °C. In a spectrophotometer, the absorbance of the standard (S), test (T), and blank (B) was measured at 630 nm.

The calculation was done as follows,

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of test (T)} \times 6}{\text{Absorbance of standard (S)}}$$

### **3.11.3. Serum albumin, globulin, A/G ratio**

#### **3.11.3.1. Albumin**

The Bromocresol Green Binding Method was used to determine albumin (Doumas *et al.*, 1971). When albumin binds to bromocresol green (BCG) in a buffered medium, the green colour produced is proportional to the albumin content.

Three test tubes bearing the labels test (T), standard (S), and blank (B) were obtained. Each tube received two ml of distilled water and one ml of buffered dye reagent. A volume of 0.01 ml of albumin standard was transferred to the standard test tube, and a volume of 0.01 ml of serum was introduced to the test tube. After thorough mixing, it was incubated for ten minutes at 37 °C. In a spectrophotometer set to 630 nm, the absorbance of standard (S) and test (T) was measured instantaneously against blank (B). The following computation was made:

$$\text{Albumin (g/dl)} = \frac{\text{Absorbance of test (T)} \times 4}{\text{Absorbance of standard (S)}}$$

#### **3.11.3.2. Globulin**

Globulin was calculated by subtracting albumin values from total protein

$$\text{Globulin (g/dl)} = \text{Total protein (g/dl)} - \text{Albumin (g/dl)}$$

#### **3.11.3.3. Albumin-globulin ratio**

A/G ratio was calculated by dividing albumin values by globulin

$$\text{A/G Ratio} = \frac{\text{Albumin (g/dl)}}{\text{Globulin (g/dl)}}$$

#### **3.11.4. Serum glucose**

Using the kit, serum glucose was calculated using the GOD-POD method (Ambade *et al.*, 1998). In an alkaline biuret reagent media, glucose contained in the plasma interacts with copper ions to form a purple colour complex, the absorbance of which is proportionate to the protein content.

Three test tubes were obtained, with the labels Blank (B), Standard (S), and Test (T). One ml of glucose reagent and one ml of distilled water were added to each tube. A volume of 0.01 ml of glucose standard was transferred to the standard test tube, and a volume of 0.01 ml of serum was added to the test tube. After thorough mixing, it was incubated for 15 minutes at 37 °C. In a spectrophotometer, the absorbance of the standard (S), test (T), and blank (B) was measured at 670 nm.

The calculation was done as follows,

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of test (T)}}{\text{Absorbance of standard (S)}} \times \text{Concentration of standard (mg/dl)}$$

#### **3.12. Statistical Analysis**

The statistical tool SPSS version 22.0 was used to analyse the data statistically. One-way analysis of variance (ANOVA) was performed on the data, and Duncan's multiple range tests were employed to identify any significant variations between the means. Analyses were conducted using a 5% probability level.

# *RESULTS*

## **4. RESULTS**

### **4.1. Physico-Chemical Parameters of Water**

Physico-chemical parameters of water such as temperature (°C), dissolved oxygen (mg/L), pH, ammonia (mg/L), were estimated and average values of all treatments are presented in Table 3.

#### **4.1.1. Temperature**

The temperature of different experimental treatments ranged from 17.90 to 18.20 °C during the experimental period.

#### **4.1.2. Dissolved oxygen**

The dissolved oxygen concentration of all the experimental groups were recorded within the range of 8.40 to 8.62 mg/L during the entire experimental period.

#### **4.1.3. pH**

The pH values were recorded within range of 8.0 to 8.15.

#### **4.1.4. Ammonia**

The total ammonia concentrations of all the experimental group were recorded before water exchange and was found to be in range of 0.06 to 0.08 mg/L.

### **4.2. Proximate composition of the Diets**

The proximate composition of the different experimental diets was given in Table 4. The dry matter in the experimental diets varied from 92.5 to 94.10%. The crude protein in the diets varied from 37.0 to 37.55%, whereas the ether extract level varied from 9.90 to 10.15%. The crude fibre in the diets varied from 4.55 to 5.55%. The total ash content of the diets varied between 4.00 to 4.75%. The nitrogen-free extract was calculated within the range of 42.90 to 44.00%. The estimated gross energy (GE) and digestible energy (DE) varied from 481.130 to 487.556 kcal/100g and 409.610 to 415.531 kcal/100g, respectively.

**Table 3: Physico-chemical parameters of different experimental groups during experimental period of 8 weeks**

Parameters	Treatments							p-value
	C	T1	T2	T3	T4	T5	T6	
<b>Temperature (°C)</b>	17.97±0.04	18.17±0.03	18.02±0.05	17.96±0.04	18.11±0.05	18.07±0.07	17.98±0.04	0.333
<b>pH</b>	8.06±0.41	8.12±0.31	8.08±0.30	8.14±0.12	8.02±0.18	8.12±0.19	8.06±0.35	0.17
<b><sup>2</sup>DO (mg/L)</b>	8.48±0.22	8.51±0.16	8.41±0.17	8.55±0.14	8.40±0.17	8.61±0.24	8.43±0.29	0.213
<b><sup>3</sup>Free CO<sub>2</sub> (mg/L)</b>	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
<b>Ammonia (mg/L)</b>	0.07±0.002	0.05±0.004	0.06±0.005	0.08±0.002	0.06±0.004	0.07±0.003	0.08±0.004	0.374

Data is expressed as mean ± SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.05).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

<sup>2</sup>DO, dissolved oxygen;

<sup>3</sup>Free CO<sub>2</sub>, carbon di-oxide

**Table 4: Proximate composition of experimental diets (% dry matter basis)**

Parameters	<sup>1</sup> Treatments						
	C	T1	T2	T3	T4	T5	T6
Dry matter (%)	93.43	94.02	92.99	93.62	92.93	93.57	92.84
Crude protein (%)	37.11	37.06	37.25	37.21	37.52	37.31	37.43
Ether extract (%)	9.97	10.12	10.06	9.95	10.13	10.11	9.98
Crude fibre (%)	5.52	5.29	5.08	5.32	5.29	5.06	4.56
<sup>2</sup> NFE (%)	42.96	42.92	43.44	42.80	42.75	43.23	43.99
Total ash (%)	4.43	4.61	4.17	4.72	4.31	4.29	4.03
<sup>3</sup> GE (kcal/100 g)	481.42	482.36	484.96	481.13	484.37	484.96	487.56
<sup>4</sup> DE (kcal/100 g)	410.05	411.02	413.27	409.61	412.26	413.17	415.53

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.05).

<sup>1</sup>C - Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

<sup>2</sup>NFE (Nitrogen free extract, %) = [100 - (crude protein%+crude lipid%+crude fibre%+ash %)];

<sup>3</sup>GE, gross energy (Kcal/100g) = [5.7  $\times$  CP% + 9.4  $\times$  EE% + 4.1  $\times$  NFE%] (Halver, 1976);

<sup>4</sup>DE, digestible energy (kcal/100g) = [4  $\times$  CP% + 9  $\times$  EE% + 4  $\times$  NFE%] (Halver, 1976).

### 4.3. Whole Body Composition of the Fish

The whole-body composition of the fish of different experimental groups is shown in Table 5. In the present study, moisture, crude protein, crude lipid, total ash and total carbohydrate were significantly different among treatments (p<0.05). The moisture (%) in experimental fishes varied from 74.23 to 74.69%, whereas crude protein varied from 15.30 to 16.39%. Total ash was in the range of 2.31 to 2.74% and total carbohydrate varied from 2.22 to 3.70%. The crude lipid ranged between 2.56 to 3.45%.

**Table 5: Body composition (on % wet weight basis) of *P. hypophthalmus* fingerlings reared at low temperature**

<sup>1</sup> Treatments	Moisture	<sup>2</sup> CP	<sup>3</sup> CL	<sup>4</sup> TA	<sup>5</sup> TC
<b>C</b>	74.68 <sup>a</sup> ±0.38	15.41 <sup>a</sup> ±0.06	3.46 <sup>c</sup> ±0.05	2.74 <sup>c</sup> ±0.15	3.70 <sup>b</sup> ±0.25
<b>T1</b>	75.26 <sup>a</sup> ±0.05	15.41 <sup>a</sup> ±0.13	3.33 <sup>c</sup> ±0.05	2.58 <sup>abc</sup> ±0.05	3.41 <sup>b</sup> ±0.07
<b>T2</b>	76.27 <sup>bc</sup> ±0.14	16.28 <sup>b</sup> ±0.15	2.57 <sup>a</sup> ±0.05	2.32 <sup>a</sup> ±0.12	2.30 <sup>a</sup> ±0.07
<b>T3</b>	75.36 <sup>b</sup> ±0.20	15.51 <sup>a</sup> ±0.09	3.35 <sup>c</sup> ±0.05	2.38 <sup>ab</sup> ±0.02	3.38 <sup>b</sup> ±0.16
<b>T4</b>	75.55 <sup>ab</sup> ±0.46	15.30 <sup>a</sup> ±0.09	3.43 <sup>c</sup> ±0.05	2.41 <sup>ab</sup> ±0.06	3.30 <sup>b</sup> ±0.37
<b>T5</b>	76.49 <sup>c</sup> ±0.15	16.39 <sup>b</sup> ±0.09	2.82 <sup>b</sup> ±0.05	2.32 <sup>a</sup> ±0.13	2.22 <sup>a</sup> ±0.05
<b>T6</b>	75.54 <sup>ab</sup> ±0.31	15.35 <sup>a</sup> ±0.06	3.36 <sup>c</sup> ±0.06	2.68 <sup>bc</sup> ±0.06	3.06 <sup>b</sup> ±0.36
<b>p-value</b>	<0.050	<0.001	<0.001	<0.050	<0.050

Data is expressed as mean ± SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.05).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

<sup>2</sup>CP- Crude protein; <sup>3</sup>CL-Crude lipid; <sup>4</sup>TA- Total ash; <sup>5</sup>TC- Total carbohydrate.

## 4.4. Feed Intake and Growth Parameters

### 4.4.1. Feed intake

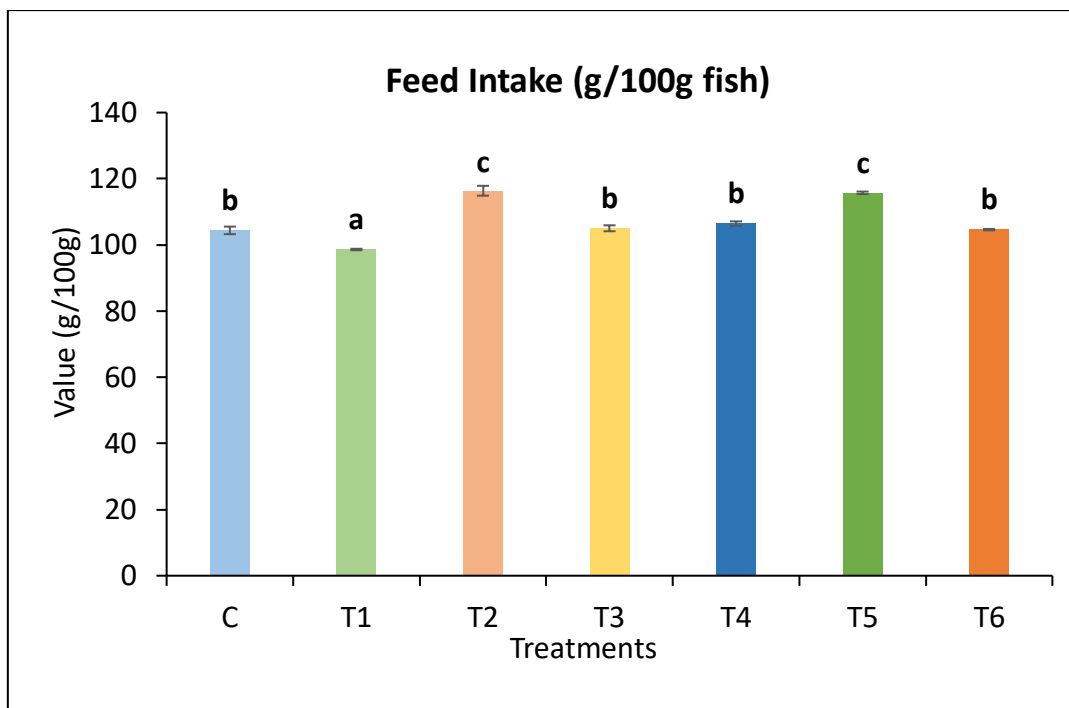
The feed intake of different experimental groups was shown in Table 6 and Fig.1 Dietary cLA and quercetin and their combination significantly affected the feed intake in striped catfish at low temperature ( $p < 0.05$ ). Maximum feed intake was observed in T2 and T5 groups ( $p < 0.05$ ) followed by control, control, T3, T4, and T6 groups ( $p > 0.05$ ) and lowest in T1 group ( $p < 0.05$ ).

**Table 6: Feed intake of different experimental groups fed with different experimental diets**

<sup>1</sup> Treatments	Feed Intake (g/100g fish)	Voluntary feed intake (%/g fish/day)
<b>C</b>	104.39 <sup>b</sup> ±1.14	1.74 <sup>b</sup> ±0.018
<b>T1</b>	98.61 <sup>a</sup> ±0.22	1.64 <sup>a</sup> ±0.004
<b>T2</b>	116.38 <sup>c</sup> ±1.49	1.94 <sup>c</sup> ±0.024
<b>T3</b>	105.03 <sup>b</sup> ±0.90	1.75 <sup>b</sup> ±0.015
<b>T4</b>	106.42 <sup>b</sup> ±0.69	1.77 <sup>b</sup> ±0.011
<b>T5</b>	115.75 <sup>c</sup> ±0.37	1.92 <sup>c</sup> ±0.006
<b>T6</b>	104.61 <sup>b</sup> ±0.21	1.74 <sup>b</sup> ±0.003
<b>P- value</b>	<0.001	<0.001

Data is expressed as mean ± SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin



**Fig. 1: Feed intake (g/100g) of *P. hypophthalmus* fed with graded levels of conjugated linoleic acid and quercetin for 8 weeks**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1- Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet + 1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

#### 4.4.2. Final body weight and weight gain

Feeding of graded levels of conjugated linoleic acid, quercetin and their combinations had a significant ( $p < 0.05$ ) effect on weight gain as shown in Table 7. The final body weight and weight gain (g) were found to be higher ( $p < 0.05$ ) in treatment T2 group fed with (1.0% cLA) followed by T5 (0.5% cLA + 0.5% quercetin) fed group that also showed a higher final weight and weight gain compared to control group (group fed with 0% cLA + 0% quercetin).

#### 4.4.3. Growth rates (weigh gain %, specific growth rate and thermal growth coefficient)

The growth rates (WG%, SGR and TGC) of different experimental groups are shown in Table 7. Dietary supplementation of conjugated linoleic acid, quercetin and their combinations had significant ( $p < 0.05$ ) effect on growth rates

(WG%, SGR and TGC). Fish fed with diet supplemented with cLA exhibited higher growth rates than control ( $p < 0.05$ ). Similarly, 0.5% dietary quercetin supplementation also had positive effect on growth rates ( $p < 0.05$ ) however, the growth rate at 1.0% quercetin showed no significance with control ( $p > 0.05$ ). 0.5% cLA and 0.5% quercetin showed higher growth rate among the combinatorial groups whereas 1% combination of both cLA and quercetin showed the growth rates non-significant with control ( $p > 0.05$ ).

## **4.5. Nutrient Utilization, Body Indices and Survival Parameters**

### **4.5.1. Feed conversion ratio (FCR)**

Dietary supplementation of conjugated linoleic acid, quercetin and their combinations had significant ( $p < 0.05$ ) effect on FCR. Fish fed with diet supplemented with cLA exhibited low FCR value than control ( $p < 0.05$ ). Similarly, 0.5% dietary quercetin supplementation showed better FCR ( $p < 0.05$ ) however, FCR at 1.0% quercetin showed no significance with control ( $p > 0.05$ ). 0.5% cLA and 0.5% quercetin combination showed better FCR than 1% combination of both cLA and quercetin showed the FCR non-significant with control ( $p > 0.05$ ).

### **4.5.2. Feed efficiency ratio (FER)**

Dietary supplementation of conjugated linoleic acid, quercetin and their combinations significantly ( $p < 0.05$ ) affected the FER. Fish fed with diet supplemented with cLA exhibited higher FER value than control ( $p < 0.05$ ). Similarly, 0.5% dietary quercetin supplementation showed better FER ( $p < 0.05$ ) however, FER at 1.0% quercetin showed no significance with control ( $p > 0.05$ ). 0.5% cLA and 0.5% quercetin showed better FER among combinatorial groups whereas 1% combination of both cLA and quercetin showed the FER non-significant with control ( $p > 0.05$ ).

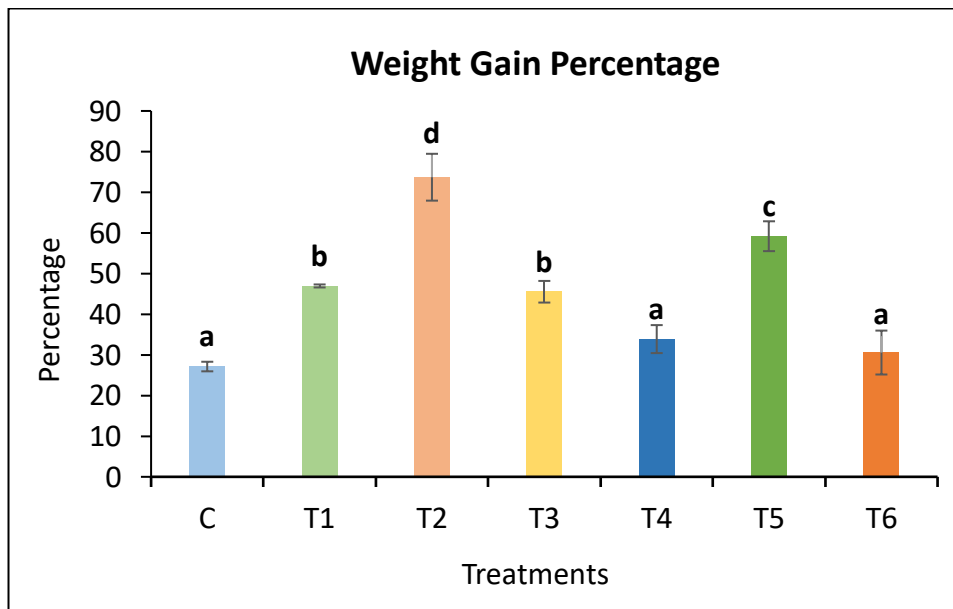
### **4.5.3. Protein efficiency ratio (PER)**

Dietary supplementation of conjugated linoleic acid, quercetin and their combinations had significant ( $p < 0.05$ ) effect on PER. Fish fed with diet supplemented with cLA exhibited higher PER value than control ( $p < 0.05$ ). Similarly, 0.5% dietary quercetin supplementation showed better PER ( $p < 0.05$ ) however, PER at 1.0%

quercetin showed no significance with control ( $p>0.05$ ). 0.5% cLA and 0.5% quercetin showed better PER among combinatorial groups whereas 1% combination of both cLA and quercetin showed the PER non-significant with control ( $p>0.05$ ).

#### 4.5.4. Lipid efficiency ratio (LER)

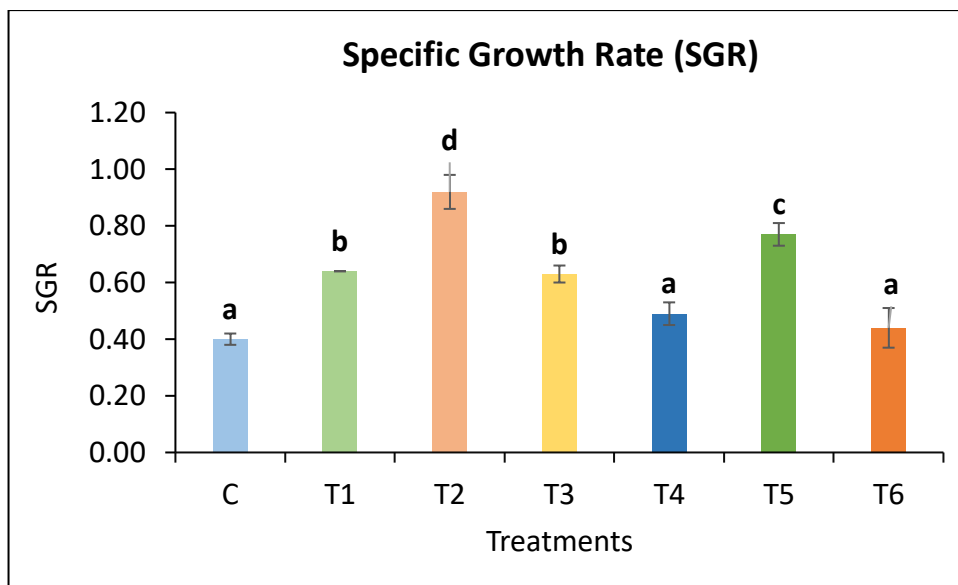
Dietary supplementation of conjugated linoleic acid and quercetin had significant ( $p<0.05$ ) effect on LER. Fish fed with diet supplemented with cLA exhibited higher LER value than control ( $p<0.05$ ). Similarly, 0.5% dietary quercetin supplementation showed better LER ( $p<0.05$ ) however, LER at 1.0% quercetin showed no significance with control ( $p>0.05$ ). 0.5% cLA and 0.5% quercetin showed better LER among combinatorial groups whereas 1% combination of both cLA and quercetin showed the LER non-significant with control ( $p>0.05$ ).



**Fig. 2: Weight gain % of *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE ( $n=3$ ). The mean value in a column under each category bearing different superscripts differ significantly ( $p<0.05$ ).

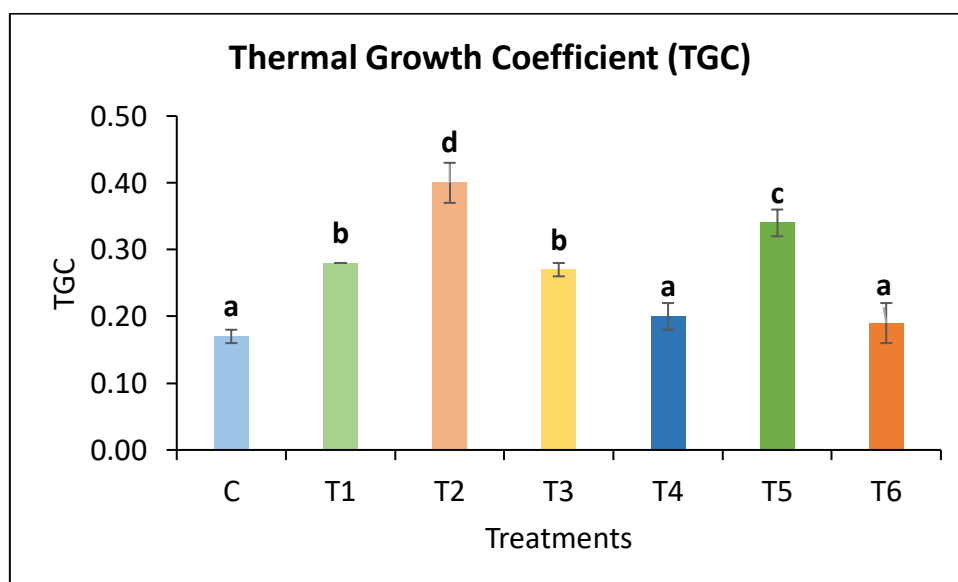
<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin



**Fig. 3: Specific growth rate (SGR) of *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1- Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin



**Fig. 4: Thermal growth coefficient (TGC) of *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1- Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

**Table 7: Growth parameters of, *P. hypophthalmus* fingerlings reared at low temperature for 8 weeks**

<sup>1</sup> Treatments	<sup>2</sup> In. wt	<sup>3</sup> Fn. wt	<sup>4</sup> WG	<sup>5</sup> WG%	<sup>6</sup> SGR	<sup>7</sup> TGC
<b>C</b>	10.29 <sup>a</sup> ±0.01	12.78 <sup>a</sup> ±0.12	2.73 <sup>a</sup> ±0.12	27.13 <sup>a</sup> ±1.18	0.40 <sup>a</sup> ±0.016	0.17 <sup>a</sup> ±0.006
<b>T1</b>	10.29 <sup>a</sup> ±0.06	14.98 <sup>b</sup> ±0.08	4.78 <sup>b</sup> ±0.02	46.95 <sup>b</sup> ±0.37	0.64 <sup>b</sup> ±0.004	0.28 <sup>b</sup> ±0.001
<b>T2</b>	11.69 <sup>d</sup> ±0.02	17.54 <sup>d</sup> ±0.52	7.44 <sup>d</sup> ±0.56	73.70 <sup>d</sup> ±5.76	0.92 <sup>d</sup> ±0.056	0.40 <sup>d</sup> ±0.026
<b>T3</b>	10.59 <sup>b</sup> ±0.16	14.67 <sup>b</sup> ±0.27	4.59 <sup>b</sup> ±0.27	45.53 <sup>b</sup> ±2.66	0.63 <sup>b</sup> ±0.031	0.27 <sup>b</sup> ±0.014
<b>T4</b>	10.64 <sup>b</sup> ±0.05	13.52 <sup>a</sup> ±0.30	3.42 <sup>a</sup> ±0.33	33.88 <sup>a</sup> ±3.44	0.49 <sup>a</sup> ±0.042	0.20 <sup>a</sup> ±0.018
<b>T5</b>	11.25 <sup>c</sup> ±0.02	16.09 <sup>c</sup> ±0.35	5.98 <sup>c</sup> ±0.36	59.19 <sup>c</sup> ±3.67	0.77 <sup>c</sup> ±0.038	0.34 <sup>c</sup> ±0.017
<b>T6</b>	10.46 <sup>ab</sup> ±0.05	13.25 <sup>a</sup> ±0.50	3.10 <sup>a</sup> ±0.54	30.57 <sup>a</sup> ±5.4	0.44 <sup>a</sup> ±0.067	0.19 <sup>a</sup> ±0.029
<b>p-value</b>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Data is expressed as mean ± SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.05).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

<sup>2</sup>In.wt- Initial weight (g); <sup>3</sup>Fn.wt- Final weight (g); <sup>4</sup>WG-Weight gain (g); <sup>5</sup>WG%- Weight gain %; <sup>6</sup>SGR-Specific growth rate (%/day); <sup>7</sup>TGC- Thermal growth coefficient

**Table 8: Nutrient utilization, body indices and survival of, *P. hypophthalmus* fingerlings reared at low temperature for a period of 8 weeks**

<sup>1</sup> Treatment	Feed Intake	<sup>2</sup> FCR	<sup>3</sup> FER	<sup>4</sup> PER	<sup>5</sup> LER
<b>C</b>	10.29 <sup>a</sup> ±0.01	3.78 <sup>b</sup> ±0.15	0.26 <sup>a</sup> ±0.011	0.71 <sup>a</sup> ±0.03	2.65 <sup>a</sup> ±0.11
<b>T1</b>	10.29 <sup>a</sup> ±0.06	2.15 <sup>a</sup> ±0.01	0.46 <sup>b</sup> ±0.002	1.25 <sup>b</sup> ±0.01	4.64 <sup>b</sup> ±0.03
<b>T2</b>	11.69 <sup>d</sup> ±0.02	1.59 <sup>a</sup> ±0.12	0.63 <sup>b</sup> ±0.050	1.71 <sup>c</sup> ±0.15	6.35 <sup>c</sup> ±0.47
<b>T3</b>	10.59 <sup>b</sup> ±0.16	2.32 <sup>a</sup> ±0.15	0.43 <sup>c</sup> ±0.022	1.17 <sup>b</sup> ±0.06	4.33 <sup>b</sup> ±0.29
<b>T4</b>	10.64 <sup>b</sup> ±0.05	3.16 <sup>b</sup> ±0.28	0.32 <sup>a</sup> ±0.030	0.86 <sup>a</sup> ±0.08	3.21 <sup>a</sup> ±0.29
<b>T5</b>	11.25 <sup>c</sup> ±0.02	1.89 <sup>a</sup> ±0.11	0.53 <sup>b</sup> ±0.026	1.43 <sup>b</sup> ±0.07	5.31 <sup>b</sup> ±0.33
<b>T6</b>	10.46 <sup>ab</sup> ±0.05	3.55 <sup>b</sup> ±0.51	0.29 <sup>a</sup> ±0.044	0.79 <sup>a</sup> ±0.12	2.95 <sup>a</sup> ±0.50
<b>p-value</b>	<0.001	<0.001	<0.001	<0.001	<0.001

Data is expressed as mean ± SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.05).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

<sup>2</sup>FCR-Feed conversion ratio; <sup>3</sup>FER- Feed efficiency ratio; <sup>4</sup>PER-Protein efficiency ratio; <sup>5</sup>LER- Lipid efficiency ratio

## 4.6. Digestive Enzyme Activities

### 4.6.1. Protease activity

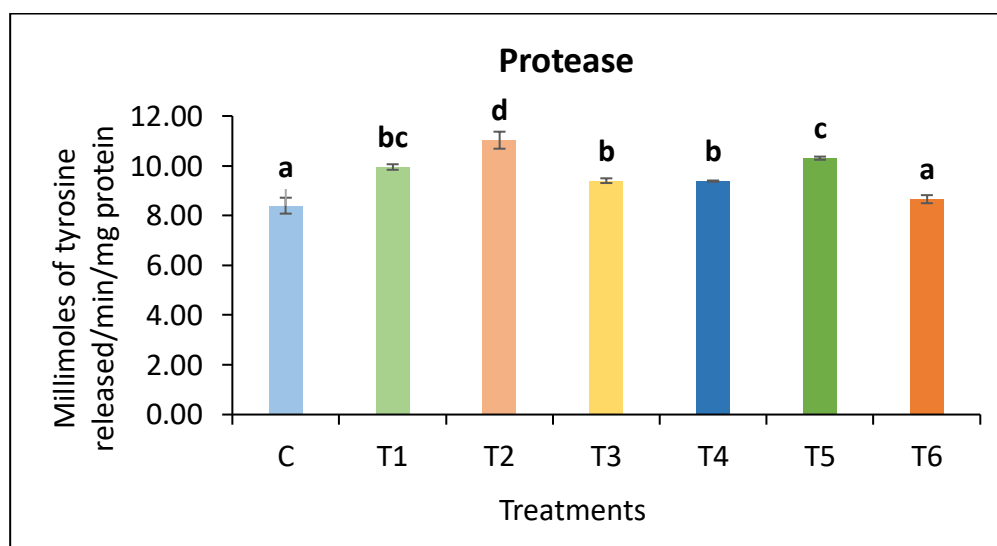
Striped cat fish exhibited different protease activities in response to dietary conjugated linoleic acid, quercetin and their combinations supplementation (p<0.05). Fish fed with diet supplemented with cLA exhibited higher protease activity than control (p<0.05). Similarly, 0.5% and 1% dietary quercetin supplementation showed significant protease activity (p<0.05). 0.5% cLA and 0.5% quercetin showed highest protease activity among combinatorial groups whereas 1% combination of both cLA and quercetin showed the protease activity non-significant with control (p>0.05).

#### 4.6.2. Amylase activity

Amylase activity was significantly influenced by conjugated linoleic acid and quercetin supplementation in diet of striped cat fish ( $p < 0.05$ ). Fish fed with diet supplemented with cLA exhibited higher amylase activity than control ( $p < 0.05$ ). Similarly, 0.5% and 1% dietary quercetin supplementation showed significant amylase activity ( $p < 0.05$ ). 0.5% cLA and 0.5% quercetin showed highest amylase activity among combinatorial groups whereas 1% combination of both cLA and quercetin showed the amylase activity non-significant with control ( $p > 0.05$ ).

#### 4.6.3. Lipase activity

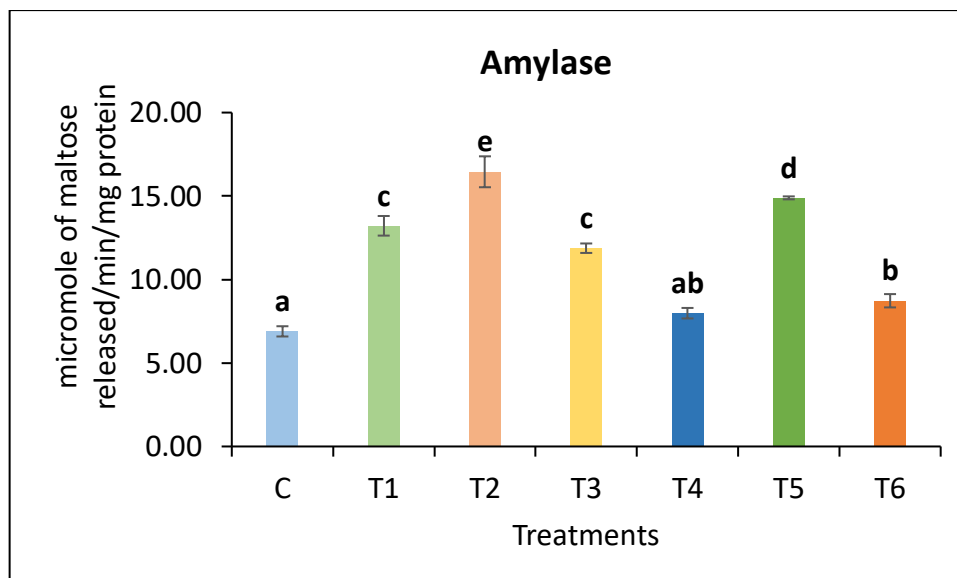
Dietary supplementation of conjugated linoleic acid and quercetin and their combinations had significant ( $p < 0.05$ ) effect on lipase activity. Fish fed with diet supplemented with cLA exhibited higher lipase activity than control ( $p < 0.05$ ). Similarly, 0.5% and 1% dietary quercetin supplementation showed significant lipase activity ( $p < 0.05$ ). 0.5% cLA and 0.5% quercetin showed highest lipase activity among combinatorial groups whereas 1% combination of both cLA and quercetin showed the lipase activity non-significant with control ( $p > 0.05$ ).



**Fig. 5: Protease activity in *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE ( $n=3$ ). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

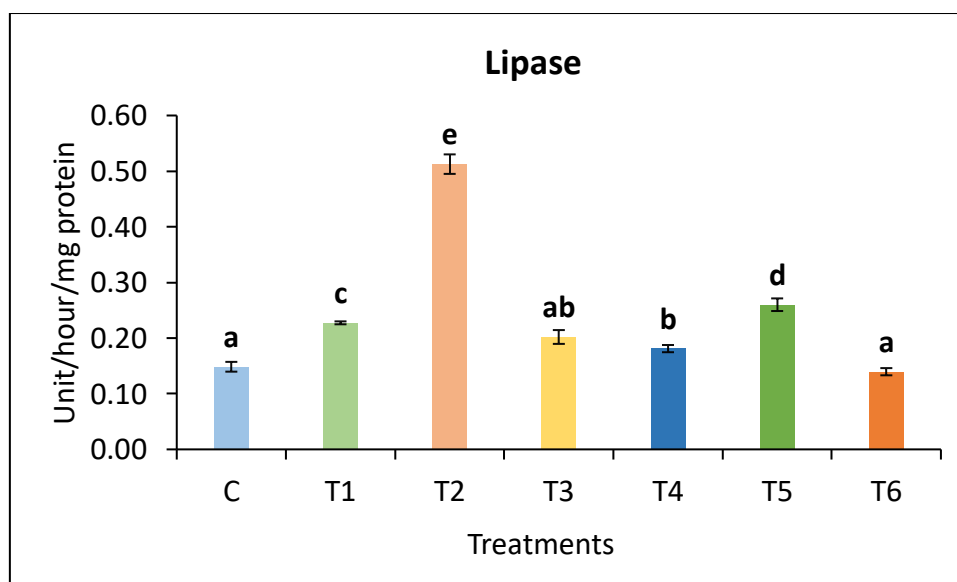
<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1- Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet + 1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin



**Fig. 6: Amylase activity in *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin



**Fig. 7: Lipase activity in *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

**Table 9: Digestive enzyme activities in the intestine of, *P. hypophthalmus* fingerlings reared at low temperature**

<sup>1</sup> Treatments	<sup>2</sup> Protease	<sup>3</sup> Amylase	<sup>4</sup> Lipase
<b>C</b>	8.39 <sup>a</sup> ±0.32	6.89 <sup>a</sup> ±0.30	0.15 <sup>a</sup> ±0.008
<b>T1</b>	9.95 <sup>bc</sup> ±0.11	13.22 <sup>c</sup> ±0.58	0.23 <sup>c</sup> ±0.003
<b>T2</b>	11.03 <sup>d</sup> ±0.34	16.45 <sup>e</sup> ±0.92	0.51 <sup>e</sup> ±0.017
<b>T3</b>	9.40 <sup>b</sup> ±0.09	11.88 <sup>c</sup> ±0.28	0.20 <sup>ab</sup> ±0.012
<b>T4</b>	9.38 <sup>b</sup> ±0.02	7.98 <sup>ab</sup> ±0.31	0.18 <sup>b</sup> ±0.006
<b>T5</b>	10.30 <sup>c</sup> ±0.06	14.89 <sup>d</sup> ±0.08	0.26 <sup>d</sup> ±0.011
<b>T6</b>	8.66 <sup>a</sup> ±0.16	8.73 <sup>b</sup> ±0.39	0.14 <sup>a</sup> ±0.006
<b>p-value</b>	<0.001	<0.001	<0.001

Data is expressed as mean ± SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.05).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

<sup>2</sup>Protease activity is expressed in millimole of tyrosine released/min/mg protein;

<sup>3</sup>Amylase activity expressed in micromole maltose released/min/mg protein;

<sup>4</sup>Lipase activity is expressed in unit/h/mg protein

## 4.7. Protein Metabolism Enzyme Activities

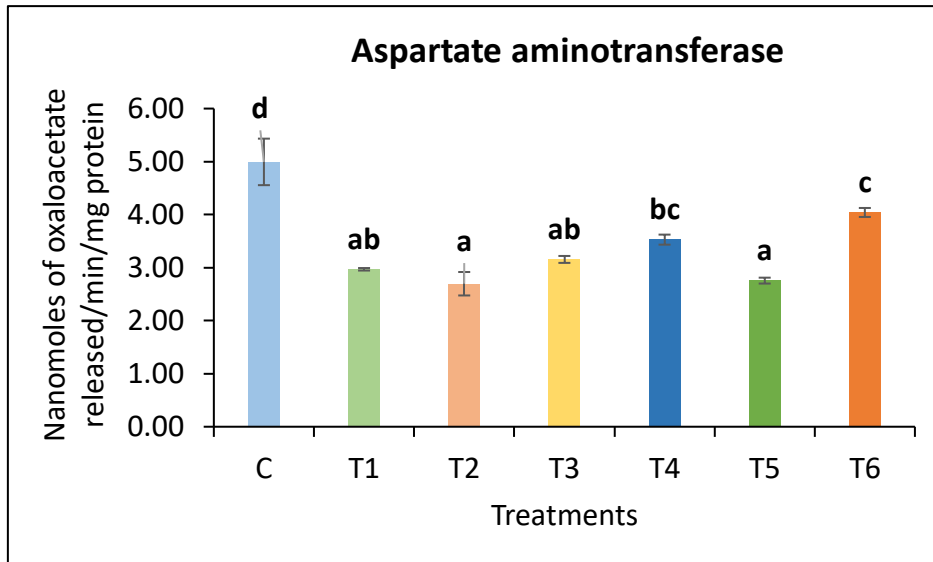
Effect of dietary conjugated linoleic acid and quercetin on the activity of aspartate aminotransferase and alanine aminotransferase in the liver of *P. hypophthalmus* fingerlings of different experimental groups is given in Table 10.

### 4.7.1. Aspartate aminotransferase activity (AST) activity

The AST activity in the liver was found to be significantly (p<0.05) different among treatment groups. The lower AST was seen in the T2 (1.0% cLA) fed group followed by T5 (0.5% cLA + 0.5% quercetin) fed group and the highest activity was seen in the control group.

### 4.7.2. Alanine aminotransferase activity (ALT) activity

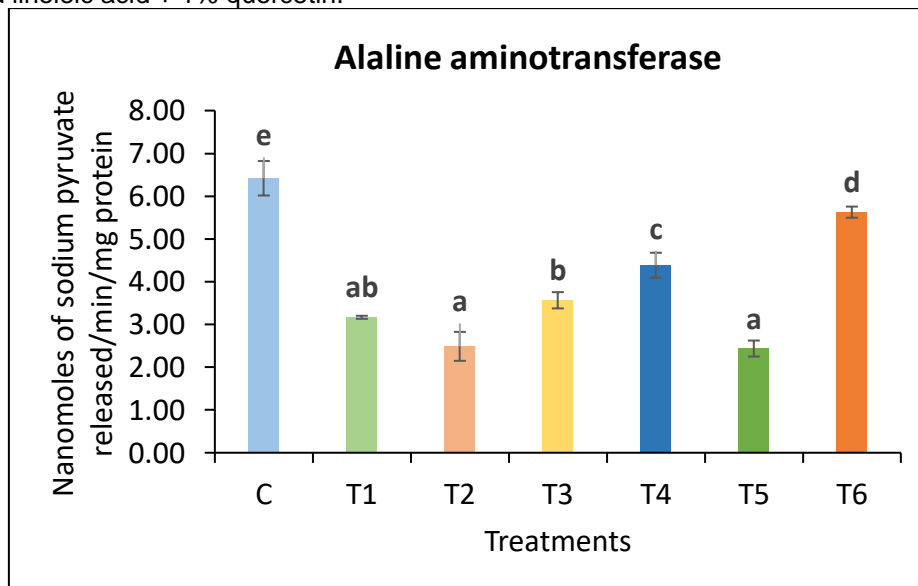
The ALT activity in the liver was found to be significantly (p<0.05) different among treatment groups. The lower ALT was seen in the T2 (1.0% cLA) fed group followed by T5 (0.5% cLA + 0.5% quercetin) fed group and the highest ALT activity was seen in the control group.



**Fig. 8: Aspartate aminotransferase activity in *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.05).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin.



**Fig. 9: Alanine aminotransferase activity in *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.05).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

## 4.8. Enzymes of Carbohydrate Metabolism

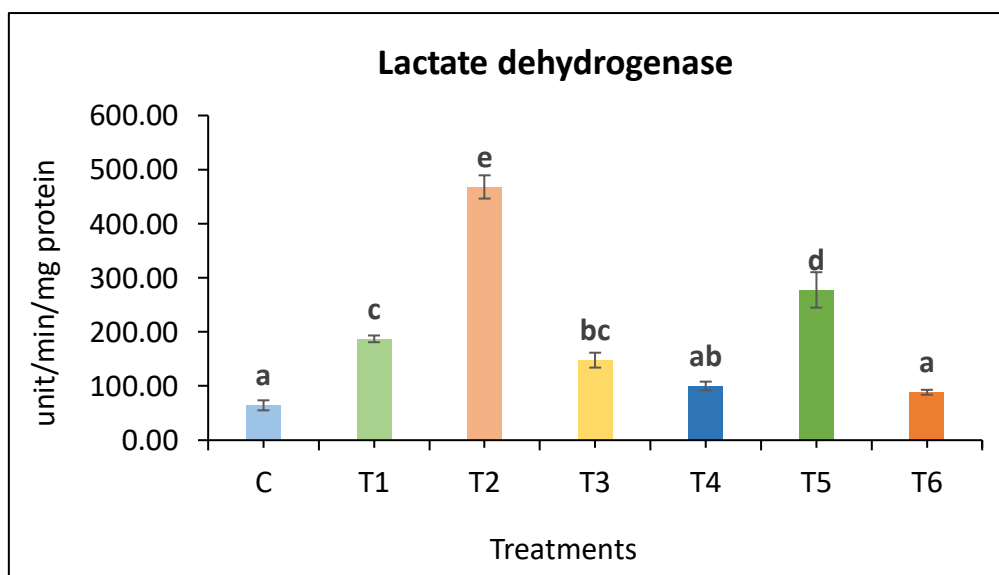
Effect of dietary conjugated linoleic acid and quercetin on the activity of lactate dehydrogenase and malate dehydrogenase in the liver of *P. hypophthalmus* fingerlings of different experimental groups is given in Table 10.

### 4.8.1. Lactate dehydrogenase (LDH)

The LDH activity was found to be significantly ( $p < 0.05$ ) different among the different experimental groups as shown in Table 10 and Fig.10. Highest effect of LDH was seen in the T2 (1.0% cLA) group ( $p < 0.05$ ) followed by T5 (0.5% cLA + 0.5% quercetin) fed group and the lowest LDH activity was reported in control. T6 (1.0% cLA and 1.0% quercetin) group showed no significance with the control ( $p > 0.05$ ).

### 4.8.2. Malate dehydrogenase (MDH)

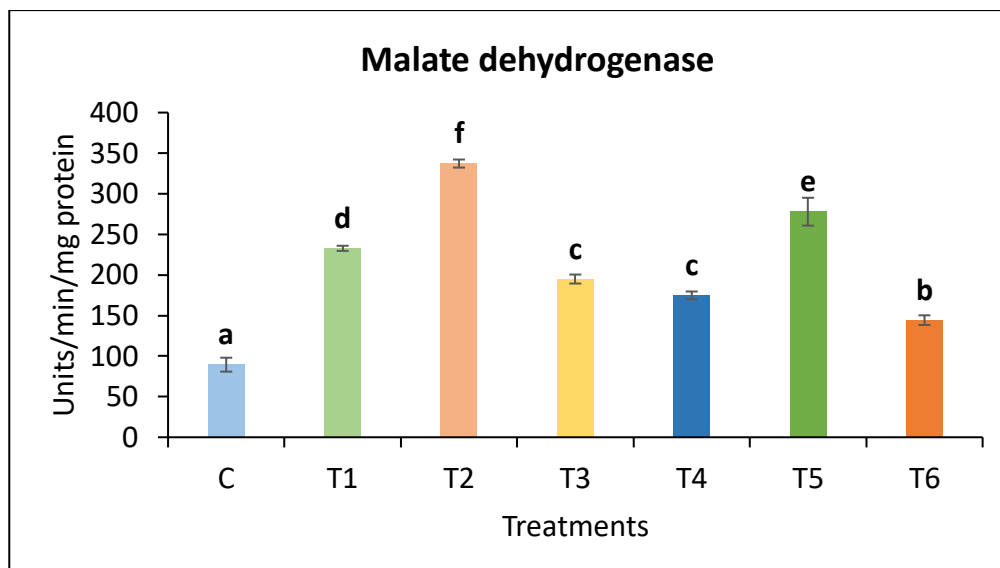
The MDH activity was found to be significantly ( $p < 0.05$ ) different among the different experimental groups as shown in Table 10 and Fig.11. Highest effect of MDH was seen in the T2 (1.0% cLA) group ( $p < 0.05$ ) followed by T5 (0.5% cLA + 0.5% quercetin) fed group and the lowest MDH activity was reported in control.



**Fig. 10: Lactate dehydrogenase activity in *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1- Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet + 1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin



**Fig. 11: Malate dehydrogenase (MDH) activity in *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1- Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet + 1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

## 4.9. Enzyme related to lipogenesis

### 4.9.1. Glucose-6-phosphate dehydrogenase (G6PDH)

The G6PDH activity was found to be significantly ( $p < 0.05$ ) different among the different experimental groups as shown in Table 10 and Fig.12. Highest effect of G6PDH was seen in the T2 (1.0% cLA) group ( $p < 0.05$ ) followed by T5 (0.5% cLA + 0.5% quercetin) fed group and the lowest G6PDH activity was reported in control. T6 (1.0% cLA and 1.0% quercetin) group showed no significance with the control ( $p > 0.05$ )

## 4.10. Oxidative Stress Enzyme Activities

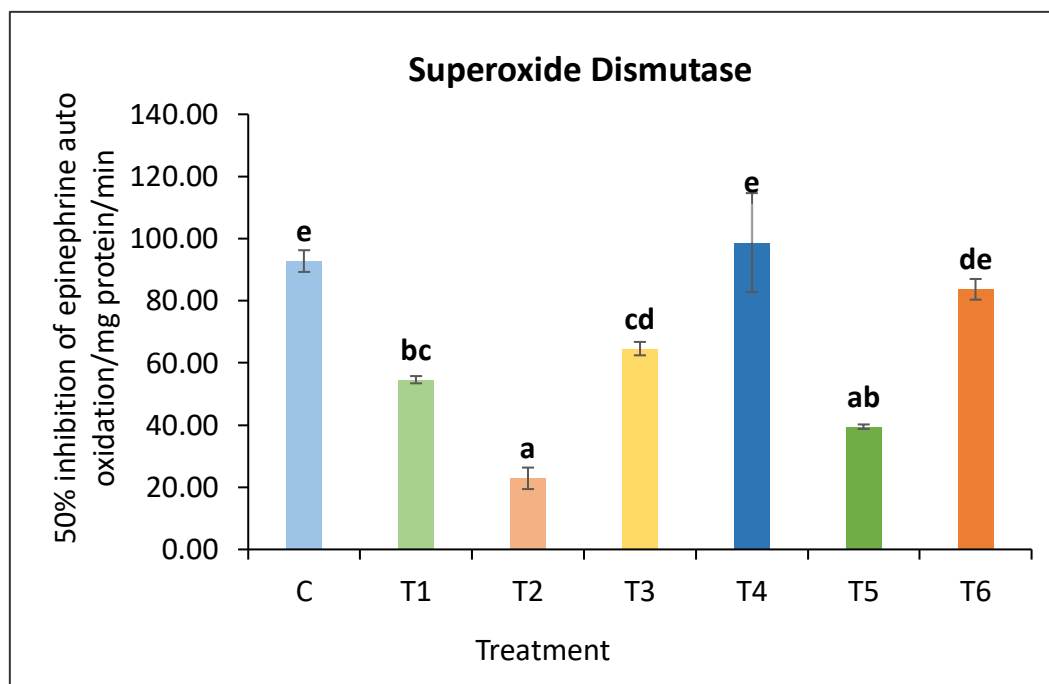
### 4.10.1. Superoxide dismutase (SOD)

The SOD activity among different experimental groups varied significantly ( $p < 0.05$ ) as shown in Table 10. Highest SOD activity was seen in control

group and lowest SOD activity was found among T2 group (1.0% cLA) followed by T5 (0.5% cLA + 0.5% quercetin).

#### 4.10.2. Catalase (CAT)

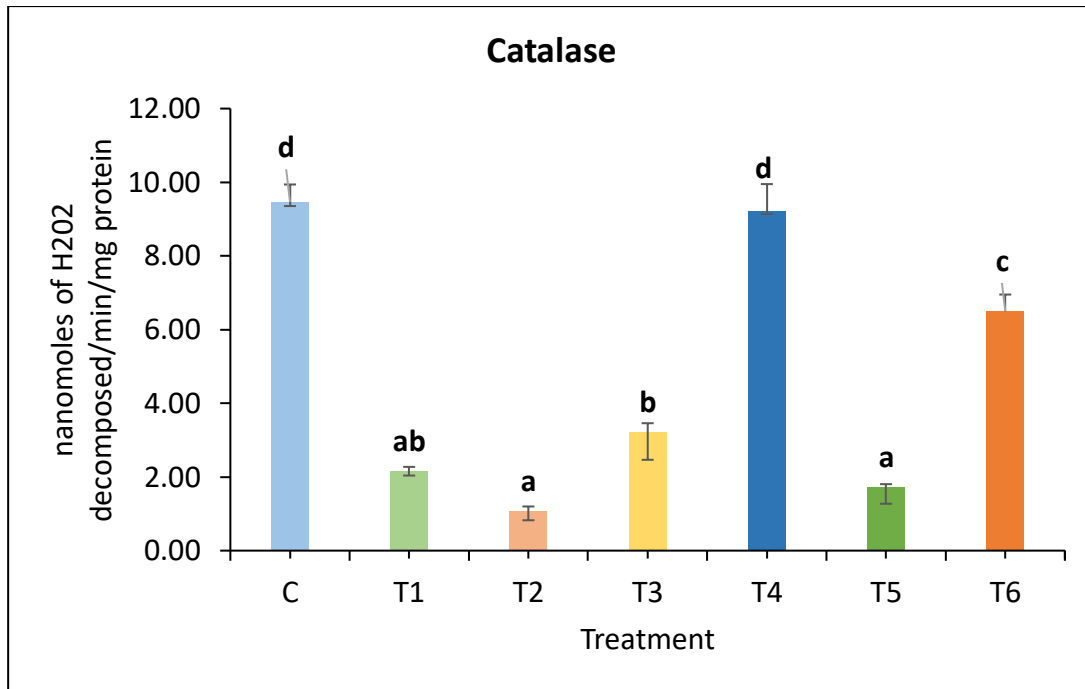
The catalase activity among different experimental groups varied significantly ( $p < 0.05$ ) as shown in Table 10. Highest catalase activity was seen in control group and lowest catalase activity was found among T2 group (1.0% cLA) followed by T5 (0.5% cLA + 0.5% quercetin). Dietary supplementation of 1.0% quercetin showed no significance with the control ( $p > 0.05$ ).



**Fig. 12: Superoxide dismutase (SOD) activity in *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1- Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet + 1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin



**Fig. 13: Catalase activity in *P. hypophthalmus* fingerlings reared at low temperature**

Data is expressed as mean  $\pm$  SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly ( $p < 0.05$ ).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

**Table 10: Metabolism and oxidative stress enzyme activities in the liver of *P. hypophthalmus* fingerlings of different experimental groups fed with experimental diets for 8 weeks**

<sup>1</sup> Treatments	<sup>2</sup> AST	<sup>3</sup> ALT	<sup>4</sup> LDH	<sup>5</sup> MDH	<sup>6</sup> G6PDH	<sup>7</sup> SOD	<sup>8</sup> Catalase
<b>C</b>	4.99 <sup>d</sup> ±0.44	6.42 <sup>e</sup> ±0.41	64.43 <sup>a</sup> ±9.25	89.44 <sup>a</sup> ±8.58	5.93 <sup>a</sup> ±0.41	92.78 <sup>e</sup> ±3.49	9.47 <sup>d</sup> ±0.47
<b>T1</b>	2.97 <sup>ab</sup> ±0.02	3.17 <sup>ab</sup> ±0.03	187.31 <sup>c</sup> ±6.23	232.77 <sup>d</sup> ±3.14	13.38 <sup>c</sup> ±0.28	54.61 <sup>bc</sup> ±1.17	2.16 <sup>ab</sup> ±0.11
<b>T2</b>	2.69 <sup>a</sup> ±0.22	2.48 <sup>a</sup> ±0.34	467.98 <sup>e</sup> ±21.49	337.24 <sup>f</sup> ±4.94	28.19 <sup>e</sup> ±1.39	22.87 <sup>a</sup> ±3.46	1.07 <sup>a</sup> ±0.12
<b>T3</b>	3.16 <sup>ab</sup> ±0.06	3.56 <sup>b</sup> ±0.18	147.91 <sup>bc</sup> ±13.79	194.94 <sup>c</sup> ±5.53	12.02 <sup>bc</sup> ±0.73	64.60 <sup>cd</sup> ±2.16	3.20 <sup>b</sup> ±0.25
<b>T4</b>	3.53 <sup>bc</sup> ±0.09	4.38 <sup>c</sup> ±0.29	100.26 <sup>ab</sup> ±7.95	174.82 <sup>c</sup> ±4.79	8.74 <sup>ab</sup> ±0.32	98.72 <sup>e</sup> ±15.92	9.21 <sup>d</sup> ±0.73
<b>T5</b>	2.76 <sup>a</sup> ±0.06	2.44 <sup>a</sup> ±0.18	277.66 <sup>d</sup> ±32.79	277.92 <sup>e</sup> ±17.14	18.41 <sup>d</sup> ±2.54	39.48 <sup>ab</sup> ±0.73	1.73 <sup>a</sup> ±0.07
<b>T6</b>	4.04 <sup>c</sup> ±0.085	5.63 <sup>d</sup> ±0.13	88.54 <sup>a</sup> ±4.56	144.30 <sup>b</sup> ±5.94	7.70 <sup>a</sup> ±0.45	83.69 <sup>de</sup> ±3.32	6.49 <sup>c</sup> ±0.46
<b>p-value</b>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Data is expressed as mean ± SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.05).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

<sup>2</sup>AST (Aspartate aminotransferase) activity expressed as nanomoles of oxaloacetate released/min/mg protein; <sup>3</sup>ALT (Alanine aminotransferase) activity expressed as nanomoles of sodium pyruvate released/min/mg protein; <sup>4</sup>LDH (Lactate dehydrogenase) activity expressed in unit/min/mg protein;

<sup>5</sup>MDH (Malate dehydrogenase) activity expressed in unit/min/mg protein; <sup>6</sup>G6PDH (Glucose-6-phosphate dehydrogenase) activity in unit/min/mg protein;

<sup>7</sup>SOD (Superoxide dismutase) activity expressed as 50% inhibition of epinephrine auto-oxidation/mg protein/min; <sup>8</sup>Catalase activity expressed as nanomoles H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein

## **4.11. Serum Parameters**

Effect of conjugated linoleic acid and quercetin on the serum parameters like serum total protein, albumin, globulin, albumin to globulin ratio, and glucose of *P. hypophthalmus* fingerlings reared at low temperature of different experimental groups is given in Table 12.

### **4.11.1. Serum total protein**

Serum total protein level of different experimental groups varied significantly ( $p < 0.05$ ) and was found to increase with major effect of above was seen in the T2 (1.0% cLA) fed group followed by T5 (0.5% cLA + 0.5% quercetin) group. Dietary supplementation of (0.5% quercetin) T3 group and (1.0% cLA and 1.0% quercetin) T6 group showed no significant difference ( $p > 0.05$ ) than control.

### **4.11.2. Serum albumin**

Serum albumin level of different experimental groups varied significantly ( $p < 0.05$ ). Major effect of above was seen in the T2 (1.0% cLA) group. T1, T3, T4 and T5 showed significant difference ( $p < 0.05$ ) as compared to control. (0.5% cLA and 0.5% quercetin) T5 showed high albumin than (1.0% cLA and 1.0% quercetin) T6, when compared among combinatorial groups.

### **4.11.3. Serum globulin**

Serum globulin level of different experimental groups varied significantly ( $p < 0.05$ ). The highest effect of above was seen in the T2 (1.0% cLA) fed group followed by T5 (0.5% cLA + 0.5% quercetin) fed group and the lowest was seen in the T6 as it showed no significant difference to control ( $p > 0.05$ ).

### **4.11.4. Serum albumin to globulin ratio (A: G Ratio)**

Serum albumin to globulin ratio of different experimental groups didn't vary significantly ( $p > 0.05$ ) as shown in Table 11.

### **4.11.5. Serum glucose**

Serum glucose level of different experimental groups varied significantly ( $p < 0.05$ ) shown in Table 11. The lower serum glucose level was found in the T2 (1.0% cLA) fed group followed by T5 (0.5% cLA + 0.5% quercetin) fed group.

**Table 11: Serum parameters in, *P. hypophthalmus* fingerlings reared at low temperature for 8 weeks**

<sup>1</sup> Treatment	<sup>2</sup> STP	<sup>3</sup> ALb	<sup>4</sup> GLo	<sup>5</sup> A: G	<sup>6</sup> GLU
<b>C</b>	7.17 <sup>ab</sup> ±0.4	2.39 <sup>ab</sup> ±0.26	4.77 <sup>abc</sup> ±0.17	0.5±0.042	95.56 <sup>c</sup> ±7.55
<b>T1</b>	8.2 <sup>bc</sup> ±0.13	3.08 <sup>cde</sup> ±0.07	5.12 <sup>bc</sup> ±0.08	0.6±0.012	79.86 <sup>ab</sup> ±2.52
<b>T2</b>	9.83 <sup>d</sup> ±0.33	3.53 <sup>e</sup> ±0.15	6.3 <sup>d</sup> ±0.41	0.57±0.059	71.46 <sup>a</sup> ±1.59
<b>T3</b>	7.47 <sup>ab</sup> ±0.56	2.88 <sup>bcd</sup> ±0.04	4.59 <sup>ab</sup> ±0.53	0.65±0.077	82.76 <sup>abc</sup> ±2.7
<b>T4</b>	6.72 <sup>a</sup> ±0.03	2.72 <sup>abc</sup> ±0.09	4 <sup>a</sup> ±0.07	0.68±0.033	82.56 <sup>abc</sup> ±4.14
<b>T5</b>	8.92 <sup>cd</sup> ±0.11	3.35 <sup>de</sup> ±0.15	5.58 <sup>cd</sup> ±0.26	0.61±0.056	74.87 <sup>a</sup> ±3.09
<b>T6</b>	7.26 <sup>ab</sup> ±0.4	2.33 <sup>a</sup> ±0.19	4.93 <sup>abc</sup> ±0.21	0.47±0.018	91.26 <sup>bc</sup> ±4.05
<b>P-value</b>	<0.001	<0.001	<0.05	0.08	0.011

Data is expressed as mean ± SE (n=3). The mean value in a column under each category bearing different superscripts differ significantly (p<0.001).

<sup>1</sup>C- Control with 0.0% conjugated linoleic acid and quercetin, T1-Basal diet + 0.5% conjugated linoleic acid, T2- Basal diet +1% conjugated linoleic acid, T3- Basal diet + 0.5% quercetin, T4- Basal diet + 1% quercetin, T5- Basal diet + 0.5% conjugated linoleic acid + 0.5% quercetin, T6- Basal diet + 1% conjugated linoleic acid + 1% quercetin

<sup>2</sup>STP: Serum Total Protein; <sup>3</sup>Alb: Albumin; <sup>4</sup>Glo: Globulin; <sup>5</sup>A: G: Albumin/Globulin; <sup>6</sup>Glu- Serum Glucose

# *DISCUSSION*

## 5. DISCUSSION

Since fish are poikilothermic, the primary abiotic element affecting their behaviour and physiological processes is environmental temperature. The most significant abiotic factor affecting numerous other factors, including water temperature, is the reason for the great variance in fish physiology and pond characteristics. Fish development, metabolism, and eating behaviour are all significantly impacted by the temperature of the pond water (Richard *et al.*, 2016). Aquaculture pond productivity, production, and the culture season are all negatively impacted by low water temperatures in the winter (O'Gorman *et al.*, 2016). Fish become inactive in the winter and stay close to the pond bottom to save energy (Khan *et al.*, 2004). Nevertheless, this may expose them to low oxygen levels and an unfavourable environment, which could impair the fish's immune and antioxidant systems and significantly affect how much it eats and how it processes its food (Khan *et al.*, 2004). In the end, this compounding impact leads to decreased growth. To properly create feed treatments to increase output, a detailed understanding of the many physiological responses that fish exhibit throughout pre-winter and winter is necessary (Khan *et al.*, 2004). Due to extended cold temperatures, fish farmers in northern India have observed that catfish, especially the smaller striped catfish, suffer from winter shock or cold shock syndrome. During the winter, it causes massive deaths of striped catfish. Numerous tropical and subtropical nations have also reported similar incidents (Kumar *et al.*, 2022). Therefore, utilizing conjugated linoleic acid and quercetin in the food, the current study assessed the feed intake, growth, physio-metabolic variation, and survival in striped catfish subjected to low temperature.

### 5.1 Physico-chemical Parameters of Water

Numerous researchers have previously documented that the temperature, pH, dissolved oxygen, free carbon dioxide, and ammonia, among other physico-chemical characteristics of water, were found to be within the optimal range. Toxic gas accumulation is more likely to happen in low-temperature environments because fish prefer to dwell below the surface. Fish development, metabolism, feed intake, and productivity are all impacted by low temperature, which also compromises

the immune system and leaves the fish more susceptible to illness (Khan *et al.*, 2004). An online chiller in a RAS setup was used in this study to keep the temperature between 17.90 and 18.25. This study was designed to study the effect of low temperature on feed intake, growth performance and survival of striped catfish, *P. hypophthalmus* fingerlings. According to Nguen (2022), pangasius grows best in temperatures between 24 to 34 °C, and its critical thermal minimum (CT<sub>min</sub>) was determined to be 15.98 °C. The current study's pH range (8.00 to 8.15) was found to be within the permitted range of pH for fish, which is 6.5–9.0, according to AbuDalo *et al.*, (2021). It was found that the dissolved oxygen content in the various experimental tubs ranged from 8.40 to 8.62 mg/L. The lower water temperature is the cause of the greater DO concentrations. The well-aerated system and circulation maintained by RAS were discovered to be the reason for the low carbon dioxide concentration and nitrogen levels of ammonia.

## **5.2. Proximate Composition of Experimental Diets**

Seven realistic iso-nitrogenous and iso-caloric diets were created for the current study. The striped catfish's nutritional needs were taken into consideration when creating the experimental diets. a diet that included 409.610 to 415.531 kcal/100g of digestible energy (DE), 9.90–10.15% ether extract, and 37.0–37.55% crude protein. The proximate composition complied with the findings of Sivaramakrishnan *et al.*, (2017) and Jayant *et al.*, (2018). Authors stated that 10.1% crude lipid and 37% crude protein were required for the growth of juvenile striped catfish, respectively. The range of the diets' crude fibre content was 4.55 to 5.55%. The nitrogen-free extract and total ash content of the diets ranged from 42.90 to 44.00% and 4.00 to 4.75%, respectively. The estimated gross energy (GE) varied from 481.130 to 487.556 kcal/100g.

## **5.3. Whole Body Composition**

Fish exposed to cold temperatures also showed an increase in membrane fluidity serves as a survival mechanism for low temperature adaptation. The increase in the amount of polyunsaturated fatty acids causes alterations in membrane fluidity (Farkas *et al.*, 2001; Tocher, 2003). In this study, body composition of striped catfish was significantly affected by dietary cLA and quercetin

supplementation at low temperature. Results revealed that T2 (1% dietary conjugated linoleic acid, cLA) and T5 (0.5% cLA and 0.5% quercetin supplementation) groups exhibited lower crude lipid content of striped catfish than control. Whereas, other treatment groups T1, T3, T4 and T6 did not show any variation in crude lipid content in striped catfish. It indicates that cLA at 1% dose and in combination of 0.5% cLA and 0.5% quercetin showed hypolipidemic properties at low temperature. cLA improves fish quality and decreases tissue fat deposition, and Chu's croaker can successfully integrate it in tissues with up to 2% dietary lipid without growth impairment (Huang *et al.*, 2017). It is observed that fish and other animals frequently exhibit homeoviscous adaptation, which increases fatty acid unsaturation to preserve membrane permeability and fluidity in order to maintain physiological balance amid extreme temperatures (Atwood *et al.*, 2003). It has been suggested that reduced body weight or muscle fat throughout the winter, as well as reduced body fat before and after, may improve growth and survival in the growing seasons that follow. According to Prchal *et al.*, (2018), the reduced fat content would favour an increase in polyunsaturated fatty acids and an omega-3 fatty acid profile. According to studies by Twibell *et al.*, (2000 & 2001); Yasmin and Takeuchi (2002), cLA lowers the overall lipid content of tissue.

#### **5.4. Feed Intake**

A number of variables, including size, age, and rearing circumstances, affect optimal feeding and feed intake (Cho *et al.*, 2003). Fish development rates and feed intake are adversely affected by low temperature exposure (Khan *et al.*, 2004). Fish in the control groups in this study consumed substantially less feed than fish in the fed groups. In this study, the feed intake was significantly affected by dietary supplementation of 1% cLA (T2) and 0.5% cLA & 0.5% quercetin (T5). Whereas, the feed intake in T1, T3, T4 and T6 were not differed from control group. One potential explanation for an increase in feed intake in T2 group (1.0% cLA) is the modification of lipid metabolism by cLA. It has been demonstrated that cLA can reduce body fat by inhibiting the enzyme stearoyl-CoA desaturase and promoting fatty acid oxidation through the peroxisome proliferator-activated receptor (PPAR) pathways. As a result, animals may experience a higher metabolic rate and energy expenditure, necessitating an increased energy intake to meet their metabolic needs. The lower

feed intake in the control group is consistent with the findings of Koskela *et al.*, (1997) and author reported a reduction in feed intake in fish being exposed to cold temperatures. Several studies have shown that dietary cLA supplementation may lead to an increase in feed intake in livestock, particularly in ruminants like cattle and sheep.

In a study conducted by Wang *et al.*, (2005), cLA supplementation in dairy cows resulted in an increase in dry matter intake (DMI), suggesting that cLA altered energy metabolism, and leading to increased feed consumption. This study also highlighted that the increase in feed intake could be a compensatory mechanism in response to reduced fat deposition or increased milk production. Another hypothesis for the increased feed intake associated with cLA is its potential effect on appetite regulation. The cLA has been suggested to interact with key hormones involved in appetite control, such as leptin and ghrelin. Leptin, a hormone produced by adipose tissue, typically reduces appetite, whereas ghrelin stimulates hunger. Some studies indicate that cLA may reduce leptin levels, potentially leading to an increase in hunger and, consequently, feed intake. Similarly, Szymczyk *et al.*, (2006) also observed that pigs fed with cLA supplemented feed exhibited reduced plasma leptin levels along with an increase in feed intake, suggesting that CLA may influence the hormonal regulation of appetite in these animals. However, these findings are still debated, and more research is needed to confirm the specific hormonal pathways through which CLA exerts this effect.

As observed that feed intake was found to increase substantially in T5 group (0.5% quercetin and 0.5% cLA). Quercetin's role in energy metabolism is another key factor in understanding its effect on feed intake. Quercetin influences energy expenditure and can modify metabolic pathways related to lipid and carbohydrate metabolism (Kelly, Cusack, and Karatzas, 2019). When energy metabolism is optimized, animals might require more energy intake to sustain higher metabolic activity, leading to an increase in feed consumption. Quercetin's impact on the regulation of hormones related to appetite control, such as ghrelin and leptin. Ghrelin is an orexigenic hormone that stimulates appetite, while leptin acts as an appetite suppressant. Quercetin has been suggested to interact with these hormonal pathways, although the precise mechanisms are still under investigation. A study by

Ibrahim *et al.*, (2019) indicated that quercetin supplementation in the diet of Nile tilapia resulted in a significant increase in plasma ghrelin levels, leading to enhanced feed intake.

## 5.5. Growth and Nutrient Utilisation

When exposed to an ambient habitat that has the ideal temperature, food circumstances, and other abiotic parameters, fish grow and reach their maximum size (Person-Le *et al.*, 2006; Oyugi *et al.*, 2011). Fish growth and feed intake are negatively impacted by exposure to cold temperatures (Koskela *et al.*, 1997). In this study, dietary supplementation of cLA and quercetin significantly influenced the growth rates, and nutrient utilization. T1, T2, T3, T4 and T5 groups showed higher growth indices than control. But growth indices did not vary between control and T6 group. Striped catfish fed with 1% cLA (T2) exhibited maximum growth rates (WG, WG %, SGR and TGC) and nutrient utilization (PER and LER) followed by and T5 group among different treatment groups. While lowest FCR was observed in T2 and T5 groups.

Higher growth in cLA supplemented groups can be explained as cLA can reduce the body fat by inhibiting the enzyme stearoyl-CoA desaturase and promoting fatty acid oxidation through the peroxisome proliferator-activated receptor (PPAR) pathways. As a result, animals may experience a higher metabolic rate and energy expenditure, necessitating an increased energy intake to meet their metabolic needs in terms of growth, feed and nutrient utilization. According to studies by Thiel-Cooper *et al.*, (2001), Wiegand *et al.*, (2002), and Dugan *et al.*, (2004), there have been contradictory findings about how cLA affects fish development and feed efficiency in various species.

Different authors reported the positive effects of quercetin on feed intake and growth in different animals (Zhai and Liu, 2013; Akrami *et al.*, 2015; Jia *et al.*, 2019; Xu *et al.*, 2019; Younes *et al.*, 2021). High doses of quercetin supplementation may encourage the growth of certain fish species (Jia *et al.*, 2019) and improve the anti-oxidant status and meat quality of grass carp, especially when supplementing with the suggested dose of quercetin (0.37 g/kg) (Xu *et al.*, 2019). 200 mg/kg quercetin supplementation enhances the growth performance and feed

conversion in Nile tilapia, *Oreochromis niloticus* (Zhai and Liu, 2013). Akrami *et al.* (2015) observed that 1% dietary onion extract significantly improved growth and nutrient utilization in beluga juvenile (*Huso huso*). Moreover, bioactive compound in onion extract i.e. quercetin was able to increase the level of ghrelin and reduce leptin hormone in the treatment groups in lambs (Akrami *et al.*, 2015). Similarly, Younes *et al.*, (2021) observed that bioactive compounds in 0.5% onion extract enhanced the growth rates and feed conversion in Nile tilapia.

## 5.6. Enzyme Assays

### 5.6.1. Digestive enzymes activities

Protease, lipase, and amylase activity are examples of digestive enzymes that are useful indicators of an organism's capacity for food digestion and adaptation to dietary changes (Gisbert *et al.*, 2009; Shamna *et al.*, 2015). Nutrient content is known to influence the activity of digestive enzymes (Kawai and Ikeda, 1972). The present investigation revealed a considerable ( $p < 0.05$ ) variation in the activities of intestinal protease, amylase, and lipase among the various treatments. The T2 (1.0% cLA) fed group showed the maximum protease activity followed by T5 (0.5% cLA and 0.5% quercetin) group. While, lowest protease activities were found in control and T6 group. In a similar vein, the activities of lipase and amylase rose, with the T2 (1.0% cLA) fed group exhibiting the largest effect, followed by the T5 (0.5% cLA + 0.5% quercetin) fed group. Increased digesting enzyme activity in cLA-fed groups may be related to the availability of substrate for the enzyme's activity. Because the groups fed cLA had higher feed consumption. It has been suggested that adding cLA to an animal's food may increase the intestinal microbiota in their guts, which in turn may improve nutritional absorption and digestion. Gopika *et al.*, (2022) observed a rising tendency for lipase, amylase, and protease in their experiment with common carp given pyrroloquinoline quinone at low temperature. Similarly, Naveen (2022) found that supplementing ashwagandha extract and powder enhanced the protease activity in rohu, *Labeo rohita* at low temperatures. It was reported that diets supplemented with cLA showed higher protease activity in Atlantic salmon (*Salmo salar*) compared to control diets (González-Rodríguez *et al.*, 2015). This enhancement could be due to cLA's role in modulating lipid metabolism, which may indirectly affect protease synthesis and secretion.

Furthermore, cLA's isomeric forms, particularly the c9, t11 and t10, c12 isomers, have been reported to influence various metabolic pathways, including those involved in protein metabolism (Chen *et al.*, 2013). Improved protease activity in fish fed cLA-enriched diets could also reflect better protein utilization, which is critical for growth performance and muscle accretion. It has been observed that quercetin, due to its antioxidant properties, can stabilize digestive enzymes, thus enhancing protease activity in fish. This might be due to the protection of proteolytic enzymes from oxidative damage, as oxidative stress can denature proteins and reduce enzyme efficiency. A study on *Oreochromis niloticus* (Nile tilapia) showed an increase in protease activity when fish were supplemented with quercetin, which was attributed to its ability to maintain enzyme stability under oxidative stress conditions (Sun *et al.*, 2020).

Amylase is an enzyme responsible for breaking down starches into simpler sugars. The relationship between cLA and amylase activity in fish is less well-studied compared to protease and lipase. However, some research indicates that fatty acid composition in the diet can influence carbohydrate metabolism. For instance, cLA may alter insulin sensitivity and glucose metabolism, which could indirectly affect amylase activity (Pariza *et al.*, 2001). In rainbow trout (*Oncorhynchus mykiss*), a study found that dietary cLA had no significant effect on amylase activity (Farhangi and Carter, 2007). This could suggest that cLA's impact on carbohydrate metabolism may not be as profound as its effect on protein and lipid metabolism. However, variations in response could be species-specific or dependent on the overall diet composition, including the ratio of carbohydrates to lipids. In a study on carp (*Cyprinus carpio*), quercetin supplementation was associated with increased amylase activity, likely due to improved gut health and nutrient absorption (Zhou *et al.*, 2017). Quercetin's role in enhancing the efficiency of glucose metabolism may indirectly stimulate the pancreas or intestinal cells to produce more amylase, thereby improving carbohydrate digestion and overall energy metabolism (Ren *et al.*, 2018). On the contrary, in some species, higher concentrations of quercetin have been associated with reduced amylase activity, suggesting a dose-dependent effect where excess flavonoid might lead to enzyme inhibition by interacting with carbohydrate substrates or enzyme cofactors (Dai *et al.*, 2020).

Lipase enzymes are crucial for the digestion of lipids, breaking down triglycerides into free fatty acids and glycerol. cLA, as a fatty acid itself, can significantly influence lipase activity. A study on tilapia (*Oreochromis niloticus*) revealed that dietary supplementation with cLA significantly increased lipase activity, leading to more efficient fat digestion (Zheng *et al.*, 2012). The increased lipase activity associated with cLA supplementation may be due to the activation of peroxisome proliferator-activated receptors (PPARs), which play a key role in lipid metabolism and can upregulate lipase gene expression (Brown and McIntosh, 2003). Moreover, cLA's ability to modulate lipid metabolism pathways, such as the oxidation of fatty acids, may result in a feedback mechanism that enhances lipase production to meet the increased demand for fatty acid oxidation (Park *et al.*, 1997). In tilapia (*Oreochromis mossambicus*), quercetin supplementation led to a significant increase in lipase activity, suggesting that quercetin might enhance fat metabolism by stimulating bile production or by increasing the secretion of lipase from the pancreas (Sun *et al.*, 2020). The ability of quercetin to reduce oxidative stress in the digestive tract could also contribute to an environment that supports optimal lipase activity (Wu *et al.*, 2019). However, in some cases, high doses of quercetin have been linked to reduce lipase activity, possibly due to the flavonoid's ability to interfere with lipid micelle formation, which is necessary for fat digestion (Li *et al.*, 2018). Additionally, quercetin has been observed to bind to free fatty acids, which may alter the substrate availability for lipase and reduce enzyme efficiency at higher concentrations (Yang *et al.*, 2019).

### **5.6.2. Enzymes of protein metabolism**

Important aminotransferase enzymes that redistribute amino-nitrogen among amino acids include aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Lehninger, 2002). The reversible transfer of an amino group from glutamate to oxaloacetate is catalysed by this enzyme. Since AST is a mitochondrial enzyme, its predominance over ALT may indicate an aerobic inclination of the tissues and increased glucose utilisation (Rastiannasab *et al.*, 2016). The interconversion of aspartate and  $\alpha$ -ketoglutarate into oxaloacetate and glutamate is catalyzed by AST. In addition to assisting in the synthesis of ATP, the deamination of amino acids results in the formation of TCA cycle intermediates (Silva *et al.*, 1995).

However, its effects in aquatic organisms like fish, especially regarding liver function as measured by aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity, remain a growing area of research. AST and ALT are key enzymes indicative of liver health, where elevated levels typically signal liver damage or stress. Hepatic AST and ALT activity in the current investigation revealed a substantial variation between the control and dietary treatment groups. Both cLA and quercetin supplementation and their combinations lowered the aminotransferase activities. Highest activities were recorded in control and lowest activities in T1, T2 and T5.

The lowest AST and ALT activity is seen T2 group (1.0% cLA) and the highest in control group. Among the combinatorial groups lowest activity is seen in T5 as compared to T6, where T5 (0.5% cLA and 0.5% quercetin) and T6 (1.0% cLA and 1.0% quercetin). And no further variation was seen among other treatment groups when compared to control. Several studies suggest that cLA supplementation may influence the activity of liver enzymes, including AST and ALT, but the results are not universally consistent across species.

AST is an enzyme found in various tissues but predominantly in the liver. It is released into the bloodstream when the liver is damaged, which makes it a useful marker for hepatic stress or injury. Studies on fish, such as rainbow trout (*Oncorhynchus mykiss*), have demonstrated that dietary cLA can lead to varying effects on AST activity depending on dosage and duration of exposure. Some reports indicate that moderate cLA supplementation may not cause significant change in serum AST levels. For instance, a study by Yildiz and Şahin (2018) observed no significant increase in AST activity in fish fed cLA-enriched diets over a period of 10 weeks, which suggests that cLA may not be hepatotoxic at certain concentrations. This could be due to cLA's potential role in improving lipid metabolism, thus reducing fatty liver accumulation—a condition known to elevate AST levels in fish (Yildiz and Şahin, 2018). However, other studies suggest that high levels of cLA could induce oxidative stress, potentially leading to liver damage and increased AST activity. For example, research on different fish species, such as zebrafish (*Danio rerio*), has shown that excessive cLA intake can result in liver cell apoptosis, potentially elevating serum AST levels as a response to hepatic injury (Jiang *et al.*, 2019).

Like AST, ALT is another critical enzyme in assessing liver health. ALT is more specific to the liver than AST and is considered a more direct marker of liver cell integrity. The influence of cLA on ALT levels in fish appears to follow a similar pattern to that of AST, though ALT levels may be more sensitive to hepatic changes due to cLA supplementation. Research indicates that cLA can either maintain or increase ALT activity depending on its dosage. Some studies have reported that low to moderate cLA levels do not significantly affect ALT activity, suggesting that liver function remains relatively unaffected under such dietary conditions (Jiang *et al.*, 2019). However, in cases where fish were subjected to higher doses of cLA or prolonged exposure, ALT activity significantly increased, correlating with liver cell stress or damage (Rahman *et al.*, 2020). This increase could be linked to cLA's role in altering lipid metabolism, possibly leading to lipid peroxidation and the subsequent release of ALT as a response to cell membrane damage. cLA is known to modulate lipid metabolism by reducing hepatic fat accumulation, a process that, in theory, should protect the liver and keep enzyme levels stable. Reduced fat accumulation may help prevent non-alcoholic fatty liver disease (NAFLD), a condition associated with elevated AST and ALT.

### **5.6.3. Enzymes of carbohydrate metabolism**

Anaerobic respiration can be reliably detected by lactate dehydrogenase, a NADH-dependent enzyme that converts pyruvate to lactate. Anaerobic respiration can occur when a cell experiences hypoxia and an increase in energy demand due to stress (Lehninger, 2002). As the amount of cLA included increased, the liver's LDH activity trended upward. A switch from aerobic to anaerobic metabolism, which produces lactic acid and, eventually, energy generation, is indicated by elevated LDH activity (Rudney, 1950; Miller *et al.*, 1953; Persons *et al.*, 1968; Hilton *et al.*, 1986). Dietary cLA and quercetin and their combination significantly enhanced the hepatic LDH and MDH activities. The highest activities of LDH and MDH were observed in T2 group (1.0% cLA) and the lowest in control group. Among the combinatorial groups higher activity was seen in T5 compared to T6 group.

The inclusion level of cLA was correlated with an increasing trend in hepatic MDH activity. This could be because the TCA cycle uses the dietary cLA as

a substrate. Kinera *et al.* (2021) observed a similar improvement in MDH activity when rohu were fed increasing amounts of lemon peel extract at a low temperature. Studies have shown that dietary supplementation of CLA in fish can alter LDH activity, although the specific effects can vary depending on species and experimental conditions. Some studies report a decrease in LDH activity in fish fed cLA or quercetin, suggesting a shift from anaerobic to aerobic metabolism, which may enhance the efficiency of energy production and reduce lactate accumulation (Xu *et al.*, 2016). This shift could be advantageous under normal oxygen availability, as aerobic pathways provide more ATP per glucose molecule than anaerobic pathways. On the other hand, in fish experiencing hypoxic conditions or subjected to intense physical activity, an increase in LDH activity in response to cLA could indicate a compensatory mechanism to meet the energy demands through glycolysis (Wang *et al.*, 2017). This modulation of LDH by cLA may suggest that cLA influences how fish respond to different environmental and physiological stressors by adjusting energy pathways. Similarly, elevated LDH activity might be related to continuous production of free reactive oxygen species due to enhanced metabolism at low temperature. Suski *et al.*, (2006) reported that reduced temperature resulted in elevated lactate concentrations in largemouth bass *Micropterus salmoides*. Similarly, LDH activity in serum was increased in rat when fed with 75 mg/kg quercetin rich diet against bisphenol-A stressor (Vanani *et al.*, 2020). The reduction in LDH activity with quercetin levels in diet (T3 to T4) reflect a protective mechanism, as quercetin reduces lactate accumulation and maintains the redox balance in tissues under stress. For instance, a study on rainbow trout (*Oncorhynchus mykiss*) reported decreased LDH activity in liver and muscle tissues following quercetin administration, pointing to a potential shift toward aerobic energy metabolism, which is more efficient and less stressful to cells under normal oxygen conditions.

MDH is involved in the citric acid cycle and plays a key role in oxidative metabolism by catalysing the conversion of malate to oxaloacetate. It is a pivotal enzyme for the regeneration of NAD<sup>+</sup>, which is essential for maintaining the energy flow through the Krebs cycle. Dietary cLA and quercetin supplementation also influenced the MDH activity, potentially enhancing oxidative metabolism. An increase in MDH activity suggests that cLA promotes aerobic energy metabolism, possibly by enhancing mitochondrial function and the efficiency of the citric acid cycle (Jiang *et*

*al.*, 2018). By improving MDH activity, cLA may contribute to better energy utilization from lipids, which could be particularly relevant in fish that store significant amounts of lipids. Furthermore, cLA's known effect on lipid metabolism may lead to increase availability of substrates for the citric acid cycle, thereby upregulating MDH activity. This increased activity would contribute to enhanced ATP production via oxidative phosphorylation, further supporting the hypothesis that CLA improves the energy efficiency of cells, especially under conditions where aerobic metabolism is favourable. Studies have indicated that quercetin enhances MDH activity, thereby promoting oxidative metabolism in fish. This suggests that quercetin encourages aerobic pathways over anaerobic ones, thus improving energy production efficiency under normal conditions. Quercetin's capacity to up-regulate the MDH activity could be linked to its ability to reduce oxidative stress, as enhanced mitochondrial function would favour the use of oxygen for energy production. For instance, in studies on zebrafish (*Danio rerio*), quercetin supplementation led to increased MDH activity, likely improving oxidative phosphorylation and thus the overall metabolic health of the fish.

#### **5.6.4. Enzyme related to lipid metabolism**

An essential component of the pentose phosphate system is glucose-6-phosphate dehydrogenase (G6PDH). Studies have shown that in species such as goldfish (Kanungo and Prosser, 1959), brook trout (Hochachka and Hayes, 1962), striped bass (Stone and Sidell, 1981), rainbow trout (Nagelkerke *et al.*, 1990), and tilapia (Naqshbandi *et al.*, 2011), there was an increased importance of HMP shunt during cold acclimation. In the current investigation, groups supplemented with 1% cLA had higher G6PDH activity than groups supplied with 0.5% cLA and 0.5% quercetin. HMP shunt serves as a source of NADPH for fatty acid production, which is integrated into membrane phospholipids (Hazel and Neas, 1982). Because of the increasing amount of polyunsaturated fatty acids in the cell membrane, it is in charge of altering the permeability of the membrane. According to Thiel-Cooper *et al.*, (2001), Tischendorf *et al.* (2002), and Yamasaki *et al.* (2003), cLA reduces body fat and increases lean body mass. Park *et al.*, (1997) reported that cLA raises the rate limiting enzyme in beta oxidation CPT activity in skeletal muscle. Yotsumoto *et al.*, (1999) and Valente *et al.*, (2007a, 2007b) additionally reported on a number of

additional research that show how cLA affects hepatic lipid metabolism. Dietary cLA has been reported to modulate G6PDH activity, with both increases and decreases observed in different studies (Kim *et al.*, 2015). The increase in G6PDH activity following cLA supplementation might be linked to an enhanced requirement for NADPH, necessary for fatty acid synthesis and detoxifying reactive oxygen species (ROS) (Chen *et al.*, 2017). Conversely, a decrease in G6PDH activity may indicate a shift away from lipogenesis, reflecting cLA's role in reducing fat deposition in fish. cLA is known for its lipid-lowering effects, and this reduction in G6PDH activity aligns with decreased fatty acid synthesis and a preference for utilizing stored fats as an energy source (Wang *et al.*, 2017). This effect could be species- and tissue-specific, depending on the lipid metabolism and storage patterns of the fish being studied.

G6PDH is a rate-limiting enzyme in the pentose phosphate pathway, crucial for maintaining cellular redox balance by producing NADPH, which is essential for antioxidant defence. Quercetin has been observed to modulate G6PDH activity in fish, often leading to its upregulation in response to oxidative stress. The increase in G6PDH activity following quercetin treatment likely reflects its role in enhancing the antioxidant defence system, as NADPH is necessary for glutathione regeneration, a key antioxidant in cells. In species such as common carp (*Cyprinus carpio*), quercetin administration has been associated with an increase in G6PDH activity, suggesting that it supports the cell's ability to manage oxidative stress by bolstering NADPH production and thus protecting the cell from ROS damage. This effect is critical in maintaining the balance between oxidation and antioxidation in metabolically active tissues like the liver and muscle, especially under environmental stressors such as pollution or temperature fluctuations.

#### **5.6.5. Enzymes of oxidative stress**

When pro-oxidant forces and reactive oxygen species overcome antioxidant defences and are not sufficiently eliminated, oxidative stress is the outcome (Sies *et al.*, 1992). Living things are shielded from ROS by a number of defence mechanisms, including antioxidant enzymes like catalase and SOD. Enzymes in the superoxide dismutase (SOD) class catalyze the breakdown of superoxide into oxygen and hydrogen peroxide. Additionally, almost all living things that are exposed to oxygen include the natural antioxidant enzyme catalase, which

catalyzes the breakdown of hydrogen peroxide into water and oxygen (Naseemashahul *et al.*, 2021). Fish are exposed to various environmental stressors that can lead to increased production of reactive oxygen species (ROS), resulting in oxidative stress. Oxidative stress can damage cellular components, including lipids, proteins, and DNA, leading to impaired physiological functions and increased mortality (Nielsen *et al.*, 2019). Antioxidant enzymes such as SOD and catalase play pivotal roles in the detoxification of ROS. SOD catalyzes the dis-mutation of superoxide radicals into hydrogen peroxide, while catalase further decomposes hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage (Halliwell & Gutteridge, 2015). Quercetin has been shown to enhance the activity of antioxidant enzymes in various species, including fish. For instance, studies demonstrate that quercetin supplementation can significantly elevate SOD and catalase levels in fish, suggesting its protective role against oxidative stress. In a study by Zang *et al.*, (2018), it was observed that quercetin administration in *Cyprinus carpio* (common carp) led to a notable increase in SOD and catalase activity, correlating with decreased levels of malonaldehyde (MDA), a marker of lipid peroxidation. This indicates that quercetin not only boosts the antioxidant defence mechanism but also mitigates oxidative damage.

The lowest activity is seen T2 group (1.0% cLA) and the highest in control group. Among the combinatorial groups lowest activity is seen in T5 as compared to T6, where T5 (0.5% cLA and 0.5% quercetin) and T6 (1.0% cLA and 1.0% quercetin). And no further variation was seen among other treatment groups when compared to control. The mechanism underlying the enhancement of SOD and catalase activity by quercetin may be attributed to its ability to modulate the expression of genes associated with antioxidant defence. Quercetin has been reported to activate nuclear factor erythroid 2-related factor 2 (Nrf2), a key transcription factor that regulates the expression of antioxidant enzymes, including SOD and catalase (Bai *et al.*, 2020). By promoting Nrf2 activation, quercetin can lead to increased transcription of antioxidant enzymes, thereby improving the fish's ability to cope with oxidative stress.

Recent studies have demonstrated that cLA supplementation can enhance SOD activity in fish, suggesting its potential role in improving the antioxidant

capacity of these organisms. For instance, a study by Figueiredo *et al.* (2022) found that feeding rainbow trout (*Oncorhynchus mykiss*) with diets enriched with cLA led to a significant increase in SOD activity compared to control groups. The authors hypothesized that the bioactive properties of cLA might stimulate the expression of antioxidant genes, thereby boosting the production of SOD. The mechanism behind this enhancement may be linked to cLA's ability to modulate oxidative stress levels. By reducing oxidative damage, cLA may indirectly increase the availability and activity of SOD, allowing fish to better cope with environmental stressors (Kumar *et al.*, 2020). This is particularly relevant for fish species raised in intensive aquaculture systems, where stressors can significantly impact health and growth.

In addition to SOD, catalase activity has also been observed to decrease with cLA supplementation. In contrast, Mardah *et al.*, (2023) reported that fish receiving cLA-enriched diets exhibited higher catalase activity, suggesting that cLA not only boosts SOD activity but also enhances the overall antioxidant defense mechanism. This dual effect is crucial, as both enzymes work synergistically to detoxify ROS and protect cells from oxidative stress. The increase in catalase activity following cLA supplementation can be attributed to several factors, including the potential anti-inflammatory properties of cLA, which may reduce the oxidative burden on fish cells. Moreover, the upregulation of catalase expression in response to cLA may reflect a broader adaptive response to enhanced oxidative stress resilience (Reyes-Cerpa *et al.*, 2019).

## **5.7. Serum parameters**

The most significant substances found in serum are proteins, which are referred to as circulating mobile proteins. Albumin and globulins are the two types of serum proteins. Albumin is produced by the liver, which also produces an osmotic force that keeps the fluid volume in the vascular space constant. Alpha, beta, and gamma globulin are the three easily distinguished groupings of components that make up serum globulin. Nearly 90% of the blood's immunologically active proteins come from the gamma globulin fraction, and globulins like gamma globulins are crucial for keeping the immune system functioning properly (Wiegertjes *et al.*, 1996). Fish in various treatments had significantly varied serum albumin and globulin

concentrations. Fish with stronger innate responses have higher serum protein, albumin, and globulin levels (Wiegertjes *et al.*, 1996).

The plasma proteins, albumin, globulin, and albumin-globulin ratio were examined in this investigation. Dietary conjugated linoleic acid and quercetin significantly affected the levels of glucose, albumin, globulin, total protein, and the A:G ratio. Significant increases were observed in total protein, albumin, and globulin. The T2 group (1% cLA) had the highest total protein values, followed by the T5 group (0.5% cLA and 0.5% Quercetin). It was discovered that the T2 group had a higher amount of albumin than the control group. The T2 and T5 groups were found to have greater serum globulin levels. With 1% cLA levels in the diet, the serum glucose levels dropped, with the T2 group having the lowest level. Other groups did not show elevated serum glucose levels. The greater energy needs to counteract cold stress may be explained by these increases in glucose and cortisol concentrations in the control diet (Atwood *et al.*, 2003). Albumin is a major plasma protein responsible for maintaining oncotic pressure and transporting various substances, including hormones, fatty acids, and drugs. Studies indicate that cLA supplementation can increase serum albumin levels in fish. For instance, a study by Hossain *et al.* (2021) demonstrated that cLA-enriched diets resulted in significantly higher albumin concentrations in the serum of Nile tilapia (*Oreochromis niloticus*). The authors suggested that cLA may enhance liver function, promoting greater albumin synthesis and secretion. Increased albumin levels may enhance the fish's ability to maintain osmotic balance and nutrient transport, thereby improving overall health and growth performance.

Globulins play crucial roles in immune responses and transport functions. Research indicates that cLA can also influence globulin levels in fish, although results may vary depending on the species and dietary formulations. For example, a study by Kim *et al.*, (2019) found that dietary cLA increased serum globulin levels in rainbow trout (*Oncorhynchus mykiss*). This increase is believed to be linked to cLA's immunomodulatory effects, potentially enhancing the fish's immune response against pathogens. Elevated globulin levels, specifically immunoglobulins, may indicate an active immune system, suggesting that cLA supplementation could help bolster fish health under stress or disease conditions.

Total serum protein levels, which reflect the sum of albumin and globulin concentrations, are critical indicators of overall health and nutritional status. Research conducted by Nascimento *et al.*, (2020) reported that fish fed cLA-supplemented diets exhibited a significant increase in total serum proteins compared to control groups. The authors proposed that the bioactive properties of cLA may stimulate protein synthesis pathways, thereby enhancing the overall protein status in fish. This enhancement in protein levels can lead to improved growth rates and better feed conversion efficiency, critical factors in aquaculture. cLA has been shown to influence lipid metabolism in fish, potentially leading to increased availability of fatty acids for protein synthesis (Park *et al.*, 2017). This process may favour albumin synthesis in the liver, where fatty acids serve as precursors for various metabolic processes.

The mechanisms underlying the effects of quercetin on serum proteins in fish may involve several pathways. Quercetin exhibits potent antioxidant activity, reducing oxidative stress and inflammation, which can impair protein synthesis and immune function (Nawaz *et al.*, 2019). By mitigating oxidative stress, quercetin may enhance the liver's ability to synthesize albumin and globulin. Additionally, quercetin may activate signalling pathways associated with protein synthesis, such as the mTOR pathway, which is crucial for growth and development in fish (Huang *et al.*, 2020).

# *SUMMARY*

## 6. SUMMARY

Fishes being poikilothermic animals, alterations in the surrounding water temperature have a pronounced effect on their physiology. Temperature, the abiotic master factor, influences feed intake, growth, metabolism and energy expenditure. Though fish live in a wide range of thermal tolerance, they have an optimum range for growth and upper and lower lethal temperatures. At low water temperature, the metabolism and feed intake of the animal are reduced. In order to conserve energy, most of the time fish restrict their movement and remain at the bottom. Low-temperature exposure is crucial in fish farming as it reduces the growing season by about four months. Feeding activity becomes almost negligible at low temperatures, but farming activities remain the same. Farmers suffer due to slow growth and high fish mortality, leading to a reduction in income during the winter period. After a decline in the winter period, farmers are unable to cope with a loss in production and are unable to produce a marketable size fish with good meat quality. Physiological, metabolic and immune disorders affecting the fish during cold are mainly manifested by voluntary fasting and impaired digestive and absorptive capacity induced by cold stress. Hence, it is essential to understand the winter physiology of fish to have a proper intervention for improving feed intake, survival and growth during the low-temperature phase in aquaculture practices.

In the present study, a feeding trial of 8 weeks was conducted to understand the combinatorial effect of dietary conjugated linoleic acid (cLA) on feed intake and growth in pangasius fingerlings reared at low temperature. There were seven isonitrogenous (37%) and isocaloric (412 kcal DE/100 g) practical diets with varying levels of conjugated linoleic acid and quercetin viz., Control (0.0% cLA + 0.0% que<sup>r</sup>), T1 (0.5% cLA + 0.0% que<sup>r</sup>), T2 (1.0% cLA + 0.0% que<sup>r</sup>), T3 (0.0% cLA + 0.5% que<sup>r</sup>), T4 (0.0% cLA + 1.0% que<sup>r</sup>), T5 (0.5% cLA + 0.5% que<sup>r</sup>) and T6 (1.0% cLA + 1.0% que<sup>r</sup>) The experiment was conducted in a low temperature ( $18 \pm 1$  °C) re-circulatory aquaculture system with a flow rate of 1.6 L/min.

With the inclusion of cLA and quercetin in diet feed intake and growth was seen to increase with respect to control fed group. Better FCR was obtained in treatment groups. Weight gain, weight gain percentage, specific growth rate and

thermal growth coefficient were seen to increase significantly in the treatment groups. Major effect of above was seen in the T2 (1.0% cLA) fed group followed by T5 (0.5% cLA + 0.5% quercetin) fed group. Survivability reported through the experimental period is 100%. Digestive enzymes like protease, amylase and lipase activities are higher in the T2 group fed with 1.0% cLA followed by T5 group fed with (0.5% cLA+ 0.5% quercetin).

Supplementation of cLA and quercetin in the diet had increased the metabolic rate, which was observed through the increased key metabolic enzyme of TCA cycle i.e. malate dehydrogenase (MDH). Higher activity was reported in T2 (1.0% cLA) fed group followed by T5 (0.5% cLA + 0.5% quercetin). Similarly, lactate dehydrogenase (LDH) activity showed higher activity in groups fed with 1% cLA followed by T5 (0.5% cLA + 0.5% quercetin). Moreover, the AST and ALT activities in the liver of T2 (1.0% cLA) and T5 (0.5% cLA + 0.5% quercetin) fed groups showed decrease in the AST and ALT value. The lipogenic enzyme glucose-6-phosphate dehydrogenase (G6PDH) activity was also higher in the 1.0% cLA fed group. The activities of both SOD and catalase were lower in T2 (1.0% cLA) followed by T5 (0.5% cLA + 0.5% quercetin) fed groups compared to the other group. Serum total protein was higher in the treatment groups than in the control group. Hence, the major observations in the study are;

- Feed intake in Control was reduced in response to low temperature exposure ( $18 \pm 1$  °C).
- Low temperature exposure reduced growth, nutrient utilization, metabolic enzyme activities and serum total protein in *P. hypophthalmus*.
- Feeding of cLA at 1.0% and combined diet with inclusion level (0.5% cLA + 0.5% quercetin) increased the feed intake.
- Feeding of cLA at 1.0% and combined diet with inclusion level (0.5% cLA + 0.5% quercetin) increased final weight, weight gain %, SGR, TGC compared to the control group.

- The protease, amylase, lipase, MDH, LDH, and G6PDH activities were improved by the dietary supplementation of cLA at 1.0% and combined diet with inclusion level (0.5% cLA + 0.5% quercetin).

The present study indicates that the effect of cLA and quercetin is beneficial to the cultured *Pangasius* in terms of feed intake, growth, survival and improved metabolic activities. This is the first study of its kind where feed intake and growth aspects are taken care during low temperature by using the combination of cLA and quercetin, paving the way for the development of winter feed for aquaculture. Hence, further studies are required to fully understand the role and effects of dietary cLA and quercetin on fish reared at low temperature for longer duration.

#### **Further recommendations**

- For a better understanding, a detailed study of the molecular aspects of the low-temperature exposed striped catfish is required.
- To have a practical utility, it is suggested to go for trials in field conditions.

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# *APPENDICES*

# APPENDICES

## Abbreviation:

ACTH: Adrenocorticotropic hormone

ADG: Average daily gain

ALT: Alanine aminotransferase

ANOVA: Analysis of Variance

AOAC: Association of Official Analytical Chemists

APHA: American Public Health Association

AST: Aspartate aminotransferase

BGC: Bromocresol green

CAT: Catalase

CF: Crude fibre

CIFE: Central Institute of Fisheries Education

Cl: Chloride ion

CL: Crude lipid

CP: Crude protein

CRD: Completely randomized design

CTmax: Critical thermal maximum

CTmin: Critical thermal minimum

CuSO<sub>4</sub>: Copper sulphate

DE: Digestible energy

dl: Deci litre

DM: Dry matter

DNPH: Dinitrophenyl hydrazine

DO: Dissolved oxygen

DORB: De-oiled rice bran

EDTA: Ethylenediaminetetraacetic acid

EE: Ether extract

FAO: Food and Agricultural Organisation

FCR: Feed conversion ratio

FDA: Food and drug administration

FER: Feed efficiency ratio

g: Gram  
GE: Gross energy  
GNOC: Groundnut oil cake  
GOD: Glucose oxidase  
H<sub>2</sub>SO<sub>4</sub>: Sulphuric acid  
HCl: Hydrochloric acid  
ICAR: Indian Council of Agriculture Research  
IMARC: Indian Market Analysis Research and Consulting  
ISI: Intestinal somatic index  
IU: International unit  
K<sub>2</sub>SO<sub>4</sub>: Potassium sulphate  
K<sup>+</sup>: Potassium ion  
kcal: Kilo calorie  
kg: Kilo gram  
L/min: Liter/minute  
LER: Lipid efficiency ratio  
MCH: Mean corpuscular haemoglobin  
MCHC: Mean cell haemoglobin concentration  
MCV: Mean corpuscular volume  
mg: Milligram  
mM: Millimole  
MUFA: Monounsaturated fatty acid  
Na<sup>+</sup>: Sodium ion  
NADPH: Nicotinamide adenine dinucleotide phosphate  
Na-K ATPase: Sodium-potassium adenosine triphosphatase  
NaOH: Sodium hydroxide  
NFE: Nitrogen Free Extract  
NRC: National Research Council  
OD: Optical Density  
PER: Protein Efficiency Ratio  
PG: Propylene glycol  
POD: Peroxidase  
ppm: Parts per million  
ppt: Parts per thousand

PUFA: Polyunsaturated fatty acid  
RAS: Re-circulatory aquaculture system  
ROS: Reactive oxygen species  
rpm: Rotations per minute  
SE: Standard Error  
SGR: Specific growth rate  
SMR: Standard metabolic rate  
SOD: Superoxide dismutase  
SOFIA: State of world fisheries and aquaculture  
TA: Total ash  
TCA: Trichloroacetic acid  
W/V: Weight/ Volume  
WG%: Weight gain percentage  
WG: Weight gain  
%: Percentage  
µg: Microgram  
µL: Microlitre  
µM: Micromole  
°C: Degree Celsius  
Δ9D: Delta-9-desaturase

**ISBN: 978-93-48499-98-1**