OPTIMIZATION OF TOMATO POMACE MEAL IN THE DIET OF *LABEO ROHITA* (HAMILTON,1822) THROUGH FERMENTATION AND EXOGENOUS ENZYME SUPPLEMENTATION

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of the requirements

for the degree of

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

by

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DEDICATED TO MY BELOVED PARENTS

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Dated: 31st August, 2023

CERTIFICATE

Certified that the dissertation entitled **"OPTIMIZATION OF TOMATO POMACE MEAL IN THE DIET OF *LABEO ROHITA* (HAMILTON,1822) THROUGH FERMENTATION AND EXOGENEOUS ENZYME SUPPLEMENTATION"** is a bonafide record of independent research work carried out by **Ms. Shalini Sundi** during the period of study from November, 2022 to August, 2023 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 award of any degree, diploma, associateship, fellowship or any other similar title.

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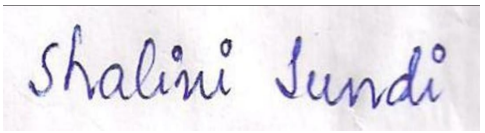

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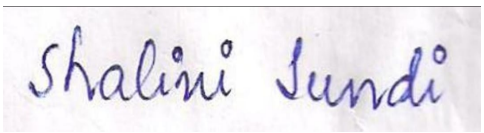

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सारांश

आहार टमाटर पोमेस भोजन (TPM), एक्सोजेनस एंजाइमों(EE) के विकास प्रदर्शन, फिजियो-चयापचय स्थिति और *Labeo rohita* अंगुलिक की प्रतिरक्षात्मक प्रतिक्रियाओं पर प्रभाव का अध्ययन करने के लिए 60-दिवसीय प्रयोग किया गया था। तीन सौ पंद्रह अंगुलिक (औसत शरीर का वजन 12.08 ± 0.5 ग्राम) को यादृच्छिक रूप से तीन प्रतियों में सात समूहों में वितरित किया गया था। इस प्रकार, 21 टैंकों में से प्रत्येक में 15 मछलियों का स्टॉक किया गया था। सात आइसो-नाइट्रोजनस (30% CP), आइसोलिपिडिक (6%) और आइसोकैलोरिक (360 किलो कैलोरी डीई / 100 ग्राम) प्रयोगात्मक आहार जैसे C, कंट्रोल (30% DORB और 0% TPM के साथ आहार), TPM 15 (50% DORB के प्रतिस्थापन में 15% TPM के साथ आहार), TPM 30 (100% DORB के प्रतिस्थापन में 30% TPM के साथ आहार), TPM 15 + EE (TPM 15 आहार के साथ पूरक 100% DORB के साथ आहार)। TPM 30+ EE (TPM 30 आहार 1: 1 पर 0.1% सेल्यूलोज-जाइलनेस मिश्रण के साथ पूरक), FTPM15 (50% DORB के प्रतिस्थापन में 15% FTPM के साथ आहार), FTPM 30 (100% DORB के प्रतिस्थापन में 30% FTPM के साथ आहार) तैयार किया गया और 60 दिनों के लिए दिन में दो बार संबंधित समूहों को खिलाया गया। WG, WG%, SGR और PER ऑफ कंट्रोल और TPM फेड समूह समान थे, लेकिन FTPM 30 समूह ने उच्चतम मूल्य दिखाए। FCR ने वृद्धि की विपरीत प्रवृत्ति दिखाई। सभी समूहों की मछलियों 86% से अधिक जीवित राहे । C,TPM 15 और ए FTPM 30 की मछलियों ने समान ISI मूल्य दिखाए, लेकिन अन्य समूहों ने काफी कम मूल्य दिखाए। FTPM 30 समूह ने नियंत्रण की तुलना में काफी अधिक प्रोटीज गतिविधि दिखाई, लेकिन अन्य समूहों ने नियंत्रण के लिए समान गतिविधि दिखाई। FTPM 30 और TPM 30 समूहों ने क्रमशः उच्चतम और सबसे कम एमाइलेज गतिविधि दिखाई। FTPM 15 और FTPM 30 समूहों में उच्चतम यकृत और मांसपेशी एए गतिविधि पाई गई । FTPM 15 और FTPM 30 समूहों ने नियंत्रण की तुलना में काफी अधिक मांसपेशी ALT गतिविधि दिखाई। TPM 15 + EE और टीपीएम 30 + EE समूहों ने नियंत्रण की तुलना में काफी कम मांसपेशी एलडीएच गतिविधि दिखाई। हेपेटिक और मांसपेशी LDH गतिविधि सभी समूहों में समान थी। FTPM 15 और FTPM 30 समूहों में नियंत्रण की तुलना में यकृत MDH गतिविधि काफी कम थी। हेपेटिक SOD गतिविधि सी, टीपीएम 15 और टीपीएम 30 समूहों में समान थी, लेकिन अन्य समूहों में नियंत्रण की तुलना में कम गतिविधि थी। टीपीएम 15 + EE और TPM 30 + EE समूहों ने नियंत्रण की तुलना में सीरम ग्लूकोज स्तर को काफी कम दिखाया। सीरम ट्राइग्लिसराइड्स, कोलेस्ट्रॉल, कुल प्रोटीन, एल्बुमिन, ग्लोब्युलिन और एल्बुमिन से ग्लोब्युलिन अनुपात के मूल्य उपचार के बीच काफी भिन्न नहीं थे। अंत में, TPM को मछली के विकास प्रदर्शन और स्वास्थ्य की स्थिति से समझौता किए बिना DORB के 100% प्रतिस्थापन के साथ 30% तक *L. rohita* फिंगरलिंग के आहार में शामिल किया जा सकता है। हालांकि, 30% आहार FTPM सबसे अच्छा प्रभाव देता है, लेकिन आहार कार्बोहाइड्रेज अतिरिक्त वृद्धि नहीं देता है।

ABSTRACT

A 60-day experiment was carried out to study the effect of dietary tomato pomace meal (TPM), exogeneous enzymes supplemented TPM and fermented TPM (FTPM) on growth performance, physio-metabolic status and immunological responses of *Labeo rohita* fingerlings. Three hundred and fifteen fingerlings (avg. body wt. 12.08 ± 0.5 g) were randomly distributed into seven groups in triplicate. Thus, 15 fish were stocked in each of 21 tanks. Seven isonitrogenous (30% CP), isolipidic (6%) and isocaloric (360 kcal DE/100 g) experimental diets such as C (Diet with 30% DORB and 0% TPM), TPM_{15} (Diet with 15% TPM in replacement of 50% DORB), TPM_{30} (Diet with 30% TPM in replacement of 100% DORB), TPM_{15}+EE (TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1), TPM_{30}+EE (TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1), $FTPM_{15}$ (Diet with 15% FTPM in replacement of 50% DORB}, $FTPM_{30}$ (Diet with 30% FTPM in replacement of 100% DORB) were prepared and fed to respective groups at satiation level twice daily for 60 days. The WG, WG%, SGR and PER of control and TPM fed groups were similar, but $FTPM_{30}$ group showed the highest values. The FCR showed the opposite trend of growth. The fish of all groups showed more than 86% survival. The fish of C, TPM_{15} and $FTPM_{30}$ showed similar ISI values, but the other groups showed significantly lower values. The $FTPM_{30}$ group showed significantly higher protease activity than control, but other groups showed similar activity to control. $FTPM_{30}$ and TPM_{30} groups showed the highest and the lowest amylase activity, respectively. The highest liver and muscle AST activity was found in $FTPM_{15}$ and $FTPM_{30}$ groups. $FTPM_{15}$ & $FTPM_{30}$ groups showed significantly higher muscle ALT activity than control. TPM_{15}+EE & TPM_{30}+EE groups showed significantly lower muscle LDH activity than control. Hepatic and muscle LDH activity was similar in all groups. The $FTPM_{15}$ and $FPTM_{30}$ groups had significantly lower liver MDH activity than control. The liver SOD activity was similar in C, TPM_{15} & TPM_{30} groups, but other groups had lower activity than control. TPM_{15}+EE & TPM_{30}+EE groups showed significantly lower serum glucose level than control. The values of serum triglycerides, cholesterol, total protein, albumins, globulin and albumin to globulin ratio did not vary significantly among treatments. In conclusion, TPM can be incorporated in the diet of *L.rohita* fingerlings up to 30% with 100% replacement of DORB without compromising the growth performance and health status of fish. However, 30% dietary FTPM gives the best effects, but dietary carbohydrase does not give extra growth.

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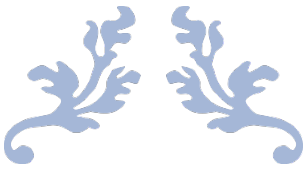


INTRODUCTION

1. INTRODUCTION

Aquaculture is considered as one of the major food producing sectors to provide nutritional security to the ever-increasing human population across the world (FAO, 2018). Aquaculture in India is slowly shifting from non-fed or extensive culture system to fed semi-intensive or intensive culture system, where feeding with artificial supplementary or complete feed is one of the most important factors for faster growth and higher yield of fish from these culture systems (Jose *et al.*, 2006; Maity and Patra, 2008). However, judicial feed management plays a vital role for profitable aquaculture as more than 50% of input cost of aquaculture is shared by feed (Tiamiyu and Solomon, 2012). Carp culture contributes more than 85 percent of total freshwater fish production in India (Laxmappa, 2015). Among the carps, rohu (*Labeo rohita*) has high market demand due to its fast growth, delicacy, and consumer preference and it shares about 35-40% of total Indian major carp (IMC) production in India (FAO, 2012). Indian carp farmers are very much reluctant to use commercial feed may be due to its high cost, thus, more than 90% carp farmers use farm-made feed prepared by using locally available plant-based ingredients. De-oiled rice bran (DORB) is the most commonly used ingredient for both farm-made and commercial carp feed preparation as it is one of the cheapest agricultural by-products available throughout the year. However, the insufficient production of rice bran in recent time could hardly meet the growing need of the present animal feed industry (Jayant *et al.,* 2020). Moreover, there is a huge competition among human food and different animal feed sectors for this raw material leading to increase in price and ultimately that leads to increased cost of aquafeed. Hence, there is a requirement of suitable DORB alternatives from cheaper non-conventional sources to make the aquaculture operation profitable and sustainable.

Recently, there were several attempts to replace DORB partially or completely in aquafeed especially by using different leaf meals alone (Antia *et al*, 2006; Osman, 2007; Adewolu, 2008; Abonyi *et al*., 2012; Vhanalakar and Muley, 2014; Meshram *et al.,* 2018; Sahoo *et al.,* 2018; Ahmad *et al*., 2019; Maiti *et al*., 2019; Nottanalan *et al.,* 2021) or in combination (Mondal *et al*., 2022). However, presence of high crude fiber (CF) and anti-nutritional factors (ANFs) could limit their optimum utilization in animal feed (Antia *et al.,* 2006). However, dietary supplementation of exogenous fiber digesting enzyme (Maiti *et al.,* 2019; Nottanalan *et al.,* 2021) and solid

state fermentation (Meshram *et al*., 2018) could improve the utilization of leaf meals in aquafeed as DORB alternatives.

As per FAO (2020), more than 20% of the fruits and vegetables by products such as peels, seeds, roots, leaf, pomace etc. are considered as wastes, which are either incinerated or just dumped in the landfill areas with the consequences of environmental pollution (Bigdeloo *et al.*, 2021). However, these wastes are rich in nutrients and bioactive compounds (Nour *et al*., 2018). Thus, instead of dumping, these wastes can be recycled for improving the quality of animal feed (Ajayi *et al.*, 2006). According to the National Horticulture Database published by the National Horticulture Board, India produced 99.07 million metric tons (MMT) of fruit in 2019-20. Globally, India stands second in total fruits and vegetables production (FA0, 2019).

Tomato (*Solanum lycopersicum L.),* belongs to 'Solanaceae' family is one of the most cultivated vegetables worldwide. Globally, about 79.52 million tons of tomato are processed in the industry for production of tomato purees (paste), sauce/ketchup etc. (EMR, 2022) with the large quantities (10-30%) of by-products as tomato pomace (TP) consisting 78% peel and pulp and 22% seeds (Sogi and Bawa, 2002; Rahmatnejad *et al.*, 2009). The world's annual tomato waste production reaches up to 11 million ton per year including 4 million tons of tomato pomace (FAO, 2016).Tomato pomace is reported to contain crude protein, lipid, total carbohydrate and total ash at the range of 16.27-19.65, 5.85-10.75, 54.79-59.03 and 3.47-4.05%, respectively (Alvarado *et al*., 1999). Besides it is rich in biologically active compounds such as phenolic compounds (gallic acid, trans-cinnamic acid and quercetin) and carotenoids (ß-carotene and lycopene) which have strong antioxidant activity as well as other various metabolic and physiological roles in the body (Schieber *et al.*, 2000; Sogi *et al.*, 2002; Knoblich *et al.*, 2005; Calvo *et al.*, 2008; Kumar *et al.*, 2018; Szabo *et al.*, 2019). Moreover, tomato pomace has been found to be devoid of anti-nutrient components (Sogi *et al.*, 2002; Del Valle *et al.*, 2006). However, presence of high Crude Fiber (CF) content may limit the inclusion level of tomato pomace meal (TPM) in the diet of animal feed. Although, the optimal dietary fiber level varies among fish species (Hansen and Storebakken, 2007; Bou *et al*., 2014; Jha and Berrocoso, 2015; Bonvini *et al.,* 2018) excessive dietary fiber levels are detrimental to fish growth and health (Altan and Korkut, 2011; NRC, 2011). Moreover, Knoblich *et al*. (2005)

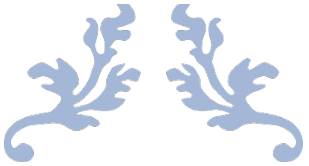
demonstrated the poor digestibility and low metabolizable energy of higher level of TPM containing poultry diet might be due to TPM derived high CF content. However, TPM could be incorporated in diet of common carp (*Cyprinus carpio*) up to 30% without deteriorating the growth performance, nutrient digestibility and body composition of fish (Amirkolaie *et al*., 2015). Studies also reported that fermented tomato pomace meals contained higher crude protein (CP) and total ash (TA) and lower crude fiber than their non-fermented counterparts (Assi and King, 2008; Azza *et al*., 2013; Roja *et al*., 2017; Yasar and Tosun, 2019). Thus, it is expected that exogenous fiber digesting enzyme supplemented or fermented TPM may be the good alternative of DORB in the fish feed (Onyimba *et al.,* 2015; Meshram *et al*., 2018;). Overall, the information on the use of TPM as DORB alternative in the diet of Indian major carp (IMC) is lacking.

Keeping the above mentioned information in mind, the present study was planned to find out the effect of dietary fermented and exogeneous enzyme supplemented tomato pomace meal on the growth, feed and nutrient utilization and physio-metabolic and immunological responses of *Labeo rohita* fingerlings with the following objectives.

Objectives:

1. To study the effect of fermented and exogeneous enzymes supplemented tomato pomace meal on growth and nutrient utilization of *Labeo rohita* fingerlings

2. To evaluate the physio-metabolic and immunological responses of *Labeo rohita* fingerlings fed with fermented and exogenous enzyme supplemented tomato pomace meal



REVIEW OF LITERATURE

2. Review of Literature

2.1. Status of Global and Indian Aquaculture

The fisheries and aquaculture sectors have been increasingly recognized for their essential contribution to global food security and nutrition in the twenty-first century (FAO, 2022). The total fish production in 2020 was 178 million metric tons, of which 88 million were from culture fisheries (FAO, 2022). The aquatic food consumption had increased at an average annual rate of 3 percent in the year 2020 (FAO, 2022). In 2020, Asian countries were the main producers, accounting for 70 percent of the total fisheries and aquaculture production (FAO, 2022). India occupies the third position in world aquaculture production. The current fish production in India (2020-21) is 16.24 MMT out of which 12.12 MMT is from inland fish production (Handbook of Fisheries statistics, 2022). The annual growth rate of inland fish production is 7.76 %, whereas the marine sector is increasing at a rate of 18.7 % (Handbook of Fisheries Statistics, GoI 2022). Andhra Pradesh leads the table with 6.3 lakh tonne inland fish production, followed by West Bengal, Karnataka, Odisha and Gujarat.

2.2. Status of Carp Farming in India

The production of major carps was 5.95 mmt in 2019-20 (Handbook of Fisheries Statistics, GoI 2022). Andhra Pradesh is the top producer among all and contributes 1.99 MMT of major carps followed by West Bengal, Uttar Pradesh, Chhattisgarh, and Bihar. IMC contributes 87% of the total freshwater aquaculture production of the country (Jayashankar, 2018). Rohu (*Labeo rohita)* is a freshwater fish belonging to the carp family of *Cyprinidae*. It possesses remarkable growth potential, wide consumer demand along with year-round culture potential (FAO, 2016). It is a column feeder and feeds mainly on plant materials including decayed vegetation (Khan and Jhingran, 1975). It is found primarily in Indian subcontinent and other South Asian countries (Menon, 1955).

2.3. Global Status of Aqua Feed

The global aquafeed market capacity was 55.71 billion $ in 2020 and is expected to catch the mark of 80 billion by the end of 2027 showing a growth rate of 5.3% (Mordor Intelligence Report, 2019). The rising popularity of aquafeed incorporated with beneficial ingredients holds steady growth opportunities for the market. Asia Pacific is a prominent market for aquafeed at present, and is expected to hold a significant proportion of the global market share during the forecast period. The Asia Pacific market has developed rapidly over the last decade, with China and India occupying more than half of the regional sales. The demand for fish feed in China and India is expected to increase tremendous in the upcoming years (Fortune Business Insights Report, 2022. The Aquaculture sector experienced a total global feed production growth of 2.7%. The top Five Aquaculture feed countries are China, Vietnam, India, Norway, and Indonesia (Alltech Agri-Food Outlook Report, 2023).

2.4. Indian Aquafeed Status

Indian aquaculture feed market was worth 1.4 billion US $ in 2020 and is expected to grow at the rate of 8% in the period of 2022-27 (EPR Report, 2020). Indian feed mills have the capacity to produce 2.88 MMT, with Andhra Pradesh being the top producer and consumer (Entrepreneur India Magazine, 2023). The aquafeed industry in India is segmented into different parts based on Ingredient (soybean, fish meal, corn, fish oil, etc.), product form (pellets, extruded, powdered, liquid), species (carp, shrimp, tilapia, catfish, etc.). The thriving aquaculture sector and the increases in domestic consumption as well as the export of fishes are the key industry trends propelling the markets growth. Aquafeed is the vital component of fish farming and accounts for 40-60% of total operational cost. The cost of finished feed is determined by the cost of conventional feed ingredient used in feed formulation. But the use of conventional feed ingredients has become a limiting factor in expansion of aquaculture because of decline in supply of these ingredients (Wu *et al.,* 1999). Hence, the use of locally available unconventional ingredients with the help of several methods (such as water soaking, moist heat treatment, and fermentation) to neutralize the anti-nutritional factors can act as a better measure (Meshram *et al*., 2018; Ahmad *et al.*, 2019).

2.5. Global Status of Waste Generation

Globally, 7-9 billion tonnes of waste are generated every year (Wilson and Velis, 2015). Among that agricultural waste accounts for approximately 2 billion tons. Losses from the fruits and vegetables sector are more than 20% (FAO, 2020). Agro-industrial by-products represent one of the most important and promising energy and protein sources (Salajegheh *et al.*, 2012). During the industrial processing of tomatoes, large quantities of wastes are generated consisting of peels, seeds, fibrous parts and pulp residues that account for 7.0–7.5% of raw materials (Nour *et al.*, 2018). Approximately, 20% of the production of fruits and vegetables in India are going waste every year (Rudra *et al.*, 2015). So as the production increased in the country, it also increased the percentage of waste produced from them. Most of these wastes are left unutilized or untreated, which cause adverse effect on environment but the composition of these waste contains large number of organic compounds. The management of tomato wastes represents a worldwide problem for both the environmental and the economic aspects and the recycling or re-usage of these by-products can reduce processing costs (Poli *et al.*, 2011). Although these wastes have no commercial value, they are a rich source of nutrients and highly biologically active compounds. This massive amount of waste that is generated either dumped in the landfill (37%) or open dumped (33%), or just incinerated (11%). Only a small fraction (19%) is reused after recycling (Bigdeloo *et al.*, 2021)

2.6. Status of Waste Generation in India

According to the 'Swachhata Sandesh Newsletter (January 2020)' by Ministry of Housing and Urban Affairs (MoHUA) India generated 147,613 metric tonnes of solid waste every day. India produces around 620 MMT of agricultural wastes annually (Patel *et al.*, 2020). India encounters a waste of close to 25 % of total vegetables and fruits produced. Another report says that almost 12 million tonnes of waste from fruits and 21 million tonnes from vegetables every year in India (Kumar *et al.,* 2020). Like the rest of the world, these wastes are also not used beneficially by humankind; instead, they are just dumped or burned their way out. To highlight the significance of nutritious fruits and vegetables, and create awareness among the public to attain sustainable development goals, the UN general assembly designated the year

2021 as the international year of fruits and vegetables (IYFV). Among the vegetables, tomato contribute a major chunk of waste as it is highly perishable.

Large amounts of by-products comprising various reusable and high value components with economic potential are generated during the industrial processing of fruits and vegetables. These by-products are high in bioactive chemicals such as phenolic compounds, flavonoids, carotenoids, and anthocyanins, which can have anticancer, antiviral, antitumor, antibacterial, and antioxidant effects (Acar *et al.,*2015; Baba *et al.,* 2016; Kesbic *et al.*, 2019).

2.7. Status of Tomato Processing Plants

Global consumption of processed tomatoes stood at 41.52 million tonnes in 2020 and is expected to grow and reach 51.93 million tonnes in 2026 (EMR report, 2020). Asia pacific is the largest producer of tomatoes, globally, accounting for nearly half the global tomato output. China and India are the leading producer in the world. It is well known that agro-industrial by-products from fruits and vegetables are rich in dietary fibers, some of which contain appreciable amounts of colorants, antioxidant compounds or other substances with positive health effects. These wastes are generated in large amounts throughout the year, and are the most abundant renewable resources on earth. They are mainly composed of sugars, fibers, proteins, and minerals, which are compounds of industrial interest. Due to the availability and composition of rich compounds that could be used in other processes, there is a great interest on the reuse of these wastes, both from economic and environmental viewpoints (Ayo *et al.*, 2016).

The major issue in reusing these wastes are they are heterogeneous in nature containing bulk of unsuitable food and fodder as they are too fibrous to be digested by man and many animals respectively (Ha *et al.*, 2014). These substances which contains 3-70% carbohydrates of various forms and proportions can be treated chemically, physically and biologically to improve their utilizations for man and animals (Fillaudeau *et al.*, 2006).

2.8. Strategies to Reduce Anti-Nutritional Factors and Fibers

Several methods are documented to neutralize the anti-nutritional factors from leaf meals such as moist heat treatments, water soaking, and fermentation etc (Campbell and Bedford,1992; Meshram *et al*., 2018; Ahmad *et al.,* 2019). Solid state fermentation (SSF) has gained renewed attention from industry because it has become a more attractive alternative to liquid fermentation for many productions. Thus, SSF was found to produce a more stable product, with less energy requirements, in smaller fermenters and smaller volumes of polluting effluents (Perez-Guerra *et al.*, 2003). Improving value of tomato waste could be possible through the development of environment-friendly technologies that convert it into new food ingredients or alternative products (Nour *et al.*, 2018).

2.9. Tomato by-products and Nutritional Composition

Tomato (*Solanum lycopersium*) is one of the vegetable crops most widely produced in the world, being either consumed directly (fresh tomato) or used to produce tomato products (processing tomato). The industrial processing of tomatoes to obtain tomato products such as paste, juice, puree, sauce, soup, ketchup, whole dried tomatoes and tomato powder is an industrial activity with growing importance on a global scale. During the processing of tomatoes, the main residue generated is the tomato pomace, a mix of tomato skin and seeds and a small fraction of the pulp (Silva *et al.,* 2019).

Tomato pomace (TP) constitutes about 5% of the processed tomato (Palomo *et al.*, 2019). The wet tomato pomace contains 33% seed, 27% skin and 40% pulp, while the dried pomace contains 44% seed and 56% pulp plus skin (Sogi and Bawa, 1998). Tomato pomace contains fiber, sugars, proteins, lipids, vitamins, and minerals (Albanese *et al.*, 2014). It is also a good source of flavonoids, carotenoids, and nucleosides (Palomo *et al.,* 2019). Tomato pomace contains antioxidant compounds including lycopene, folate, vitamin C, β-carotene, αtocopherol, phenolics, and flavonoids (Sahin *et a.,* 2008; Selim *et al.*, 2013).Tomato waste is rich in bioactive components such as ß-carotene and lycopene, which have been shown to exhibit strong antioxidant activity in many studies (Szabo *et al.*, 2019).

Tomato seeds are considered a potential natural source of antioxidants because of their rich phytochemical profile. They are a reservoir of bioactive phenolic compounds (PCs) and carotenoids that play a crucial role in managing various metabolic and physiological processes in the body. Particularly, PCs (flavonoids, phenolic acids), carotenoids (β-carotene, lycopene) and nucleosides (guanidine, inosine, adenosine) are present in tomato seeds (Meyer *et al.*, 2020).

During the processing of tomato products, up to 30% of their original weight are turned into waste, still the left part is considered to contain some nutritive values (Edenharder *et al.,* 1994). Large amounts of by-products comprising various reusable and high value components with economic potential are generated during the industrial processing of fruits and vegetables. These by-products are high in bioactive chemicals such as phenolic compounds, flavonoids, carotenoids and anthocyanins which can have anticancer, antiviral, antitumour and antioxidant effects (Acar *et al.,* 2015; Baba et al., 2016; Acar et al., 2018; Kesbic *et al.,* 2019).

Table 1. Proximate composition (% dry matter) of Dried tomato pomace

Composition	Dried tomato pomace (%)
Dry matter	91.90
Crude protein	17.32
Ether extract	8.65
Crude fiber	28.2
Crude Ash	4.14
Starch	2.50
Sugar	3.16
ME (Kcal/kg)	1681.00

Source-(Ayhan and Aktan *et al*., 2004)

Although studies on the use of tomato-derived products and extracts in the nutrition of livestock for different purposes, limited knowledge has been reported the effects of wastes of tomato-using industries on fish. Therefore, this study focuses on investigating the potential effects of tomato pomace on growth, in fish.

2.10. Bioactive compound in Tomato Pomace Meal

A huge amount of waste is produced during the processing of tomatoes; therefore, its utilization becomes difficult and poses a significant threat to the environment. Although some of the waste is utilized as animal feed, there is a need for further exploitation of tomato waste, including both peels and seeds

Tomato waste is rich in bioactive components such as ß-carotene and lycopene, which have been shown to exhibit strong antioxidant activity in many studies (Szabo *et al.*, 2019). Tomato seeds are considered a potential natural source of antioxidants because of their rich phytochemical profile.

Similarly, tomato skin and pulp also contain good number of bioactive components including flavonoids, phenolic acids, lycopene, β-carotene etc. Among these components, PCs exhibit several bioactivities, such as anticancer, antimicrobial, antimutagenic, anti-inflammatory, anti-neurodegeneration, antiplatelet, and cardioprotective properties (Kumar *et al.,* 2021). Several agro-food by-products contain significant amount of proteins, carbohydrates, lipids and other bioactive compounds including phenolics, dietary fibers, alkaloids and pigments (Fernandez *et al.*, 2018; Reidah *et al.,* 2017).

Recovery of valuable compounds from the masses of by-products can play a role in the global food sustainability, environment and economy (Kumar *et al.*, 2018). Recently, protein recovery techniques from plant-origin by-products is trending among the scientists working in various fields (Gorgus *et al.,* 2019; Zardo *et al.,* 2019) especially in the developed and developing countries. Plant-based proteins have advantages over animal-based proteins as they are cholesterol-free, have lower saturated fatty acids and are abundant (Richter *et al.*, 2015).

2.11. Effect of Tomato pomace Meal on Growth of fish and Other Animals

Amirkolaie *et al.* (2015) evaluated growth performance, nutrients digestibility and body composition in common carp (*Cyprinus carpio*) fed diet containing different levels of tomato pomace (10%, 20% and 30%). Results showed that 10% inclusion of tomato pomace significantly increased the final weight and specific growth rate of common carp compared to the control diet. Feed conversion ratio and weight gain were also improved after 10% tomato pomace diet was given to the experimental fish. Dietary addition of 10% tomato pomace also improved apparent digestibility coefficients (ADCs) of dry matter and fat. However, protein ADC was decreased with increasing of tomato pomace inclusion. Hence, conclude that common carp can utilize the tomato pomace up to 30% with no negative impact on growth.

Another study on the effect of supplementation of tomato puree at the rate of 0.5 %-1% showed that feed intake, weight gain, and feed conversion were improved at 0.5% level. Whereas, at 1 % level, the total antioxidant capacity increased and malondialdehyde (MDA) concentration reduced in heat stressed broilers (Selim *et al.,* 2013)

The effect of supplementation ratio of the tomato pomace to-broiler diet reflected the increases in the live body weight and feed consumption in broilers fed a tomato pomace supplemented diet for periods up to 21 days of continuous feeding (Persia *et al.*, 2003).

Mohammed *et al.* (2021) reported that two broilers breeds fed the diets with 0, 4, or 6% sun-dried tomato pomace (SDTP) for 42 consecutive days showed that inclusion up to 6% SDTP in the diet of IR or Cobb chickens had no negative impact on growth performance.

2.12. Effect of Tomato Pomace Meal on Physio-metabolic Parameters

Amirkolaie *et al.* (2015) studied the effect of feeding tomato pomace meal containing diet in physio-metabolic changes of common carp. The result indicated that blood cholesterol level of the carp was elevated by feeding of dietary tomato pomace. Apparently, fiber content of tomato pomace might not have affected the absorption of intestinal cholesterol reflecting in the elevated blood cholesterol. In addition, a larger fat ADC in tomato pomace diets associated with greater values of blood cholesterol may support the idea that tomato fiber does not block cholesterol absorption in the fish intestine.

Kesbic *et al.* (2022) examined different growth performances and blood parameters of common carp (*Cyprinus carpio*) fed with tomato paste by-product extracts (TPEs). Five diets with different concentrations of TPEs, 0% , 0.5% , 1% , 2%, and 5% were supplied to 300 common carp TPE considerably increased the erythrocyte count (RBC), hemoglobin content (Hb), and hematocrit (Hct). The blood biochemical findings indicate that using 1% or more extracts considerably reduced the serum glucose, cholesterol, and triglyceride ratios while significantly increasing the total protein, albumin, and globulin ratios in common carp. Based on the findings of the study, it was concluded that the 2% extract generated from tomato paste by-products in common carp diets could be utilized as a growth-promoting product without any negative effects on blood parameters linked to feeding in carp.

Salajegheh *et al.* (2012) studied the effects of dietary inclusion of dried tomato pomace (DTP) on growth performance, egg quality and serum metabolites in laying hens. Birds were fed either a basal diet or the basal diet supplemented with 150, 170 or 190 g/kg of DTP. As dietary DTP increased from 0 to 19%, Yolk colour score significantly increased from 7.25 to 9.67 and 7.25 to 9.83 in first and second periods, respectively. Total serum protein, cholesterol, LDL, HDL, albumin, glucose and triglyceride levels were not significantly affected by DTP addition. In summary, DTP can be used as an alternative feedstuff in laying hen diets at inclusion levels up to 190 g/kg without any negative impact on performance and egg quality traits.

El- Medany *et al.* (2008) studied the effects of using dried tomato pomace (DTP) at different levels on growth performance, digestibility of nutrients, blood parameters, carcass traits. fed diets containing 15, 30 and 45% DTP, respectively in growing rabbit. The results showed that there were no significant effects of dietary DTP levels on total plasma proteins, albumin, globulin, A/G ratio and urea-N concentration compared with control group. Total plasma cholesterol was significantly reduced when rabbit fed with 30.0% of DTP in the diet. On the other hand, rabbits fed diet containing 45% DTP recorded higher value for total plasma lipids compared with 0, 15 and 30% DTP groups. Liver enzyme activities were significantly reduced in groups fed either 15 or 30% DTP than those fed control and 45%DTP diets.

Rahmatnejad *et al.* (2009) studied the effect of dried tomato pomace in poultry diet and at an inclusion rate of 24% in broiler chicken diet increased total serum protein. The dietary levels of 16 and 24% caused significant increase in the mean values of high-density lipoproteins (HDL) and a decrease in serum cholesterol and LDL. Tomato pomace can considered as promising alternative protein and energy sources employed for monogastric animal feed industry (Mansoori *et al.*, 2008; Peiretti *et al.*, 2013). This ingredient has a potential to be included as an aquafeed ingredient in fish diets (Nengas *et al.*, 1995; Hotfman *et al.*, 1997).

Hence, the present study was planned to find out the effect of dietary fermented and exogeneous enzyme supplemented tomato pomace meal on the growth, feed and nutrient utilization and physio-metabolic and immunological responses of *Labeo rohita* fingerlings.

MATERIAL AND METHODS

3. MATERIAL AND METHODS

An experiments was conducted to evaluate the nutritional potential of raw, fermented and fiber digesting enzyme supplemented tomato pomace meal (TPM) in the diet of *Labeo rohita* fingerlings.

3.1. Collection of Tomato Pomace and Preparation of TPM

Tomato pomace (TP) was collected from Malas Agro Processing Private limited, Nashik, Maharashtra, India and fully dried in a tray drier at 60 ^{0}C for 2 days and then finely ground and sieved to get TPM.

3.2. Preparation of Inoculum for SSF

The pure strain of fungi (*Aspergillius niger,* MTCC 281) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The culture was maintained at 4 ^{0}C on potato dextrose agar (PDA, Himedia). Spores from 7 day-old cultures grown at room temperature (28 ^{0}C) were used as inoculum for SSF of TPM.

3.3. Solid State Fermentation (SSF) of TPM

SSF of TPM was conducted in a 250 ml conical flasks. Dry TPM (50 g) (moisture <10 %) was added to 40 ml of water to achieve the final moisture content of 50% in the mixture, which was then thoroughly mixed using a sterile glass rod, autoclaved for 15 min at 121 ^{0}C and allowed to cool at room temperature. After cooling, the mixture was inoculated with 0.5 ml of *Aspergillus niger* spore suspension (3 x 10^8 CFU/ml) prepared by mixing of scrapped 7-days old slant culture grown on potato dextrose agar (PDA) with 0.1% (v/v) Tween 80. It was then mixed thoroughly under sterile conditions and incubated for 7 days at room temperature followed by drying and grinding to obtain fermented TPM (FTPM).

Fig.1. Tomato Pomace

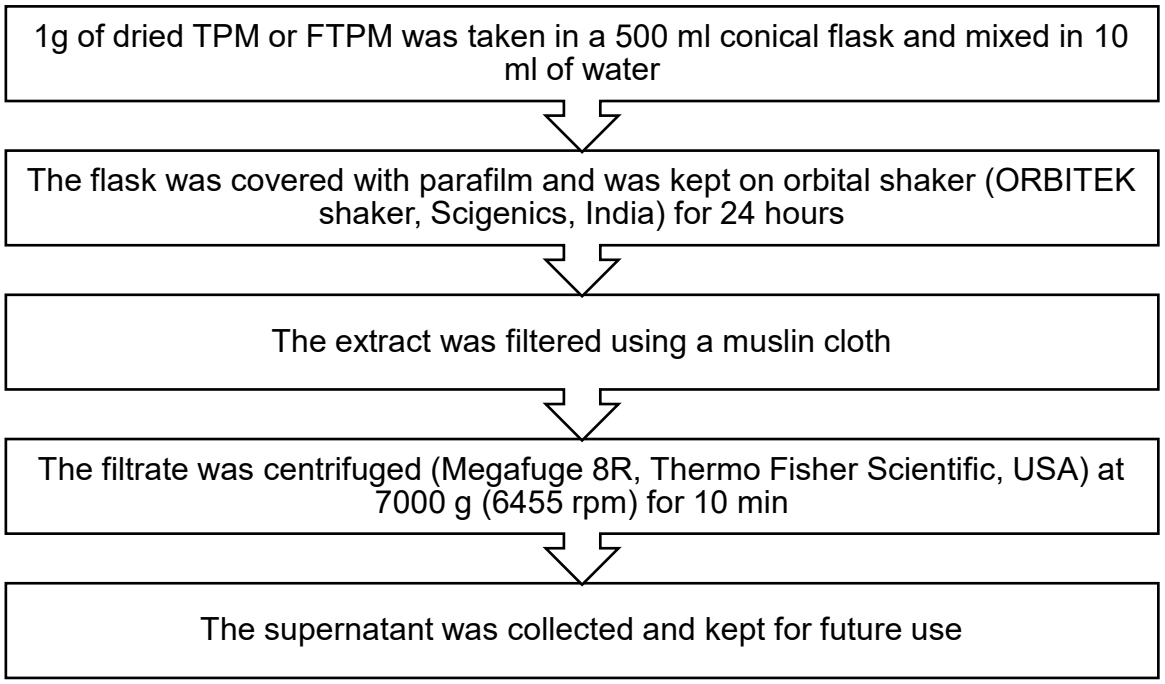
Fig. 2. Ground Tomato Pomace Meal

Fig.3. Fermented Tomato Pomace Meal

3.4. Qualitative Analysis of Bioactive Compound in TPM and FTPM

3.4.1. Preparation of TPM and FTPM Extracts

The aqueous extracts of TPM or FTPM were prepared according to Fawole *et al*. (2020). The detailed extraction process was as follows:



3.4.2. Estimation of Flavonoids

2 ml of TPM or FTPM extract was mixed with 2 ml of 2% NaOH solution. An intense yellow colour was formed that further turned colourless after addition of few drops of diluted acid to indicate the presence of flavonoids (Deyab *et al*., 2016).

3.4.3. Estimation of Tannins

2 ml of TPM or FTPM extract was taken and three drops of $FeCl_3$ diluted solution was added. There was appearance of a blue or greenish-black colour, which clearly indicated the presence of tannins (Evans, 2002).

3.4.4. Estimation of Phenols

1 mL of TPM or FTPM extract was added to 2 mL of distilled water followed by addition of few drops of 10% ferric chloride. The appearance of blue / green colour indicated the presence of phenols (Gunwantrao *et al*., 2016).

3.4.5. Estimation of saponins

The presence of saponins was determined by Frothing test. The TPM or FTPM extract (1 mL) was vigorously shaken with distilled water (2 mL) and was kept

to stand for 10 min. The appearance of stable froth in the solution indicated the presence of saponins (Parekh and Chanda, 2007).

3.4.6. Estimation of coumarins

For coumarins estimation, 1 mL of 10 % NaOH was added to 1 mL of TPM or FTPM extract. Appearance of yellow colour indicated the presence of coumarins (Deyab *et al.,* 2016).

3.5. Site of the Experiment

The experiment was conducted for 60 days from 26th May to 26th July in the wet lab of Freshwater Fish Farm (FWFF), Balabhadrapuram, Kakinada Centre of ICAR-CIFE, Andhra Pradesh. The various Laboratory analysis were done at the laboratory of Fish Nutrition, Biochemistry and Physiology Division, ICAR- Central Institute of Fisheries Education (CIFE), Mumbai.

3.6. Chemicals and Glasswares

The glasswares 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.

3.7. Procurement and Acclimatization of Experimental Animals

Animals used for the experimental purpose were fingerlings of Rohu, *Labeo rohita*. The fish were procured from Venkateswara Fishing Nursery, East Godavari, Balabhadrapuram, Andhra Pradesh and transported to the wet lab in oxygenated water filled HDPE bags and carefully released to rectangular cemented tank (previously cleaned with $KMnO_4$ solution at the concentration of 4mg L^{-1}) and left undisturbed the whole night. In order to alleviate the handling stress, the fishes were treated with salt solution (20 g L^{-1}) for 2 min followed by treatment with $KMnO_4$ solution (4 mg L^{-1}) until stressed on the next day. The stock was acclimatized under aerated condition for 15 days. During this period the fish were fed with commercial feed (30% crude protein and 6% lipid) on satiation basis twice daily.

3.8. Experimental Design and Experimental Set-up

Three hundred and fifteen (315) acclimatized fingerlings of *Labeo rohita* (average body weight 12.08 ± 0.5 g) were randomly distributed in seven distinct experimental groups *viz.,* C, Control {Diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM)}, TPM_{15} (Diet with 15% TPM in replacement of 50% DORB), TPM_{30} (Diet with 30% TPM in replacement of 100% DORB), TPM_{15}+EE (TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1), TPM_{30}+EE (TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1), $FTPM_{15}$ {Diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB}, $FTPM_{30}$ (Diet with 30% FTPM in replacement of 100% DORB) in triplicate following completely randomized design (CRD) with the stocking density of 15 fish/tank. Thus, the setup consisted of 21 FRP circular tanks (100 L capacity and 75 L water volume). The tanks were initially washed and filled with $KMnO_4$ solution (4 mg L^{-1}) and left overnight for disinfection. In the next day, the $KMnO_4$ solution was removed and the tanks were washed again with clean water to remove $KMnO_4$ residues. The total volume of the water in each tank was maintained constant throughout the experimental period of 60 days. Round-the-clock aeration was provided to each tank. The top of tanks was covered multipurpose green colour net to prevent the escaping of fish.

Fig.4. Experimental setup

Table 2: Experimental design for feeding trial of 60 days

Treatments	Particulars	Replication		
C	Control (Diet with 30% DORB and 0% TPM)	R1	R2	R3
TPM_{15}	Diet with 15% TPM in replacement of 50% DORB	R1	R2	R3
TPM_{30}	Diet with 30% TPM in replacement of 100% DORB	R1	R2	R3
TPM_{15}+EE	TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1	R1	R2	R3
TPM_{30}+EE	TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1	R1	R2	R3
$FTPM_{15}$	Diet with 15% FTPM in replacement of 50% DORB	R1	R2	R3
$FTPM_{30}$	Diet with 30% FTPM in replacement of 100% DORB	R1	R2	R3

3.9. Formulation and Preparation of Experimental diets

Seven isonitrogenous (30% CP), isolipidic (6%) and isocaloric (360 kcal DE/100gm) experimental diets were formulated (Table 3). In this formulation, de-oiled soybean meal, groundnut oil cake, and mustard oil cake were used as protein sources, wheat flour, corn flour, de-oiled rice bran (DORB) and fermented and non-fermented tomato pomace meals were used as carbohydrate sources, soybean oil and fish oil in 1:1 ratio were used as lipid sources, carboxymethylcellulose, choline chloride and butylated hydroxy toluene (BHT), vitamin and mineral mixture and cellulase and xylanase mixture (1:1) were used as additives.

For diet preparation, all the ground ingredients were weighed as per the formula and kept separately in a plastic tray. The ingredients except oils and additives were then mixed uniformly and required level of water was added to form a dough. The dough was then put in a heat resistant plastic bag and steam cooked in a pressure for 20 minutes. The pressure cooker was then removed from the flame and kept aside for cooling. The cooled dough was taken out, spread and the oils and additives were

added to it, mixed uniformly and a new dough was remade. The dough was then pressed through a mechanical pelletizer to prepare pellets of 1 mm dia. The pellets were then dried in room temperature under fan for one day followed by oven drying at 40 ^{0}C until achieving the moisture content of around 10%. The dried pellets were then broken to smaller size (about 70% of mouth size of experimental fish), packed in polythene bags, sealed airtight, labelled according to the treatments and stored in refrigerated condition (4 ^{0}C) until fed.

3.10. Rearing Condition

The fish were again acclimated in experimental tanks for 7 days through feeding with control diet before commencement of the experimental feeding. No measures were taken to stimulate or control the environmental condition. The experimental conditions were kept same throughout the experimental period. The body weight was measured at 15 days' interval to assess the growth and calculate the daily ration. The fish were starved overnight every time before measuring the body weight. The 25% water of each tank was exchanged with clean freshwater at 3 days' interval. Daily mortality of fish was counted for entire experimental period to calculate the survival percentage.

3.11. Experimental Feeding

The fish of different treatments were fed with respective experimental diets on satiation basis twice daily at 10:00 am in the morning and 17:00 pm in the evening throughout the feeding trial of 60 days.

3.12. Cleaning and Siphoning

The experimental tanks were cleaned manually and the faecal matter was siphoned out every day with replenishment of equal volume of siphoned water by clean freshwater throughout the experimental period. During digestibility trial, the faecal matter was collected carefully.

Table 3: Ingredient Composition (% dry matter) of different experimental diets fed to *Labeo rohita* fingerlings reared for the period of 60 days

Ingredients (%)	Diets/Treatments[1]						
	C	TPM_{15}	TPM_{30}	TPM_{15}+EE	TPM_{30}+EE	$FTPM_{15}$	$FTPM_{30}$
De-oiled soybean meal	20.00	20.00	20.00	20.00	20.00	17.00	15.00
Groundnut oil cake	22.00	22.00	22.00	22.00	22.00	22.00	22.00
Mustard oil cake	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Wheat flour	5.00	5.00	5.00	5.00	5.00	8.00	10.00
Corn flour	5.33	6.33	7.33	6.23	7.23	6.33	7.33
DORB[2]	30.00	15.00	0.00	15.00	0.00	15.00	0.00
TPM[3]	0.00	15.00	30.00	15.00	30.00	0.00	0.00
FTPM[4]	0.00	0.00	0.00	0.00	0.00	15.00	30.00
Sunflower oil	2.50	1.50	0.50	1.50	0.50	1.50	0.50
BHT[5]	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Vit-min mix[6]	1.50	1.50	1.50	1.50	1.50	1.50	1.50
CMC[7]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Choline chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Stay C[8]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL-methionine	0.70	0.70	0.70	0.70	0.70	0.70	0.70
L-lysine	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Cellulase-xylanase mix[9]	0.00	0.00	0.00	0.10	0.10	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB

[2]DORB, De-oil rice bran; [3]TPM, Tomato pomace meal; [4]FTPM, Fermented tomato pomace meal; [5]BHT, Butylated hydroxytoluene [6]Composition of vitamin-mineral mixture (quantity/kg): Vitamin A, 550,000 IU; Vitamin D3, 110,000 IU; Vitamin B2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Vitamin B6, 1000 mg; Vitamin B12, 6 mcg; Calcium Pantothenate,2500 mg; Nicotinamide, 10g; Choline Chloride,150 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 L-lysine, 10 g; DL-Methionine, 10 g; Selenium 50 ppm; [7]CMC, Carboxymethylcellulose; [8]Stay C, Protected vitamin C; [9]Cellulase-xylanase mix, Cellulase and xylanase mixture at 1:1

3.13. *In vitro* Protein Digestibility of Experimental Diets

In vitro protein digestibility study was carried out according to the method of Ali *et al*. (2009). The intestine was dissected out from acclimatized *Labeo rohita* and tissue homogenate was prepared under ice cold condition and diluted with distilled water (1:10 w/v). The crude digestive enzyme was extracted by centrifuging the homogenate at 12000 rpm for 15 min at 4 ^{0}C. An equivalent amount of finely powdered experimental feed that provided 160 mg of crude protein was weighed and mixed with 20 mL of distilled water and 2 mL of crude enzyme extract to obtain 8 mg crude protein per millilitre and the pH was adjusted to 8 (Eutop pH tutor, Thermo Fisher Scientific, Singapore). The pH drop was recorded at one-minute interval for 10 min and casein was used as the reference protein. The relative protein digestibility (RPD) percentage was calculated using the following formula.

$$RPD\% = (-\Delta pH \text{ of experimental feed}/-\Delta pH \text{ of casein}) \times 100.$$

3.14. *In vivo* Digestibility Trials for Experimental Diets

A digestibility trial was conducted for a period of 10 days after the feeding trial was over. The digestibility study was carried out by indirect method using the chromic oxide (Cr_2O_3) marker in the experimental diet (Fawole *et al*., 2016). Accordingly, the experimental diets for digestibility trial were formulated with addition of 0.5% chromic oxide (Cr_2O_3) in existing formula (Table 3) at the cost of corn flour and then the diets were prepared. During the trial, the fish were fed once daily on satiation basis at 10:00 AM. The faecal matter was collected by siphoning once daily at about 17.00 PM and subjected for drying. Accordingly, dried faecal samples of 10 days from each treatment was pooled and stored at -20 ^{0}C until analysis (Shiau and Liang, 1994; Usmani *et al*., 2003). The dried faecal samples were then analysed for nutrients (crude protein and lipid) by following the methods of AOAC (1995). Quantification of chromic oxide content of feed and faecal matters were carried out according to the method of Furukawa and Tsukahara (1966). The apparent digestibility coefficients (ADCs) of dry matter and individual nutrient were calculated as follows (Cho and Slinger, 1979):

$$\text{ADC of dry matter} = 100\left(1-\frac{\%\text{ of chromic oxide in diet}}{\%\text{ of chromic oxide in feces}}\right)$$

$$\text{ADC of nutrients} = 100\left(1 - \frac{\%\text{ of chromic oxide in diet} \times \%\text{ of nutrient in feces}}{\%\text{ of chromic oxide in feces} \times \%\text{ of nutrient in feed}}\right)$$

3.15. Analysis of Proximate Composition and Energy

Proximate analysis of ingredients, experimental diets and whole body of fish and energy determination were carried out as per the standard methods of the Association of Official Analytical Chemists (AOAC, 1995)

3.15.1. Moisture

The moisture content of the ingredients, experimental diets and whole body of fish were determined by taking a known weight of the sample in the petri dish and drying it in a hot air oven at 100 ^{0}C till a constant weight was achieved. The difference in weight of the sample gave the moisture content, which was calculated by using the following formula

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

3.15.2. Crude Protein (CP)

The crude protein content (N x 6.25) was determined by the Kjeldahl method and the assay comprised acid digestion (concentrated sulphuric acid), alkali distillation (40 % NaOH) using an auto Kjeldahl System (Kelplus auto digester and distillation unit; Pelican, Chennai, India) followed by titration (SI Analytics TitroLine® 5000 titrator; Xylem Analytics, Germany). The crude protein percentage was obtained by multiplying the nitrogen percentage by a factor of 6.25.

Crude protein (%) = Total nitrogen (%) X 6.25

3.15.3. Ether Extract (EE)

The ether extract content of samples was estimated by the solvent extraction method (Socsplus, SCS-08-As, Pelican equipment, Chennai, India) and the assay comprises extraction of lipid with an organic solvent (diethyl ether) at 40-60 ^{0}C temperature in Soxhlet apparatus.

The calculation was made as follows.

$$\text{EE (\%)} = \frac{\text{Dry weight of lipid(g)}}{\text{Dry weight of sample (g)}} \times 100$$

3.15.4. Total Ash (TA)

Total ash of the experimental diets and whole body of fish was determined by incinerating the moisture-free sample at 550 ^{0}C for 6 h in a muffle furnace. The calculation was done as follows:

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

3.15.5. Crude Fibre (CF)

The determination of crude fiber of experimental diets was carried out by acid digestion (1.25 % sulphuric acid) of a fat-free sample, washing with distilled water and then alkali digestion (1.25 % sodium hydroxide) followed by incineration in the muffle furnace. The difference in weight after calculation indicates the quantity of fibre present in the sample.

$$\text{Crude fibre (\%)} = \frac{\text{Dry weight of crude fibre}}{\text{Dry weight of fat free sample}} \times 100$$

3.15.6 Nitrogen Free Extract (NFE)

Nitrogen free extract of diet was determined by subtraction method as follows:

NFE (%) = 100- {CP (%) + EE (%) + TA (%) + CF (%)}

3.15.7 Total Carbohydrate (TC)

The whole body total carbohydrate of fish was determined by subtraction method as follows:

TC (%) = 100- {CP (%) + EE (%) + TA (%)}

3.15.8. Gross energy (GE)

The gross energy of the diets was calculated by using energetic values of protein, lipid and total carbohydrate (NFE+CF).

GE (kcal/100g) = {CP (%) ×5.65} + {EE (%) × 9.45} + {TC (%) × 4.2}

3.15.9. Digestible energy (DE)

The digestible energy values of experimental diets were calculated based on standard physiological values (Halver, 1976) as per the following formula.

DE (kcal/100g) = {CP (%) x 4} + {EE (%) x 9} + {NFE (%) x 4}

3.16. Physico-chemical parameters of water

Water quality parameters viz. temperature, pH, dissolved oxygen, free carbon dioxide, total hardness, ammonia-N, nitrite-N and nitrate-N were recorded during the experimental period of 60 days.

3.16.1. Temperature

The water temperature of all the experimental tanks was recorded using thermometer (MERCK, Germany).

3.16.2. pH

The water pH of all the experimental tanks was measured by a digital pH meter (LABINDLA).

3.16.3. Dissolved Oxygen

The dissolved oxygen in water of all the experimental tanks was measured by membrane electrode method using dissolved oxygen meter (MERCK, Germany).

3.16.4. Free Carbon Dioxide

The dissolved free carbon dioxide in water of all experimental units was measured by titrimetric method (APHA, 1998)) and calculated using the following formula.

CO_2 (mg L^{-1}) = A x N x 44 x 1000/Volume of sample (ml)

Where, A = Volume of titrant (NaOH)

N = Normality of titrant (N/44)

3.16.5. Total Hardness

The total carbonate hardness of experimental water of all units was estimated by carbonate hardness test kit (Carbonate hardness test, MERCK, Germany).

3.16.6. Ammonia-N (NH_3-N)

The un-ionized ammonia nitrogen concentration in water of all experimental units was estimated spectrophotometrically at 635 nm wavelength by phenate method (APHA, 1998) and compared with standard graph. The concentration was expressed as mg L^{-1}.

3.16.7. Nitrite-N (NO_2-N)

The nitrite nitrogen concentration in water of all experimental tanks was estimated spectrophotometrically at 543 nm wavelength (APHA, 1998) and compared with standard graph. The concentration was expressed as mg L^{-1}.

3.16.8. Nitrate nitrogen (NO_3-N)

The nitrate nitrogen concentration of experimental water of all units was estimated spectrophotometrically at 220 nm to obtain NO_3 reading and at 275 nm to obtain interference due to dissolved organic matter and compared with standard graph. Concentration was expressed as mg L^{-1}.

3.17. Growth, Feed Utilization and Nutrient Utilization

Initial and final body weight of fish was measured using an electronic weighing balance. Fish were starved overnight before taking the weight.

3.17.1. Weight Gain (WG)

The weight gain was calculated using the following formula.

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

3.17.2. Weight Gain Percentage (WG %)

The percent weight gain was calculated using the following formula.

$$WG\ (\%) = \frac{\text{Final wet weight(g)} - \text{Initial wet weight(g)}}{\text{Initial Wet weight(g)}} \times 100$$

3.17.3. Specific Growth Rate (SGR)

The specific growth rate was calculated using the following formula.

$$SGR = \frac{\text{Ln of final wet weight} - \text{Ln of initial wet weight}}{\text{Experimental period in days}} \times 100$$

3.17.4. Thermal Growth Coefficient (TGC)

Thermal growth coefficient was calculated by the following formula.

$$\text{TGC} = \frac{(\text{Final wet weight})^{1/3} - (\text{Initial wet weight})^{1/3}}{\text{Water temperature in degree centigrade} \times \text{Experimental period in days}} \times 100$$

3.17.5. Feed Conversion Ratio (FCR)

The feed conversion ratio was calculated using the following formula.

$$\text{FCR} = \frac{\text{Dry feed or dry matter intake (g)}}{\text{Wet body weight gain (g)}}$$

3.17.6. Feed Efficiency Ratio (FER)

The feed efficiency ratio was calculated using the following formula.

$$\text{FER} = \frac{\text{Wet body weight gain(g)}}{\text{Dry feed intake (g)}}$$

3.17.7. Protein Efficiency Ratio (PER)

The protein efficiency ratio was calculated using the following formula.

$$\text{PER} = \frac{\text{Wet weight gain(g)}}{\text{Dry protein intake (g)}}$$

3.18. Body Indices

At the end of experiment, fish were starved for one day and 3 fish from each experimental tank were collected, anaesthetized by clove oil (50 µL L^{-1}) and weighed. Then the liver and intestine were dissected out and weighed in electronic balance. Body indices such as hepato-somatic index and intestinal somatic index were calculated by standard formulae.

3.18.1. Hepatosomatic Index (HSI)

The hepatosomatic index was calculated using the following formula.

$$\text{HSI (\%)} = \frac{\text{Wet weight of liver(g)}}{\text{Wet weight of fish(g)}} \times 100$$

3.18.2. Intestinal Somatic Index (ISI)

The intestinal somatic index was calculated as follows:

$$\text{ISI (\%)} = \frac{\text{Wet weight of intestine(g)}}{\text{Wet weight of fish(g)}} \times 100$$

3.19. Survival Rate %

At the end of the experiment, the number of the experimental fish in each tank was counted and using the following formula the survival percentage was calculated.

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

3.20. Assays of enzymes

3.20.1. Preparation of tissue homogenate

At the end of the experiment, 4 fishes were randomly collected from each tank, anaesthetized with clove oil (50µl/L) and muscle, liver, gill and intestine were dissected out carefully in ice cold condition and weighed. The 5% homogenate of each tissue was prepared in chilled sucrose solution (0.25 M) in a glass tube using tissue homogenizer (MICCRA D-9, ART Prozess and Labotechnik, Germany). The tubes were continuously kept in flake ice during the whole operation to preserve the enzymatic activity. The homogenate was then centrifuged at 5000 rpm (4200 rcf) for 10 min at 4 ^{0}C in a cooling centrifuge machine (Megafuge 8r, Thermo Fisher Scientific, USA). The supernatant was collected in 2 ml Eppendorf tubes and stored at -20 ^{0}C until used.

3.20.2. Tissue Protein Estimation

Total protein of each tissue homogenate was estimated by Bradford method (Bradford, 1976). Tissue homogenate (5µl) was taken in a 96-well flat bottom micro-titre plate and 250 µl previously prepared 'Bradford reagent' {100 mg Coommassie blue G250 dissolved in 50 ml 95% ethanol mixed with 100 ml of 85% (w/v) phosphoric acid and made to 1L} was added into it and kept for 5 min. A blank containing distilled water instead of tissue sample was also kept. Following the same procedures, a protein standard curve was prepared using bovine serum albumin (BSA) as standard. The absorbance of sample was taken at 595 nm in micro-plate reader (Epoch microplate spectrophotometer, BioTek, Mumbai, India) and the protein content

of the sample was determined against the standard curve. The protein content was expressed in mg/g wet tissue.

3.20.3. Activities of Digestive Enzymes

3.20.3.1 Protease Activity

Protease activity was determined by the casein digestion method (Drapeau, 1974). The enzyme reaction mixture containing 1% casein in 0.05 M Tris PO4 buffer (pH 7.8) was incubated for 5 min at 37 ^{0}C. Then, the intestinal tissue homogenate (0.1 ml) was added to the enzyme mixture and after ten minutes, the reaction was stopped by adding 10% TCA and then the whole content was filtered. The reagent blank was made by adding tissue homogenate just before stopping the reaction and without incubation. One unit of enzyme activity was defined as the amount of enzyme needed to release acid soluble fragments equivalent to Δ0.001A280 per minute at 37^{0}C and pH 7.8. Thus, the enzyme activity was expressed as milli-mole of tyrosine released/min/mg protein.

3.20.3.2. Amylase activity

The reducing sugars produced due to the action of gluco-amylase and α-amylase on carbohydrate was estimated using di-nitro-salicylic acid (DNS) method (Rick and Stegbauer, 1974). The reaction mixture containing 1% (w/v) starch solution, 1.8 ml phosphate buffer (pH 6.9) and the intestinal tissue homogenate (0.1 ml) was incubated at 37 ^{0}C for 30 min. 2 ml DNS was added after incubation and kept in boiling water bath for 5 min. After cooling, the reaction mixture was diluted to 10 ml with distilled water and absorbance was measured at 540 nm. Instead of tissue 35 homogenate distilled water (0.1 ml) was used for blank. Maltose was used as the standard. The amylase activity was expressed as micromole of maltose released/min/mg protein.

3.20.3.3 Lipase activity

The lipase activity was assayed following the method of Cherry and Crandall (1932), which is based on the measurement of fatty acids released by the enzymatic hydrolysis of triglycerides present in a stabilized emulsion of olive oil. In the clean, dry and properly labelled test tubes, 3 ml of distilled water and 1ml of intestinal tissue homogenate were added and mixed well and kept in boiling water for 5min.

After cooling the tubes, 0.5 ml of phosphate buffer and 2 ml of olive oil emulsion were added to all the tubes. Further, the tubes were shaken well and incubated at 37 ^{0}C for 24 h, after which the reaction was stopped by adding 3 ml of 95% ethyl alcohol. Titration with N/20 (w/v) NaOH solution was performed after adding 2 drops of phenolphthalein as indicator. The activity was calculated based on the following formula

Lipase activity (U/mg protein/h) = Volume of NaOH x wet weight of tissue sample x mg of protein per gm of tissue x 24 h

3.20.4. Activities of Protein Metabolic Enzymes

3.20.4.1 Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

The AST and ALT activities were assayed in different tissue homogenates as described by Wooten (1964). For AST activity, the substrate comprised of 0.2M D, L-aspartic acid and 2mM α-ketoglutarate in 0.05M phosphate buffer (pH 7.4). In the experimental and control tubes, 0.5ml of substrate was added. The reaction was started by adding 0.1ml of tissue homogenate. The assay mixture was incubated at 37^{0}C for 60 min. The reaction was terminated by adding 0.5 ml of 1mM 2, 4 dinitrophenyl hydrazine (DNPH). In the control tubes the enzyme source was added after DNPH solution. The tubes were held at room temperature for 20 min with occasional shaking. Then 5 ml of 0.4 N NaOH solution was added and the contents were thoroughly mixed. After 10 min, the OD was recorded at 540nm against the blank. The procedure followed for ALT activity was same as for AST activity except the substrate, which was L- alanine instead of aspartic acid. The AST activity was expressed as nanomole of oxaloacetate released/min/ mg protein and the ALT activity is expressed as nanomole of pyruvate formed/min/mg protein.

3.20.5. Activities of Carbohydrate Metabolic Enzymes

3.20.5.1 Lactate Dehydrogenase (LDH) and Malate Dehydrogenase (MDH)

The LDH activity was assayed in different tissues by the method of Wroblewski and Ladue (1955), while the MDH activity was assayed in different tissues by the method of Ochoa (1955). For both the cases, a reaction mixture of 3 ml prepared out of 2.7 ml 0.1M phosphate buffer (pH 7.5), 0.1ml of NADH solution (2 mg NADH dissolved in 1ml of phosphate buffer solution), 0.1ml of tissue homogenate and

0.1ml of sodium pyruvate or oxaloacetate. The reaction was started after addition of substrate (sodium pyruvate for LDH and oxaloacetate for MDH). The OD was recorded at 340nm at 30 sec intervals for 3min. Both the LDH and MDH activity was expressed as Unit/mg protein/min, where 1 unit was equivalent to ∆0.01OD/ min.

3.20.6 Activities of Oxidative Stress Enzymes

3.20.6.1 Superoxide Dismutase (SOD)

Superoxide dismutase was assayed according to the method described by Misra and Fridovich (1972) based on the oxidation of epinephrine-adrenochrome transition by the enzyme. 50µl of the sample was taken in the cuvette and 1.5 ml 0.1M carbonate-bicarbonate buffer containing 57 mg EDTA/dl (pH 10.2) and 0.5 ml epinephrine (3mM) was added and mixed well. Change in optical density at 480 nm was recorded immediately at 30 sec for 3 min in a spectrophotometer (BioGenix Microprocessor UV-vis Double beam spectrophotometer, New Delhi, India). One unit of SOD activity was the amount of protein required to give 50% inhibition of epinephrine auto oxidation. Thus, the SOD activity was expressed as 50% inhibition of epinephrine auto-oxidation/mg protein/min.

3.20.6.2 Catalase (CAT)

Catalase activity was assayed according to the method described by Takahara et al. (1960). To a reaction mixture of 2.45 ml phosphate buffer (50 mM, pH 7.0), 50 µl tissue homogenate were added and the reaction was started by the addition of 1.0 ml of 0.3 % H_2O_2 solution. The decrease in absorbance was measured at 240nm at 15sec intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml distilled water instead of H_2O_2 solution. The CAT activity was expressed as nanomoles of H_2O_2 decomposed/min/mg protein.

3.21. Haemato-biochemical and Immunological Parameters

3.21.1. Collection of Serum

At the end of the experiment, 3 fishes from each tub were randomly collected, anaesthetized with clove oil (50µl/L) and subjected for the collection of blood from a caudal vein using a medical syringe without using any anticoagulant. Collected blood was then transferred immediately to a dried Eppendorf tube. The tubes were allowed to stand in tilted position at room temperature for an hour which allows the

blood to clot. Then it was centrifuged at 5000 rpm (4200 rcf) for 10 min at 4 ^{0}C in a cooling centrifuge machine (Megafuge 8r, Thermo Scientific, Mumbai, India). The yellow straw coloured supernatant (serum) was carefully collected and transferred to another Eppendorf tube and stored at -20^{O}C until used.

3.21.1.1 Serum Glucose

Serum glucose content was estimated (Trinder, 1969) using Liquixx Glucose kit, (Erba® Diagnostic Mannheim, Transasia Bio-medicals Ltd, Solan, HP, India). In the presence of glucose oxidase, glucose will be oxidised to gluconic acid and hydrogen peroxide. The enzyme peroxidase catalyses the oxidative coupling of phenol with 4- aminopyridine to yield a coloured quinonimine complex which is measured at 505 nm. As the concentration of this complex is proportional to glucose present in the sample, the quantification of glucose was done using the following formula.

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

3.21.1.2. Serum Total Protein

Serum total protein was estimated according to biuret method (Reinhold, 1953) using Liquixx total protein kit (Erba® Diagnostic Mannheim, Transasia Bio-medicals Ltd, Solan, HP, India). Protein present in the serum binds with copper ions in an alkaline medium of the biuret reagent and produce a purple coloured complex, whose absorbance is proportional to the protein concentration. Three test tubes labelled as Blank (B), standard (S) and test (T) were taken. Into all the tubes, 1 ml of biuret reagent and 2 ml of distilled water were added. 0.05 ml of protein standard was taken in the test tube labelled as standard and 0.05 ml of serum was added in test tube labelled as test. It was mixed well and incubated at 37°C for 10 min. The absorbance of standard (S) and test (T) were measured against blank (B) in a spectrophotometer at 546 nm. The calculation was done as follows:

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

3.21.1.3. Serum Albumin

Serum albumin was estimated by spectrophotometric method as described by Doumas et al. (1971) by using ERBA KIT. Albumin at pH 4.2 binds with bromocresol green (BCG) and produces a blue-green colour, whose absorbance is proportional to the albumin concentration. Three test tubes labelled as Blank (B), 40 Standard (S) and Test (T) were taken. Into all the tubes, 1 ml of buffered dye reagent and 2 ml of distilled water were added. 0.01 ml of albumin standard was taken in the test tube labelled as standard and 0.01 ml of serum was added into test tube labelled as test. It was then mixed well and incubated at 37 ^{0}C for 10 min. The absorbance of test (T) and standard (S) were measured immediately against blank (B) in a spectrophotometer (BioGenix Microprocessor UV-vis Double beam spectrophotometer, New Delhi, India) at 630 nm. The calculation was done as follows:

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

3.21.1.4. Serum Globulin

Serum globulin concentration was calculated by subtracting the serum albumin value from serum total protein value as shown below.

Globulin (g/dl) = Total protein (g/dl) – Albumin (g/dl)

3.21.1.5. Serum Albumin to Globulin Ratio (A/G)

The serum albumin value was divided by serum globulin value to calculate serum A/G as shown below.

A/G = Albumin (g/dl)/Globulin (g/dl)

3.22. Statistical Analysis

The data were analyzed by one-way analysis of variance (ANOVA) using a SPSS 22.0 for windows. Duncan's multiple range test was used for post hoc comparison of means among different experimental groups. All data presented in the text, figures and tables as mean ± standard error (SE) and statistical significance for all statistical tests were set at 5% probability level ($P< 0.05$).

RESULTS

4. RESULTS

4.1. Proximate Composition of Tomato Pomace Meal (TPM) and Fermented Tomato Pomace Meal (FTPM)

The proximate composition of TPM and FTPM is shown in Table 4. The crude protein (CP) and crude fiber (CF) contents of the TPM and FTPM were found to be 17.90 and 24.60% and 28.40 and 13.00%, respectively. However, ether extract (EE), total ash (TA), nitrogen free extract (NFE), gross energy (GE) and digestible energy (DE) of TPM and FTPM were 7.78 and 8.25%, 14.40 and 16.00%, 31.52 and 38.15%, 426.32 and 431.78 kcal/100 g and 267.70 and 325.25 kcal/100 g, respectively.

Table 4. Proximate Composition (on % dry matter basis) of Tomato Pomace Meal and Fermented Tomato Pomace Meal

Proximate Composition	Tomato Pomace Meal	Fermented Tomato Pomace Meal
Moisture (%)	13.50	10.00
Crude Protein (%)	17.90	24.60
Ether Extract (%)	7.78	8.25
Crude Fiber (%)	28.40	13.00
Total ash (%)	14.40	16.00
NFE[1] (%)	31.52	38.15
TC[2] (%)	59.92	51.15
GE[3] (kcal/100g)	426.32	431.78
DE[4] (kcal/100g)	267.70	325.25

All values are expressed as means of triplicate
[1]NFE, Nitrogen free extract
[2]TC, Total carbohydrate
[3]GE, Gross energy (kcal/100g) = [{5.65 × CP (%)} + {9.45 × EE (%)} + {4.2 × TC (%)}] (Blaxter, 1989)
[4]DE, Digestible energy (kcal/100g) = [{4 × CP (%)} + {9 × EE (%)} + {4 × NFE (%)}] (Halver, 1976).

4.2. Qualitative Assessment of Bioactive Compounds in TPM and FTPM

A qualitative assessment of bioactive compound in TPM and FTPM is shown in Table 5. The aqueous extracts of TPM and FTPM contained the flavonoids, coumarins, phenols, tannins and saponins.

Table 5: Bioactive Compounds of Tomato Pomace Meal and Fermented Tomato Pomace Meal

Bioactive compound	Tomato pomace meal	Fermented tomato pomace meal
Flavonoids	+	+
Coumarins	+	+
Phenols	+	+
Tannins	+	+
Saponins	+	+

+ sign indicates the presence of bioactive compounds

4.3. Physico-chemical parameters of water

The ranges of water quality parameters such as temperature (^{0}C), pH, dissolved oxygen (mg L^{-1}), free carbon dioxide (mg L^{-1}), total hardness (mg L^{-1}), ammonia-N (mg L^{-1}), nitrite-N (mg L^{-1}) and nitrate-N (mg L^{-1}) of different treatments are given in Table 6.

4.3.1. Temperature

The water temperature of the different treatments varied from 28.5 to 32.9 ^{0}C during the experimental period of 60 days.

4.3.2. pH

The pH values of different units were found to be within the range of 7.0-8.3.

4.3.3. Dissolved Oxygen (DO)

The DO concentrations of all the experimental units were recorded within the range of 6.0-7.9 mg L^{-1} during the entire experimental period.

4.3.4. Free Carbon Dioxide

The free carbon dioxide was not detectable level in any experimental unit during the experimental period of 60 days.

4.3.5. Total Hardness

The total carbonate hardness of 0065perimental units was found to be within the range of 231.0-242.0 mg L^{-1} during the experimental period of 60 days.

4.3.6. Ammonia-N

The ammonia nitrogen content of all the experimental tanks was recorded before water exchange and found to be ranged from 0.02 to 0.09 mg L^{-1}.

4.3.7. Nitrite-N

The nitrite nitrogen content of all experimental units was found to be in the range of 0.04 - 0.09 mg L^{-1}.

4.3.8. Nitrate-N

The nitrate nitrogen content of all experimental units was found to be ranged from 0.65 - 0.96 mg L^{-1}.

4.4. Proximate Composition of the Experimental Diets & Fish

The proximate composition of the different experimental diets is given in the Table 7. The moisture content of experimental diets was found within the range of 8.29-8.53%. The CP, EE, GE and DE of the experimental diets varied from 30.04 to 30.42%, 6.09 to 6.18%, 458.37 to 462.85 kcal/100 g and 360.95 to 371.14 kcal/100 g, respectively. However, the CF, TA and NFE ranged from 7.05 to 8.68%, 7.89 to 8.98% and 46.36 to 48.85%, respectively. The P: E was found to vary from 80.91 to 83.67 mg CP/kcal DE.

The whole body proximate composition of fish of different experiment groups is depicted in Table 8. The whole body moisture, crude protein, lipid or crude fat, total ash and total carbohydrate contents did not vary significantly ($p>0.05$) among the fish of different dietary groups.

Table 6: Physico-chemical Parameters of Water during the Experimental Period of 60 days for different Experimental Groups

Treatments[1]	Temperature (°C)	Dissolved oxygen (mg L^{-1})	pH	Free CO_2[2] (mg L^{-1})	Total hardness (mg L^{-1})	Ammonia-N[3] (mg L^{-1})	Nitrite-N[4] (mg L^{-1})	Nitrate-N[5] (mg L^{-1})
C	28.7-32.5	6.0-7.1	7.4-8.2	ND	231.0-242.0	0.05-0.09	0.05-0.07	0.70-0.96
TPM_{15}	28.5-32.2	6.2-7.5	7.6-8.3	ND	233.0-241.0	0.04-0.08	0.04-0.08	0.66-0.82
TPM_{30}	28.6-32.7	6.0-7.7	7.3-8.1	ND	234.0-242.0	0.05-0.08	0.06-0.09	0.82-0.91
TPM_{15}+EE	29.4-32.4	6.1-7.9	7.2-7.9	ND	233.0-241,0	0.06-0.09	0.06-0.08	0.84-0.92
TPM_{30}+EE	29.0-32.4	6.3-7.8	7.0-7.8	ND	233.0-238.0	0.05-0.08	0.05-0.09	0.87-1.01
$FTPM_{15}$	29.2-32.9	6.2-7.5	7.3-8.0	ND	233.0-239.0	0.02-0.07	0.05-0.09	0.65-0.92
$FTPM_{30}$	29.1-32.3	6.0-7.4	7.6-8.2	ND	236.0-242.0	0.06-0.09	0.04-0.08	0.78-0.85

ND, Not detected

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); $TPM_{15,}$ diet with 15% TPM in replacement of 50% DORB; $TPM_{30,}$ diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15,}$ diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30,}$ diet with 30% FTPM in replacement of 100% DORB

[2]CO_2, Carbon dioxide; [3]Ammonia-N, Ammonia nitrogen; [4]Nitrite-N, Nitrite nitrogen; [5]Nitrate-N, Nitrate nitrogen

Table 7: Proximate Composition (on % dry matter basis) of different Experimental Diets fed to *Labeo rohita* fingerlings during the experimental period of 60 days

Proximate Composition	Diets/Treatments[1]						
	C	TPM_{15}	TPM_{30}	TPM_{15}+EE	TPM_{30}+EE	$FTPM_{15}$	$FTPM_{30}$
Moisture (%)	8.34	8.29	8.53	8.46	8.39	8.44	8.39
Crude protein (%)	30.04	30.21	30.38	30.25	30.21	30.03	30.42
Ether extract (%)	6.15	6.11	6.12	6.13	6.14	6.18	6.09
Crude fiber (%)	8.68	8.21	8.43	7.95	8.01	7.05	7.14
Total ash (%)	8.77	8.36	8.45	8.00	8.15	7.89	8.98
NFE[2] (%)	46.36	47.11	46.62	47.67	47.49	48.85	47.37
GE[3] (kcal/100g)	459.01	460.77	460.69	462.45	461.81	462.85	458.37
DE[4] (kcal/100g)	360.95	364.27	363.08	366.85	366.06	371.14	365.97
P:E[5] (mg protein/kcal DE)	83.22	82.93	83.67	82.46	82.53	80.91	83.12

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB

[2]NFE, Nitrogen free extract; [3]GE, Gross energy; [4]DE, Digestible energy; [5]P: E, Protein to energy ratio

4.5. *In vitro* Relative Protein Digestibility

The in-vitro relative protein digestibility values of TPM and FTPM were found to be 76.32±3.20 and 93.09±2.27%, respectively (Table 9).

4.6. *In vivo* Dry Matter and Nutrient Digestibility

Although the apparent dry matter digestibility coefficient (ADMDC) significantly varied ($p<0.05$) among dietary groups, the values of apparent crude protein digestibility coefficient (ACPDC) and apparent lipid digestibility coefficient (ALDC) were not significantly ($p>0.05$) different (Table 9). TPM30+EE, FTPM15 and FTPM30 groups showed significantly higher ($p<0.05$) ADMDC than TPM_{15}, TPM_{30} and TPM_{15}+EE groups and similar value ($p>0.05$) to control group.

Table 8: Whole body proximate composition (on %wet weight basis) of *Labeo rohita* fingerlings fed with different experimental diets for the experimental period of 60 days

Treatments[1]	Moisture (%)	Crude Protein (%)	Lipid (%)	Total Ash (%)	Total Carbohydrate (%)
C	74.88 ±0.49	14.19 ±0.12	4.07 ±0.18	3.48 ±0.30	3.37 ±0.35
TPM_{15}	74.75 ±0.32	14.03 ±0.15	4.09 ±0.13	3.22 ±0.13	3.91 ±0.66
TPM_{30}	73.73 ±0.32	13.98 ±0.10	4.03 ±0.10	3.37 ±0.27	4.89 ±0.61
TPM_{15}+EE	74.60 ±0.32	14.08 ±0.10	4.11 ±0.14	3.32 ±0.21	3.89 ±0.43
TPM_{30}+EE	75.04 ±0.49	14.09 ±0.14	4.12 ±0.17	3.49 ±0.39	3.27 ±0.61
$FTPM_{15}$	75.32 ±0.33	14.11 ±0.28	4.15 ±0.14	3.60 ±0.54	2.82 ±0.88
$FTPM_{30}$	75.11 ±0.12	14.23 ±0.16	4.10 ±0.13	3.62 ±0.52	2.93 ±0.46
p-value	*0.123*	*0.929*	*0.998*	*0.984*	*0.266*

Data are expressed as mean ± S.E, n=3; Mean values in the same column without superscripts did not differ significantly ($p > 0.05$)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB

Table 9: *In vitro* Relative Protein Digestibility of TPM and FTPM and *In vivo* Apparent dry matter and nutrient digestibility coefficients of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Treatments[1]	IVRPD[2](%)	ADMDC[3]	ACPDC[4]	ALDC[5]
TPM[6]	76.32±3.20			
FTPM[7]	93.09±2.27			
C		59.75^{bc}±0.97	81.11±0.79	93.26±1.11
TPM_{15}		57.50^{ab}±1.10	81.16±1.29	93.03±1.30
TPM_{30}		55.69^{a}±0.55	78.76±1.31	93.06±1.44
TPM_{15}+EE		58.02^{ab}±0.81	80.92±1.23	93.84±1.41
TPM_{30}+EE		61.36^{c}±0.68	80.39±0.93	94.92±1.00
$FTPM_{15}$		62.02^{c}±0.92	83.20±0.92	95.87±1.15
$FTPM_{30}$		61.92^{c}±0.78	82.01±0.93	95.67±1.09
p-value		*<0.001*	*0.213*	*0.453*

Data are expressed as mean ± S.E, n=3; Mean values in the same column with different superscripts differ significantly ($p < 0.05$)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB

[2]IVRPD, *In vitro* relative protein digestibility; [3]ADMDC, apparent dry matter digestibility coefficient; [4]ACPDC, apparent crude protein digestibility coefficient; [5]ALDC, apparent lipid digestibility coefficient; [6]TPM, Tomato pomace meal; [7]FTPM, Fermented tomato pomace meal

4.7. Growth, Feed and Nutrient Utilization and Survival Rate

The parameters related to the growth, feed and nutrient utilization and survival rate are shown in Table 10.

4.7.1. Weight Gain (WG)

The WG of *Labeo rohita* fingerlings was significantly ($p<0.05$) different among the treatments (Table 10; Fig 5). The highest ($p<0.05$) WG was observed in $FTPM_{30}$ group, which was however statistically similar ($p>0.05$) to $FTPM_{15}$ group. However, all other groups including control showed similar ($p>0.05$) WG. Moreover, there was no significant difference ($p>0.05$) of WG between control and $FTPM_{15}$ groups.

4.7.2. Weight Gain Percentage (WG%)

The WG% of *Labeo rohita* fingerlings was significantly ($p<0.05$) different among the treatments (Table 10; Fig 6). The highest ($p<0.05$) WG% was observed in $FTPM_{30}$ group, which was statistically similar ($p>0.05$) to $FTPM_{15}$ group. However, all other groups including control showed similar ($p>0.05$) WG. Moreover, there was no significant difference ($p>0.05$) of WG between control and $FTPM_{15}$ groups.

4.7.3. Specific Growth Rate (SGR)

The SGR of *Labeo rohita* fingerlings were significantly ($p<0.05$) different among the treatments (Table 10; Fig 7). The highest ($p<0.05$) SGR was observed in $FTPM_{30}$ group, which was statistically similar ($p>0.05$) to $FTPM_{15}$ group. However, all other groups including control showed similar ($p>0.05$) SGR. Moreover, there was no significant difference ($p>0.05$) of SGR between control and $FTPM_{15}$ groups.

4.7.4. Feed Conversion Ratio (FCR)

The *Labeo rohita* fingerlings of $FTPM_{30}$ group showed significantly lower ($p<0.05$) FCR value than control, TPM_{15} and TPM_{30} groups and similar ($p>0.05$) value to TPM_{15} + EE, TPM_{30} + EE and $FTPM_{15}$ groups (Table 10; Fig 8).

4.7.5. Protein Efficiency Ratio (PER)

The *Labeo rohita* fingerlings of $FTPM_{30}$ group showed similar ($p>0.05$) PER value to TPM_{30} + EE and $FTPM_{15}$ groups and significantly higher ($p<0.05$) PER value than other groups (Table 10; Fig 9). Moreover, the fish of control group showed significantly lower ($p<0.05$) PER than $FTPM_{15}$ group, but similar ($p>0.05$) value to TPM and enzyme supplemented TPM fed groups.

4.7.6. Survival Rate %

Survival percentage *Labeo rohita* fingerlings did not vary significantly ($p>0.05$) among dietary groups (Table 10).

Table 10: Growth, Feed and Nutrient Utilization and Survival Rate of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Treatments[1]	WG[2](g)	WG(%)[3]	SGR[4]	FCR[5]	PER[6]	Survival (%)
C	$12.11^{ab} \pm 0.60$	$100.77^{ab} \pm 4.93$	$1.16^{ab} \pm 0.04$	$2.10^{bcd} \pm 0.05$	$1.59^{ab} \pm 0.04$	86.67±3.85
TPM_{15}	$11.32^{a} \pm 0.37$	$94.07^{a} \pm 3.23$	$1.11^{a} \pm 0.03$	$2.21^{d} \pm 0.08$	$1.51^{a} \pm 0.05$	88.89±2.22
TPM_{30}	$11.70^{a} \pm 0.65$	$96.81^{a} \pm 5.30$	$1.13^{a} \pm 0.05$	$2.15^{cd} \pm 0.08$	$1.55^{ab} \pm 0.06$	91.11±2.22
TPM_{15}+EE	$12.31^{ab} \pm 0.43$	$101.66^{ab} \pm 3.97$	$1.17^{ab} \pm 0.03$	$2.06^{abcd} \pm 0.08$	$1.62^{abc} \pm 0.06$	95.55±2.22
TPM_{30}+EE	$12.82^{ab} \pm 0.46$	$105.50^{ab} \pm 3.84$	$1.20^{ab} \pm 0.03$	$1.93^{abc} \pm 0.09$	$1.73^{bcd} \pm 0.07$	93.33±3.85
$FTPM_{15}$	$13.82^{bc} \pm 0.65$	$115.13^{bc} \pm 5.37$	$1.27^{b} \pm 0.04$	$1.87^{ab} \pm 0.10$	$1.80^{cd} \pm 0.09$	95.55±2.22
$FTPM_{30}$	$14.98^{c} \pm 0.57$	$123.93^{c} \pm 4.25$	$1.34^{c} \pm 0.03$	$1.82^{a} \pm 0.05$	$1.84^{d} \pm 0.05$	97.78±2.22
p-value	*0.004*	*0.004*	*0.006*	*0.017*	*0.013*	*0.051*

Data are expressed as mean ± S.E, n=3; Mean values in the same column with different superscripts differ significantly ($p < 0.05$)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB

[2]WG, Weight gain, [3]WG%, Weight gain percentage; [4]SGR, Specific growth rate; [5]FCR, Feed conversion ratio; [6]PER, Protein efficiency ratio

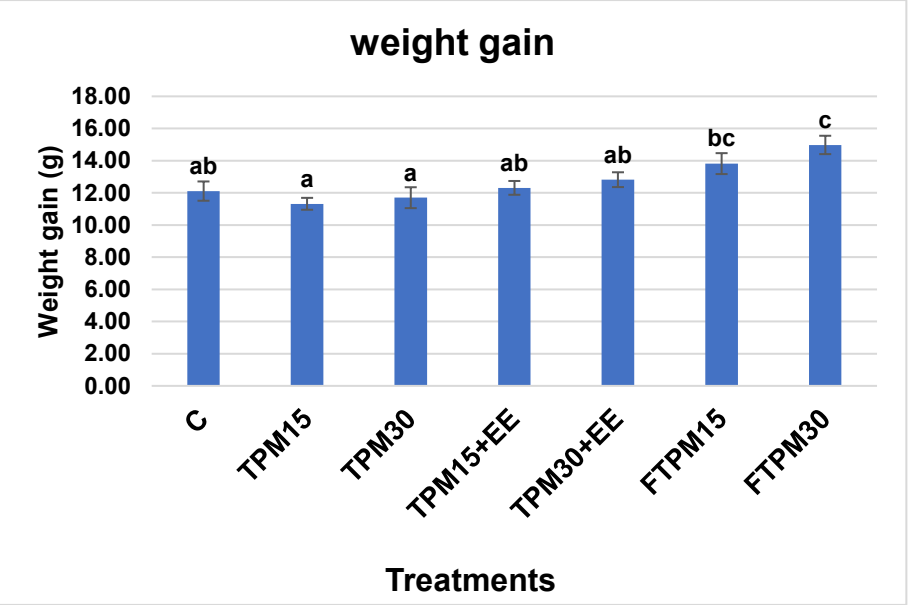


Fig 5: Weight gain of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript differ significantly (P<0.05)

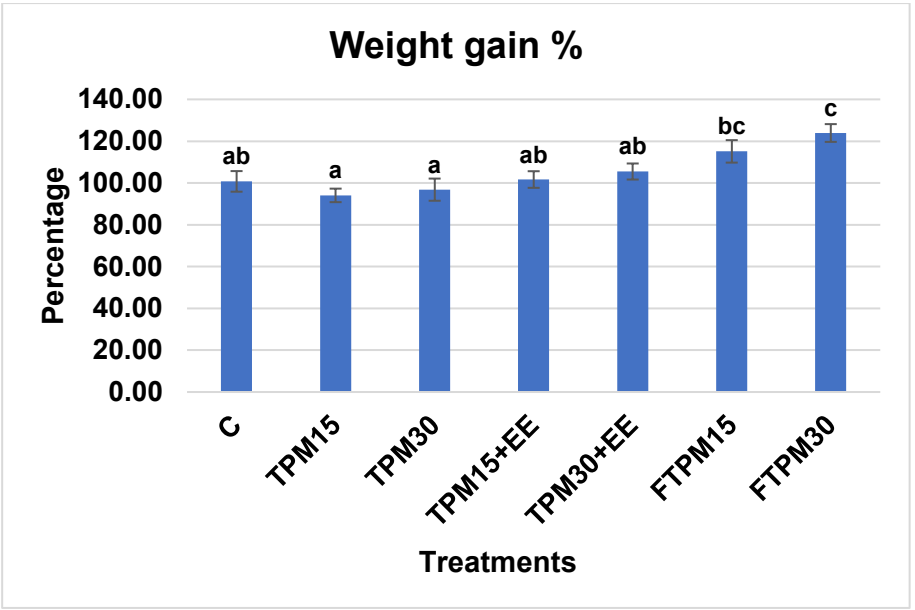


Fig 6: Weight gain percentage of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

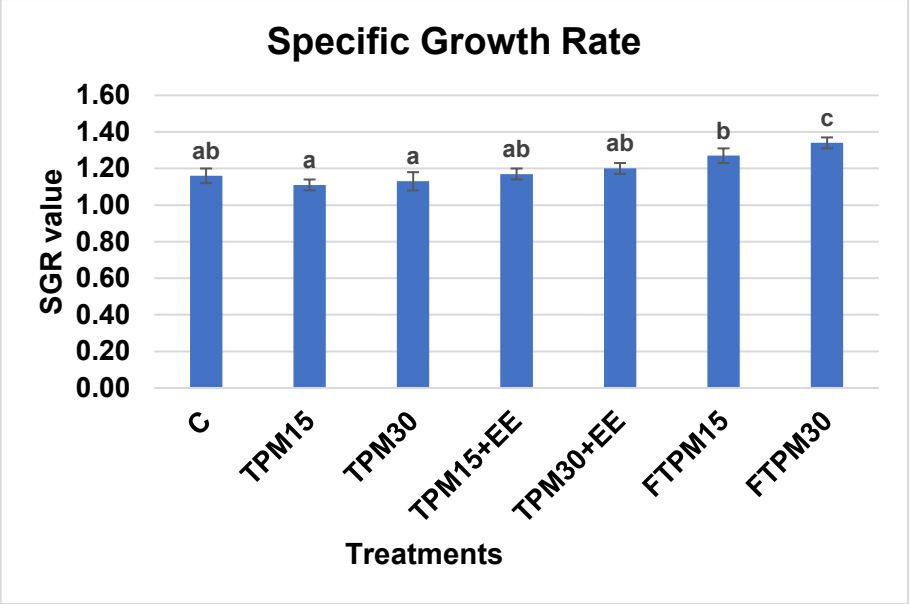


Fig 7: Specific growth rate of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

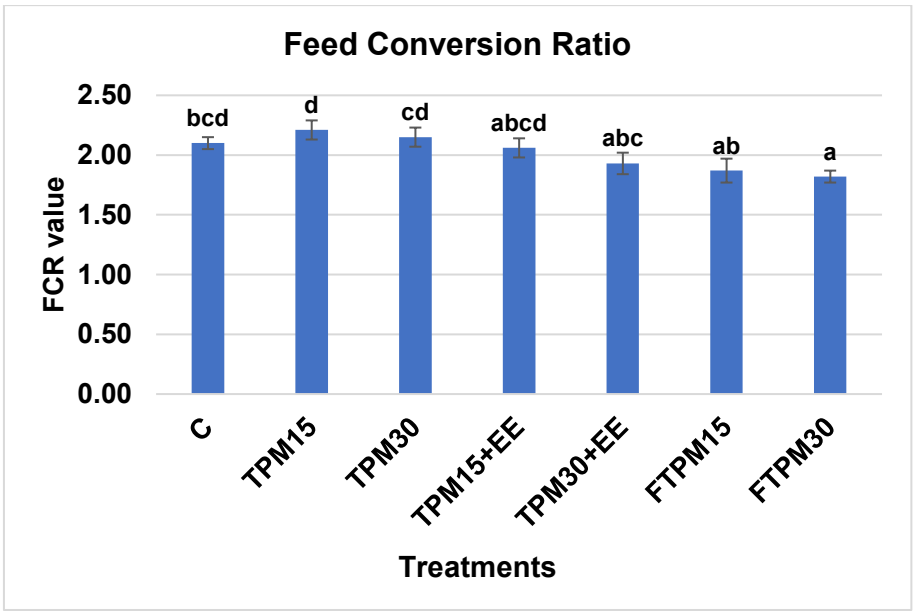


Fig 8: Feed conversion ratio of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

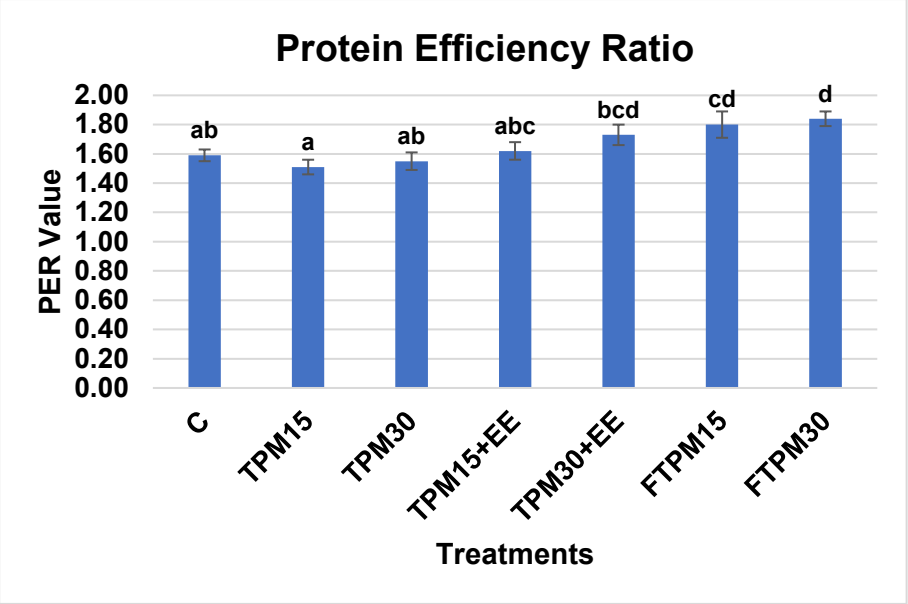


Fig 9: Protein efficiency ratio of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

4.8. Body Indices

4.8.1. Hepatosomatic Index (HSI)

The HSI (%) of *Labeo rohita* fingerlings of different treatments are given in Table 11. The HSI value did not vary significantly ($p<0.05$) among *Labeo rohita* fingerlings of different treatments.

4.8.2. Intestinal Somatic Index (ISI)

The ISI (%) of *Labeo rohita* fingerlings of different treatments is given in Table 11. The fish of TPM_{15} and $FTPM_{30}$ groups showed similar ($p>0.05$) ISI value to control, but the other groups showed significantly lower ($p<0.05$) ISI value than control group.

Table 11: HSI and ISI values of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Treatments[1]	HSI[2] (%)	ISI[3] (%)
C	0.94 ±0.12	3.11^{b}±0.27
TPM_{15}	0.92 ±0.09	2.95^{ab}±0.24
TPM_{30}	0.85 ±0.17	2.89^{a}±0.19
TPM_{15}+EE	0.94 ±0.12	2.84^{a}±0.13
TPM_{30}+EE	1.03 ±0.10	2.63^{a}±0.30
$FTPM_{15}$	1.05 ±0.17	2.73^{a}±0.29
$FTPM_{30}$	0.97 ±0.15	2.92^{ab}±0.29
p-value	*0.944*	*0.014*

Data are expressed as mean ± S.E, n=3; Mean values in the same column with different superscripts differ significantly ($p < 0.05$)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); $TPM_{15,}$ diet with 15% TPM in replacement of 50% DORB; $TPM_{30,}$ diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15,}$ diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30,}$ diet with 30% FTPM in replacement of 100% DORB

[2]HSI, hepatosomatic index; [3]ISI, intestinalsomatic index

4.9. Digestive Enzyme Activities

The activities of intestinal protease, amylase and lipase of *Labeo rohita* fingerlings of different treatments are given in Table 12.

4.9.1. Protease

The intestinal protease activity was significantly different ($p<0.05$) among the treatments (Table 12; Fig.10). The $FTPM_{30}$ group showed significantly higher ($p<0.05$) protease activity than control, but the activity of this enzyme of other groups was similar ($p>0.05$) to control group. Moreover, protease activity of TPM_{15}+EE, TPM_{30}+EE, $FTPM_{15}$ and $FTPM_{30}$ was statistically similar ($p>0.05$).

4.9.2. Amylase

The intestinal amylase activity varied significantly ($p<0.05$) among *Labeo rohita* fingerlings of different treatments (Table 12; Fig. 11). Significantly the highest ($p<0.05$) amylase activity was found in $FTPM_{30}$ group followed by $FTPM_{15}$ and TPM_{15}+EE groups. TPM_{30} group showed the lowest ($p<0.05$) amylase activity. Although the amylase activity of TPM_{15} was similar ($p>0.05$) to control, the fibre digesting enzyme supplemented and fermented TPM fed groups showed significantly higher ($p<0.05$) amylase activity than control group.

4.9.3. Lipase

The lipase activity did not vary significantly ($p>0.05$) among *Labeo rohita* fingerlings of the different treatments (Table 12).

Table 12: Intestinal digestive enzyme activities of *Labeo rohita* fingerlings fed with different experimental diets for the experimental period of 60 days

Treatments[1]	Protease[2]	Amylase[3]	Lipase[4]
C	0.24^{ab}±0.02	5.63^{b}±0.13	0.24±0.01
TPM_{15}	0.21^{ab}±0.008	5.45^{b}±0.07	0.19±0.01
TPM_{30}	0.23^{ab}±0.006	5.01^{a}±0.11	0.19±0.02
TPM_{15}+EE	0.25^{abc}±0.02	6.98^{d}±0.13	0.20±0.02
TPM_{30}+EE	0.26^{abc}±0.008	6.33^{c}±0.08	0.23±0.02
$FTPM_{15}$	0.27^{bc}±0.01	7.65^{e}±0.12	0.23±0.01
$FTPM_{30}$	0.29^{c}±0.007	8.17^{f}±0.13	0.24±0.01
p-value	*0.019*	*<0.001*	*0.078*

Data are expressed as mean ± S.E, n=3; Mean values in the same column with different superscripts differ significantly ($p < 0.05$)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); $TPM_{15,}$ diet with 15% TPM in replacement of 50% DORB; $TPM_{30,}$ diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15,}$ diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30,}$ diet with 30% FTPM in replacement of 100% DORB

[2]Protease activity is expressed in millimole of tyrosine released/min/mg protein

[3]Amylase activity is expressed in micromole maltose released /min/mg protein

[4]Lipase activity is expressed in millimoles/hour/mg protein

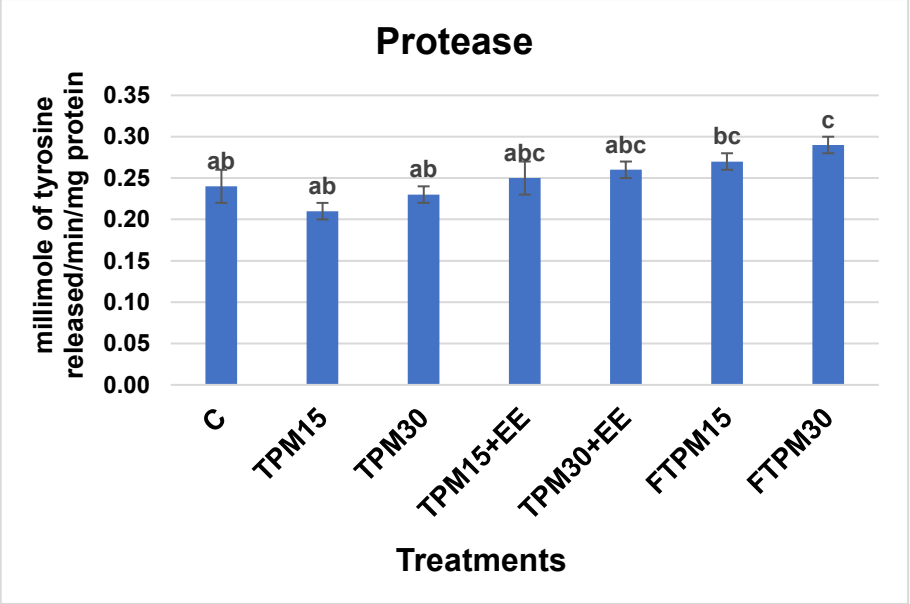


Fig 10: Protease activity in *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

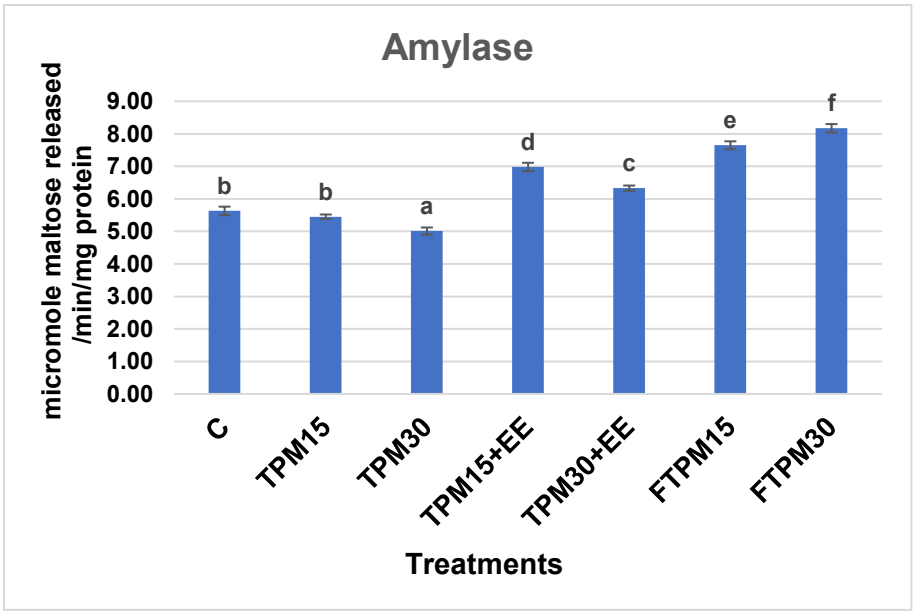


Fig 11: Amylase activity of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

4.10. Enzymes of Protein Metabolism

4.10.1. Aspartate aminotransferase (AST) activity

The hepatic and muscle AST activity of *Labeo rohita* fingerlings fed with different experimental diets is shown in Table 13 and Fig 12. Though the fish of TPM_{15} group showed significantly lower ($p<0.05$) hepatic AST activity, control and TPM_{30} groups showed similar ($p>0.05$) activity of this enzyme. The hepatic AST activity of exogenous enzyme supplemented and fermented TPM fed groups was significantly higher than control group with the highest activity in $FTPM_{15}$ and $FTPM_{30}$ groups. The TPM, enzyme supplemented TPM and fermented TPM fed groups showed significantly higher muscle AST activity than control group with the highest activity in $FTPM_{15}$ and $FTPM_{30}$ groups followed by TPM_{15} + EE and TPM_{30} + EE groups.

4.10.2. Alanine aminotransferase (ALT) activity

The hepatic and muscle ALT activity of *Labeo rohita* fingerlings fed with different experimental diet is shown in Table 13. The liver ALT activity did not vary significantly ($p>0.05$) among the groups. The muscle ALT activity (Fig 9) of fermented TPM fed groups ($FTPM_{15}$ & $FTPM_{30}$) was significantly higher ($p<0.05$) than control group, the muscle ALT activity of other groups was similar ($p>0.05$) to control group. However, the muscle ALT activity did not vary significantly among $FTPM_{15}$, $FTPM_{30}$ and TPM_{30} + EE groups.

Table 13: Activities of protein metabolic enzymes in *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Treatments[1]	AST[2]		ALT[3]	
	Liver	Muscle	Liver	Muscle
C	8.12^{b}±0.14	9.02^{a}±0.19	10.99±0.30	13.52^{a}±0.22
TPM_{15}	7.01^{a}±0.12	10.18^{b}±0.10	11.02±0.26	13.63^{ab}±0.17
TPM_{30}	8.25^{b}±0.16	11.20^{c}±0.12	11.05±0.44	13.76^{ab}±0.15
TPM_{15}+EE	10.02^{d}±0.21	12.05^{d}±0.22	11.08±0.24	13.54^{a}±0.17
TPM_{30}+EE	9.06^{c}±0.19	13.05^{e}±0.20	11.16±0.21	14.04^{abc}±0.14
$FTPM_{15}$	11.02^{e}±0.20	14.19^{f}±0.19	11.53±0.15	14.14^{bc}±0.12
$FTPM_{30}$	11.28^{e}±0.21	14.48^{f}±0.25	11.60±0.23	14.40^{c}±0.18
p-value	*<0.001*	*<0.001*	*0.559*	*0.015*

Data are expressed as mean ± S.E, n=3; Mean values in the same column with different superscripts differ significantly ($p < 0.05$)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB

[2]AST, aspartate aminotransferase, the activity is expressed as micromoles of oxaloacetate formed/mg protein/min

[3]ALT, alanine aminotransferase, the activity is expressed as micromoles of pyruvate formed/mg protein/min

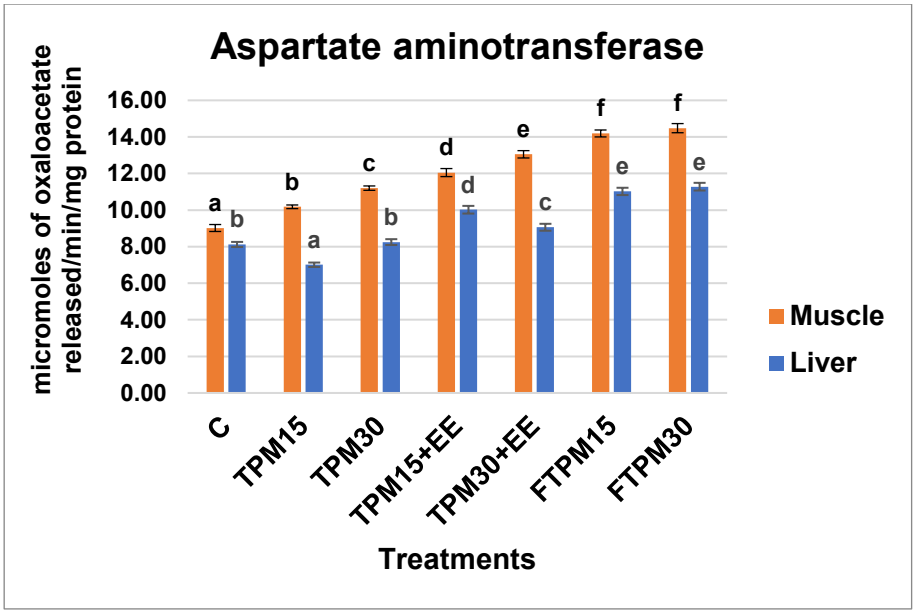


Fig 12: The AST activity in liver and muscle of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

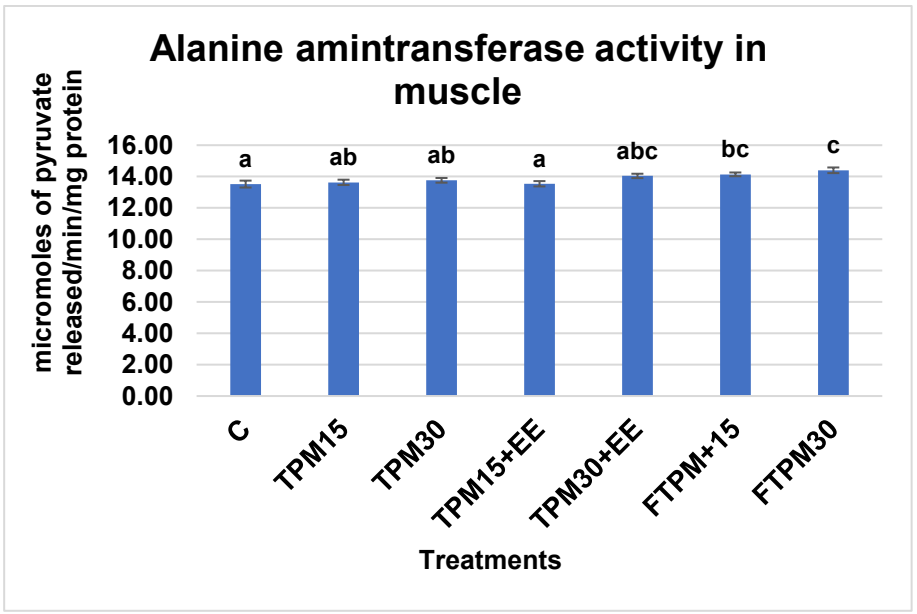


Fig 13: ALT activity in muscle of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscripts on the top differ significantly (P<0.05)

4.11. Enzymes of Carbohydrate Metabolism

4.11.1. Lactate Dehydrogenase (LDH) Activity

The LDH activity in the muscle and liver of *Labeo rohita* fingerlings of the different experimental groups are presented in Table 14 and Fig 14. The fish of TPM_{15}, TPM_{30}, $FTPM_{15}$ and $FTPM_{30}$ groups showed similar ($p>0.05$) muscle LDH activity to control group. However, exogenous enzyme supplemented groups (TPM_{15}+EE & TPM_{30}+EE) showed significantly lower ($p<0.05$) muscle LDH activity than control group. All of TPM, exogenous enzyme supplemented TPM and fermented TPM fed groups showed similar ($p>0.05$) hepatic LDH activity to control, but exogenous enzyme supplemented TPM and fermented TPM fed groups had significantly lower ($p<0.05$) hepatic LDH activity than TPM fed groups.

4.11.2. Malate Dehydrogenase (MDH) Activity

The MDH activity in liver and muscle of *Labeo rohita* fingerlings is presented in Table 14 and Fig 15. All of TPM, exogenous enzyme supplemented TPM and fermented TPM fed groups showed similar ($p>0.05$) muscle MDH activity to control, but TPM_{30}+EE, $FTPM_{15}$ and $FPTM_{30}$ groups showed significantly lower ($p<0.05$) muscle MDH activity than control group. The fish of TPM and exogenous enzyme supplemented TPM fed groups showed similar ($p>0.05$) hepatic MDH activity to control, but fermented TPM fed groups ($FTPM_{15}$ and $FPTM_{30}$) had significantly lower ($p<0.05$) hepatic MDH activity than control group. However, the exogenous enzyme supplemented TPM and fermented TPM fed groups had significantly lower ($p<0.05$) hepatic MDH activity than TPM fed groups.

Table 14: The activities of carbohydrate metabolic enzymes of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Treatments[1]	LDH[2]		MDH[3]	
	Muscle	Liver	Muscle	Liver
C	8.94^{bc}±0.48	5.59^{ab}±0.24	1.04^{abc}±0.04	0.82^{cd}±0.02
TPM_{15}	9.62^{c}±0.35	6.12^{b}±0.31	1.17^{bc}±0.15	0.90^{d}±0.01
TPM_{30}	9.01^{c}±0.39	6.00^{b}±0.37	1.21^{c}±0.02	0.95^{d}±0.03
TPM_{15}+EE	4.28^{a}±0.50	3.52^{a}±0.47	1.11^{abc}±0.01	0.76^{c}±0.03
TPM_{30}+EE	4.12^{a}±0.30	3.20^{a}±0.48	0.98^{ab}±0.03	0.72^{bc}±0.02
$FTPM_{15}$	6.71^{b}±0.39	3.70^{a}±0.29	0.90^{a}±0.04	0.64^{ab}±0.03
$FTPM_{30}$	6.04^{b}±0.41	3.41^{a}±0.41	0.98^{ab}±0.04	0.59^{a}±0.04
p-value	*<0.001*	*<0.001*	*0.044*	*<0.001*

Data are expressed as mean ± S.E, n=3; Mean values in the same column with different superscripts differ significantly ($p < 0.05$)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB

[2]LDH, Lactate dehydrogenase, the activity is expressed in unit/mg protein/min at 37^0C

[3]MDH, Malate dehydrogenase, the activity is expressed in unit/mg protein/min at 37^0C

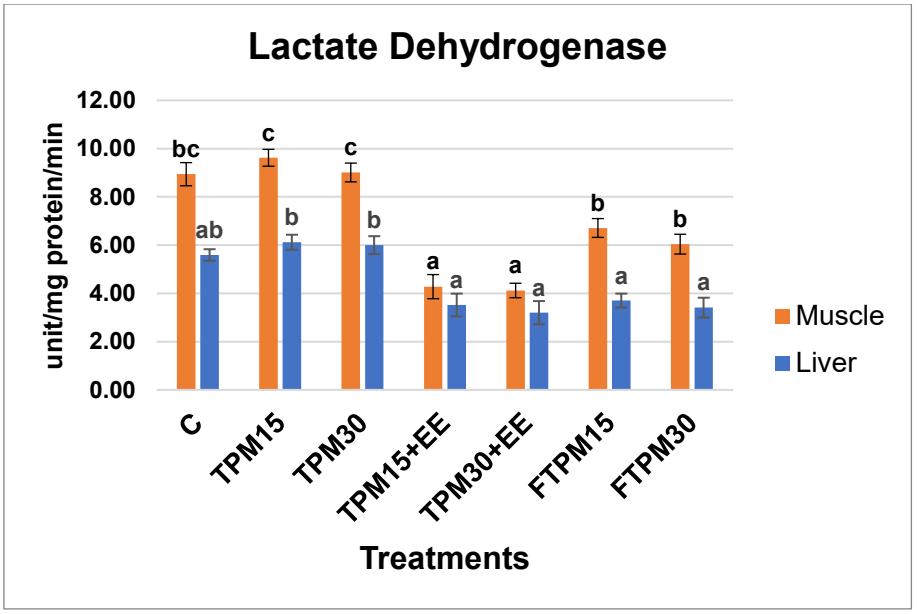


Fig 14: The LDH activity in liver and muscle of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

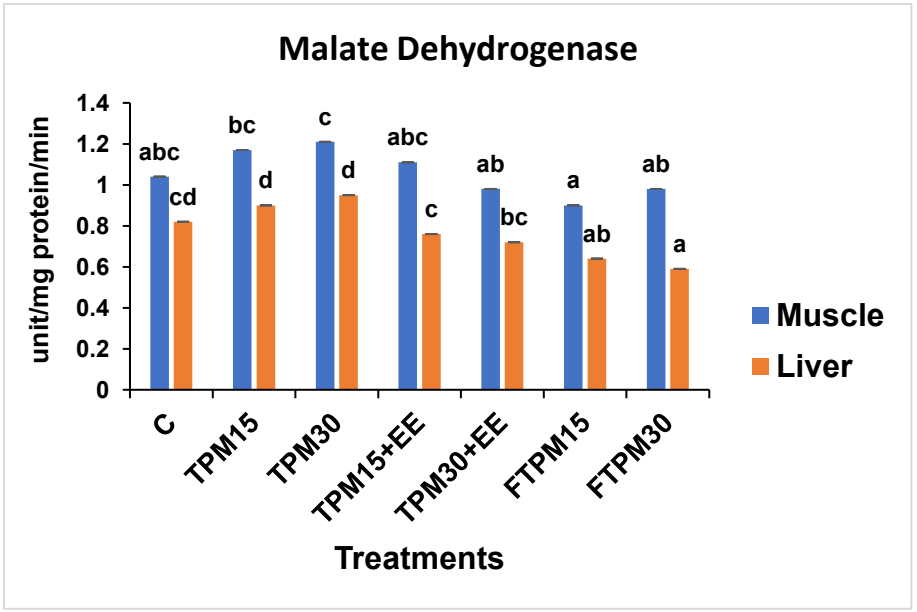


Fig 15: The MDH activity in liver and muscle of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

4.12. Enzyme of Oxidative Stress

4.12.1. Superoxide Dismutase (SOD) Activity

The SOD activity in the liver of *Labeo rohita* fingerlings fed with different experimental diet is shown in Table 15 and Fig 16. The hepatic SOD activity of TPM fed groups (TPM_{15} & TPM_{30}) was statistically similar ($p>0.05$) to control group. However, the exogenous supplemented and fermented TPM fed groups (TPM_{15}+EE, TPM_{30}+EE, $FTPM_{15}$ & $FTPM_{30}$) showed significantly lower hepatic SOD activity than control group. Moreover, hepatic SOD activity did not vary significantly ($p>0.05$) among TPM, exogenous enzyme supplemented TPM and fermented TPM fed groups.

4.12.2. Catalase (CAT) Activity

The hepatic CAT activity of *Labeo rohita* fingerlings did not vary significantly ($p>0.05$) among different treatments (Table 15).

Table 15: Oxidative stress enzymes activity in liver of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Treatments[1]	Liver SOD[2]	Liver CAT[3]
C	18.14^{b}±0.42	13.21±0.46
TPM_{15}	17.10^{ab}±0.50	13.11±0.57
TPM_{30}	17.01^{ab}±0.58	13.97±0.45
TPM_{15}+EE	16.04^{a}±0.55	13.24±0.48
TPM_{30}+EE	16.01^{a}±0.57	13.17±0.55
$FTPM_{15}$	15.92^{a}±0.59	13.14±0.41
$FTPM_{30}$	15.60^{a}±0.33	13.20±0.60
p-value	*0.038*	*0.896*

Data are expressed as mean ± S.E, n=3; Mean values in the same column with different superscripts differ significantly ($p < 0.05$)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB

[2]SOD, Superoxide dismutase, the activity is expressed as 50% inhibition of epinephrine auto-oxidation/mg protein/min

[3]CAT, Catalase, the activity is expressed as nanomoles of H_2O_2 decomposed/min/mg protein

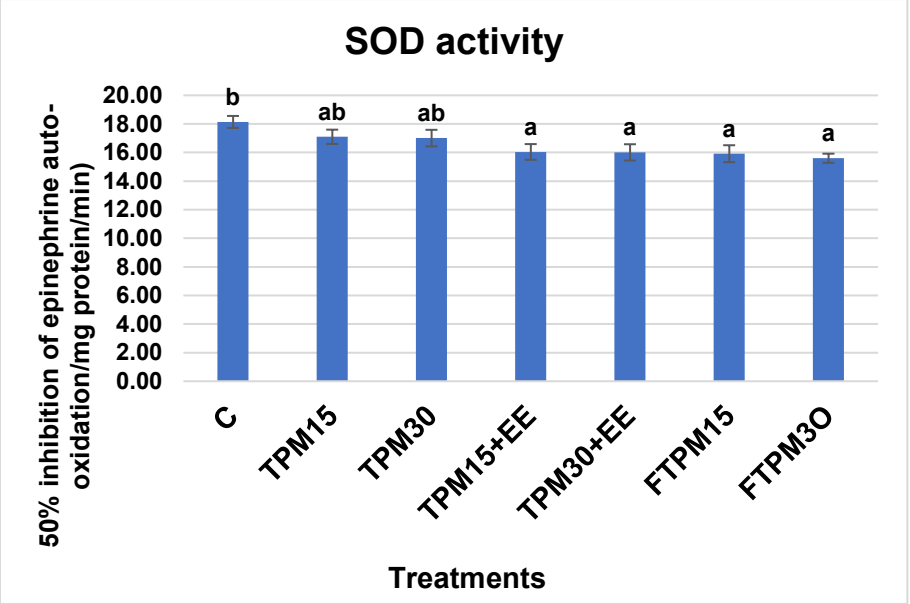


Fig 16: SOD activity in liver of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Bars with different superscript on the top differ significantly (P<0.05)

4.13. Haemato-Biochemical Status

4.13.1. Serum Glucose

The serum glucose level of *Labeo rohita* fingerlings of the different experimental groups is shown in Table 16 and Fig 17. The fish of TPM_{15}, TPM_{30}, $FTPM_{15}$ and $FTPM_{30}$ groups showed similar ($p>0.05$) serum glucose level to control group. However, exogenous enzyme supplemented groups (TPM_{15}+EE & TPM_{30}+EE) showed significantly lower ($p<0.05$) serum glucose level than control group.

4.13.2. Serum Triglycerides

The serum triglycerides level of the different experimental groups is shown in Table 16. There was no significant difference ($p>0.05$) of the serum triglycerides level among the fish of different experimental groups.

4.13.3. Serum Cholesterol

The serum cholesterol level of the different experimental groups is given in Table 16. There was no significant variation ($p>0.05$) of serum cholesterol level among the fish of different experimental groups.

Table16: Serum biochemical status of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Treatments[1]	Serum glucose (mg/dl)	Serum cholesterol (mg/dl)	Serum triglycerides (mg/dl)
C	99.24^{c}±3.69	92.32±1.95	68.06±1.42
TPM_{15}	96.12^{bc}±3.48	91.15±1.33	66.31±1.41
TPM_{30}	95.22^{bc}±3.90	88.47±1.44	65.22±1.44
TPM_{15}+EE	79.05^{a}±3.51	84.16±1.57	62.02±1.45
TPM_{30}+EE	86.48^{ab}±3.41	86.61±2.60	63.21±1.40
$FTPM_{15}$	94.22^{bc}±3.92	89.74±2.20	64.40±1.48
$FTPM_{30}$	92.55^{bc}±3.93	88.02±0.68	67.24±1.38
p-value	*0.026*	*0.068*	*0.091*

Data are expressed as mean ± S.E, n=3; Mean values in the same column with different superscripts differ significantly ($p < 0.05$)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB

4.13.4. Haemato-Immunological Profile

The serum total protein, albumins, globulins and albumin to globulin ratio of *Labeo rohita* fingerlings of different dietary groups are given in Table17. The values of serum total protein, albumins, globulin and albumin to globulin ratio did not vary significantly ($p>0.05$) among different experimental groups.

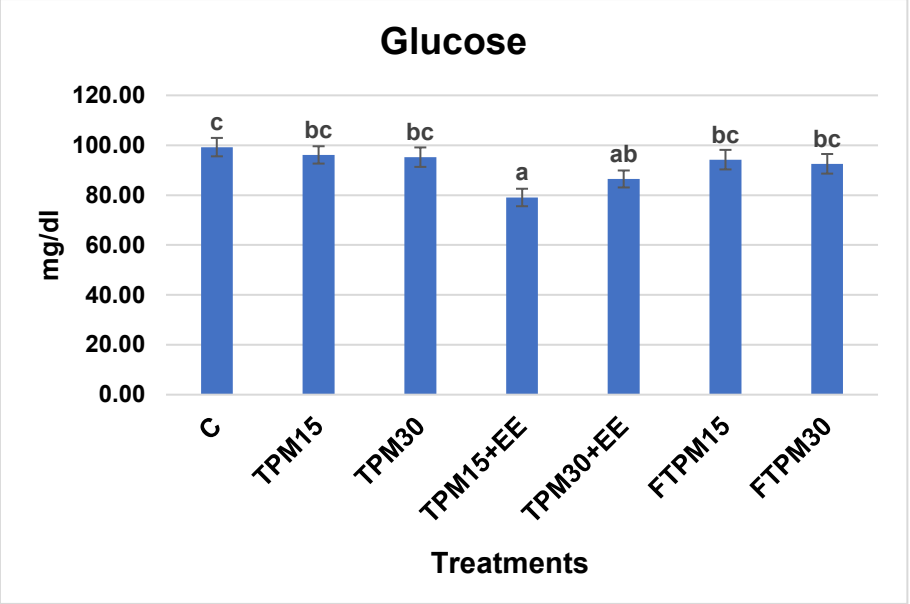


Fig 17: Serum glucose level of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

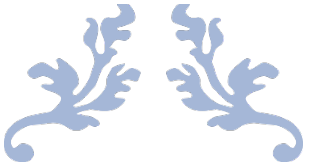
Bars with different superscript on the top differ significantly (P<0.05)

Table 17: Serum immunological profile of *Labeo rohita* fingerlings fed with different experimental diets for the period of 60 days

Treatments[1]	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G[2]
C	3.88±0.16	1.33±0.17	2.55±0.03	0.52±0.14
TPM_{15}	3.86±0.14	1.39±0.23	2.47±0.10	0.56±0.13
TPM_{30}	3.81±0.16	1.36±0.37	2.45±0.23	0.55±0.14
TPM_{15}+EE	3.82±0.15	1.37±0.50	2.46±0.40	0.56±0.10
TPM_{30}+EE	3.85±0.14	1.38±0.48	2.47±0.33	0.56±0.15
$FTPM_{15}$	3.99±0.14	1.48±0.36	2.51±0.22	0.59±0.14
$FTPM_{30}$	3.94±0.19	1.34±0.45	2.60±0.35	0.52±0.10
p-value	*0.980*	*1.000*	*1.000*	*0.791*

Data are expressed as mean ± SE. n=3; Mean values in the same column without superscript do not differ significantly (p>0.05)

[1]C, Control, diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM); TPM_{15}, diet with 15% TPM in replacement of 50% DORB; TPM_{30}, diet with 30% TPM in replacement of 100% DORB; TPM_{15}+EE, TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; TPM_{30}+EE, TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1; $FTPM_{15}$, diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB; $FTPM_{30}$, diet with 30% FTPM in replacement of 100% DORB



DISCUSSION

5. DISCUSSION

A feeding trial of 60 days was conducted to assess the effect of dietary TPM, exogenous enzymes supplemented TPM and fermented TPM on growth, feed and nutrient utilization, physio-metabolic and immunological responses of *Labeo rohita* fingerlings. Accordingly, the fish were distributed in 7 groups *viz.,* C, Control {Diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM)}, TPM_{15} (Diet with 15% TPM in replacement of 50% DORB), TPM_{30} (Diet with 30% TPM in replacement of 100% DORB), TPM_{15}+EE (TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1), TPM_{30}+EE (TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1), $FTPM_{15}$ {Diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB}, $FTPM_{30}$ (Diet with 30% FTPM in replacement of 100% DORB) in triplicate and fed with respective experimental diet daily during entire experimental period.

5.1. In-vitro Relative Protein Digestibility of Tomato Pomace Meal and Fermented Tomato Pomace Meal

The digestibility of nutrients is one of the most important criteria for evaluating the new feed ingredients to be used in feed formulation and varies from species to species. Enhanced *in vitro* protein digestibility of fermented tomato pomace meal in the present study in comparison to its non-fermented counterpart.

5.2. Proximate Composition of the Experimental Diets

In the present study, the diets used were isonitrogenous (30.03 to 30.42%), isolipidic (6.09 to 6.18%) and isocaloric (363.08 to 371.14 kcal DE/100g) as the basis of new ingredient evaluation study. To corroborate the present dietary values, Anwar and Jafri (2001) and NRC (2011) suggested that optimum protein and lipid requirement of *Labeo rohita* could be in the ranges of 30-45% and 5-6%, respectively. The digestible energy values were supported by Rostagno (2000) and NRC (2011).

5.3. Physico-chemical Parameters of Water

In the present study, all the physico-chemical parameters of water such as temperature, pH, dissolved oxygen, free carbon dioxide, total hardness, ammonia-N, nitrite-N and nitrate-N were within the optimum range for aquaculture as suggested by many authors. These findings could indicate that water quality did not produce stress on fish. Indian major carps can thrive well at a temperature range of 18-38^{0}C (Jhingran, 1991), which supports the range observed in the present study (28.5 to 32.9 ^{0}C). Similarly, the pH of water in all the experimental groups ranged from 7.0 to 8.3, which was within the acceptable range (6.5-9.0) as suggested by Swingle (1967). The dissolved oxygen levels in different experimental tanks were recorded within the range of 6.0 to 7.9 mg L^{-1}. This level was found to be within the optimum range (6-8 mg L^{-1}) for cyprinids as suggested by Huet (1975). This was achieved probably due to proving of proper aeration in the experimental units throughout the experimental period. The free carbon dioxide concentration was found to be negligible, as the biomass was optimum and water exchange was done regularly. Hence, it did not have any adverse effect on the survival and growth performance of the *Labeo rohita* fingerlings. The total hardness was found to be within 231-242 mg L^{-1} during the experimental period. Schapercdaus (1933) suggested that water total hardness of 250 mg L^{-1} could be satisfactory for carp culture. The suggested value for ammonia nitrogen in water ranges from 0 to 0.1 mg L^{-1} (Jhingran, 1991), which could support the range (0.02 to 0.09 mg/L) obtained in the present study. In this study, the Nitrite concentration range of 0.04 to 0.09 mg L^{-1} could be within the permissible range for pond aquaculture (Boyd and Tucker, 1998). The nitrate-N level in a productive pond can be within 0.5 to 1.1 mg L^{-1} (Boyd & Tucker, 1998). In the present study, nitrate-N was below the toxic level and did not adversely affect the fish.

5.4. In-vivo Digestibility of Experimental Diets

In the present study, inclusion of 30% TPM in the diet of *Labeo rohita* fingerlings significantly decreased the apparent dry matter digestibility coefficient (ADMDC) in comparison to DORB fed control group. However, the fish fed with 30% exogenous enzyme supplemented TPM or *Aspergillus niger* fermented TPM could show comparable ADMDC with control group. Similar kind of result reported by Ahmad

et al. (2014), Amirkolaie *et al*. (2015) and Maiti *et al*. (2019) during new ingredient evaluation study in the diet of *Labeo rohita* fingerlings.

On the other hand, the apparent crude protein digestibility coefficient (ACPDC) and apparent lipid digestibility coefficient (ALDC) of TPM, exogenous enzyme supplemented TPM and fermented TPM fed groups were similar to the control group. These observations corroborated the findings of Adewolu (2008) in *Tilapia zilli* fingerlings and Shi *et al*. (2017) in crucian carp (*Carassius auratus*).

5.5. Whole Body Proximate Composition of fish

In this study, the whole body proximate composition of *Labeo rohita* did not vary significantly ($p>0.05$) among the different dietary groups. With the agreement of the present findings, Adewolu (2008) and Maiti *et.al*. (2019) could not find variation of carcass composition respectively in *Tilapia zilli* fingerlings and *Labeo rohita* fingerlings during alternate ingredients evaluation study.

5.6. Growth, Feed Utilization and Nutrient Utilization

In the present study, The *Labeo rohita* fingerlings fed with TPM up to 30% in replacement of 100% DORB without or with supplementation of exogenous fiber digesting enzyme showed similar growth (WG, WG% and SGR), feed utilization (FCR) and nutrient utilization (PER) to control. The higher growth (WG, WG% and SGR) and PER and lower FCR in $FTPM_{30}$ and $FTPM_{15}$ group could indicate a better growth in these groups. This might be due to fermentation mediated reduced fiber content. Similarly, Meshram *et al*. (2018) reported better growth performance of fermented sweet potato fed *Labeo rohita* fingerlings than the DORB fed control group. Similarly, Manna *et al*. (2018) observed significantly higher growth performance in *Labeo rohita* fingerlings fed with 30% fermented *moringa oleifera* leaf meal.

Results indicated that supplementation of TPM containing diet with exogenous cellulose-xylanase mixture (1:1) at 0.1% level did not give any extra benefit of fish in relation to growth performance. In corroboration with this finding, Maiti *et al*. (2019) reported that feeding of exogenous fiber digesting enzyme supplemented *Hygrophila spinosa* leaf meal did not affect the growth performance of *Labeo rohita*. Adeoye et *al*. (2016) also reported the similar findings in *Orechromis niloticus* fed with

exogenous enzyme (combination of protease, phytase and xylanase) supplemented diet.

Up to date there is no published information on the incorporation of tomato pomace meal in fish diets as a replacer of DORB or any other ingredients. However, tomato pomace meal could be used as a feed ingredient fed with inclusion rate of 30% in the diet of *Labeo rohita* fingerlings Amirkolaie *et al.* (2018) and of 28% in the diet of Nile Tilapia without adverse effect on growth and nutrient utilization.

5.7. Survival rate %

Overall survivability of rohu, *Labeo rohita* fingerling in rearing system ranges from 60 to 70% (FAO, 2009). However, in the present study, though the survivability of *Labeo rohita* fingerling did not vary significantly among dietary groups, it ranged from 86 to 97%, which could be considered as good survival for carps.

5.8. Hepato-Somatic Index (HSI) and Intestinal-Somatic Index (ISI)

The study of intestinal-somatic and hepato-somatic indices has an important role in the metabolism of fishes, related to digestion and absorption of nutrients, synthesis and secretion of digestive enzymes and carbohydrate metabolism Ighwela *et al.* (2014). In the present study, the HSI values of different experimental groups did not vary significantly. However, the ISI values were higher in the control, TPM and FTPM groups which indicate the physiological wellbeing of digestive system in these groups. From a physiological point, a longer intestinal somatic index would facilitate an increase in digestibility and retention time by enhancing the contact time of the digestive enzymes and the feed components, resulting in increase in the digestion and absorption which support the better feed ulitization by fish in the present study. The result corroborates with the findings of kumar *et al*. (2010).

5.9. Enzyme assays

5.9.1. The activities of digestive enzymes

The protease, amylase and lipase are the digestive enzymes which help in breaking down macro nutrients like protein, carbohydrates and fat, respectively from the feed after ingestion by the fish. In the present study, protease and amylase activity increased in TPM, enzyme supplemented TPM and fermented TPM fed groups in comparison to control group.

Protease is a digestive enzyme, which hydrolyzes the peptide bonds between the adjacent amino acids in the proteins. In the present study, the protease activity in the intestine was significantly different ($P<0.05$) among the different treatments and feeding with 30% FTPM increased the protease activity might be due to fermentation mediated decrease in fiber content (Anand *et al*., 2020; Raman *et al*., 2022).

Amylase activities varied significantly ($P<0.05$) among different the treatments and feeding 30% FTPM supplemented group increased the amylase activity significantly in fish. This might be due to improved digestible carbohydrate availability probably mediated by the externally supplemented fiber degrading carbohydrases. Similarly, Manna *et al*. (2018) found that fermented *Moringa oleifera* leaf meal fed group of *Labeo rohita* showed increased amylase activity. Similar findings also reported by Mondal *et.al*. (2012) in *Labeo bata*.

Lipase activity showed no significant ($P>0.05$) difference among the treatments. It has been observed by Debnath *et al*. (2007) that lipase activity of *Labeo rohita* fingerlings was not altered with respect to the varied dietary composition.

5.9.2. Enzymes of protein metabolism

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are important aminotransferase enzymes in the liver and muscle of fishes that evidently supports in new amino acid synthesis followed by body protein synthesis and growth of fish Jiang *et.al*. (2014). If there is shortage of non-protein energy sources or the fish

are in stress condition, the newly synthesized amino acids, instead of taking part in body protein synthesis, are oxidatively deaminated for energy production at the cost of growth (De Silva and Anderson, 1995). Thus, enhanced tissue AST and ALT may indicate the improved growth or energy production at the cost of growth depending on the situation.

In the present study, both ALT and AST were studied in the muscle and liver of fishes. AST activity in liver has showed a significantly higher values among the treatment groups than control and highest activity was found in $FTPM_{30}$ group. AST activity in muscle also showed a similar trend.

In liver both AST and ALT activity are significantly higher in $FTPM_{15}$ and $FTPM_{30}$ group which are in similar trend with weight gain percentage of the fishes. This might be due to increased utilization of dietary protein derived non-essential amino acids for synthesis of other amino acids for body protein synthesis and growth of fish. Similarly, Hassan *et al*. (2015) showed that ALT and AST levels were increased in Nile Tilapia fed with high level of fermented soyabean meal containing diet.

5.9.3. Enzymes of carbohydrate metabolism

Lactate dehydrogenase is the terminal enzyme of the glycolytic pathway. LDH converts lactate to pyruvate in the presence of coenzyme NADH, which is converted to NAD+. Thus, lactate dehydrogenase helps in maintaining the glycolysis cycle by supplying NAD+. In the presence of enough oxygen, pyruvate enters the Krebs's cycle, but when there is an oxygen shortage in the tissue, pyruvate is converted to lactate (Murray *et al*., 2003). However, in the present study, liver and muscle LDH activities of enzyme supplemented TPM groups were lower than control but other groups showed similar activity of this enzyme to control group. Similar results were observed by Maiti *et al*. (2018), who observed a lower LDH activity in both liver and muscle of rohu due to feeding of carbohydrase supplemented *Hygrophila spinosa* leaf meal.

Malate dehydrogenase (MDH) is an enzyme of the citric acid cycle catalyzes the conversion of malate into oxaloacetate and vice versa. In the present study, fishes fed with fermented tomato pomace meal group showed significant lower value ($p<0.05$) in liver and muscle than other treatment groups and it is in agreement

with results reported by Shamna *et al*. (2014). The author reported that lower activity of MDH in liver and muscle in *Labeo rohita* fingerlings had been related with reduced stress as MDH activity is related with energy demand. In present study it was found that the group with significantly higher growth showed relatively less MDH activity as compared to the control group.

5.9.4. Enzyme of oxidative stress

Oxidative stress arises when the organism has elevated levels of reactive oxygen species (ROS) (Schieber and Chandel, 2014). ROS are byproducts of aerobic metabolism including immune processes, and are chemically reactive for different biological targets. Living organisms are protected from the ROS by several defence mechanisms, including antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase etc. As such, they are an important antioxidant defence in nearly all cells exposed to oxygen. The activity of superoxide dismutase (SOD) comprises the dismutation of superoxide into oxygen and hydrogen peroxide. Catalase is also an endogenous antioxidant enzyme found in nearly all living organisms that are exposed to oxygen, where it catalyzes the decomposition of hydrogen peroxide to water and oxygen. When acclimating to increased levels of oxidative stress, SOD and catalase concentrations typically increase with the degree of stress conditions.

In the present study, significantly lower SOD activities in liver was observed in fish fed with enzyme supplemented TPM and FTPM than control. The lower SOD in enzyme supplemented group probably through reducing the high CF mediated stress. Lower SOD in FTPM fed groups indicates that the free radicals were probably scavenged by the multiple bioactive compounds or anti-oxidative compounds like phenolic compounds, flavonoid and tannins etc. present in FTPM to protect the lipid membrane and other compounds being oxidized or destroyed (Popeskovic *et al*., 1980). In corroboration, Raman *et al*. (2022) reported that group fed with fermented *sesbania* leaf meal show reduced SOD activity than the group fed with raw *Sesabania aculeata* leaf meal. Thus, fish fed with the fermented tomato pomace has improved antioxidant status. However, the catalase activity was found to be non-significant among the control and other treatments.

5.10. Hemato-Biochemical and Immunological Status

Glucose is one of the important source of energy for the animal and its high concentration in serum is considered as indicator of stress (Demeal, 1978; Manush *et al*., 2004). Nakono and Tomison (1967) observed that secretion of catecholamine increased during stress condition, leading to the breakdown of glycogen and enhances blood glucose level. Serum glucose concentration again depends on intestinal absorption, hepatic production, and tissue uptake of glucose. The maintenance of blood glucose concentrations occurs via hepatic glycogenolysis, and gluconeogenesis Guo *et al*. (2012). In the present study, enzyme supplemented TPM fed groups resulted in lower serum glucose level than control might be due to lowering the CF mediated stress, but other groups showed similar serum glucose concentration to control. This result was supported by the findings of Mahmoud *et al*. (2014), who reported that supplementation of exogenous enzymes with plant-based diet significantly reduces serum glucose level than control fed with fish meal based diet. Similar result was also reported by Jahanbakhshi *et al*. (2013) in Great sturgeon (Huso huso).

In the present study, serum triglycerides and cholesterol level did not vary significantly among treatments. A similar result was also observed by Mahmoud *et al*. (2014) in Nile tilapia fed exogenous enzyme supplemented plant-based diet.

Serum protein level is an essential indicator of the non-specific immune system and health status of fish species. Serum proteins are synthesized in liver and play an important role in the immune response. It comprises two major proteins *viz.* the albumins and globulins. Of which, albumins play an important role in homeostasis; it creates an osmotic force that maintains the fluid volume of the vascular space. The serum globulins comprised of alpha, beta and gamma globulins. The gamma globulin fraction is shown to have immune functions in blood and essential for the functional immune status of the fish. However, in the present study, the serum total protein, albumin, globulin and A:G ratio did not vary significantly among the *Labeo rohita* fingerlings of different treatments. These findings indicate that feeding of TPM, exogenous carbohydrase supplemented TPM and FTPM up to 30% level cannot alter the immune status of *Labeo rohita* fingerlings.

SUMMARY

6. SUMMARY

Aquaculture becoming the fastest growing food-producing sector in the world can mitigate future global supply-demand gap for aquatic food, whereas capture fishery production become relatively static since the late 1980s. Aquaculture in India with a growth rate of 7.3% per annum is expected to have greater expansion in the near future through promoting inland culture of diverse species including rohu (*Labeo rohita*). This expansion is possible through feed-based intensified aquaculture practices, which further generate an unremitting higher pressure on ingredients like rice bran, oil cakes and fish meal. While, de-oiled rice bran (DORB) is the major ingredient in fish feed especially carp feed in India that is fed either singly or in combination with other ingredients to carps. DORB production in India is presently 5.5 MMT and not going to support the demand for carp feed. It is expected that in the near future there will be a higher demand for the DORB as expecting aquaculture production will be double by 2025. Hence, there is need to focus on cheaper, readily available and good quality alternative sources of feed ingredients for the partial or complete replacement of the DORB. There is an increasing research effort to evaluate the nutritive value of agro-industrial wastes and by-products to replace the costly ingredients in the fish feed. However, use of these agro-industrial wastes has been limited due to the presence of high crude fiber and anti-nutritional factors. However, supplementation of exogenous enzymes and using a simple technique of solid state fermentation may reduce the ill effect of these ingredients and maximise its utilisation in aqua-feed. In this regard, tomato pomace (TP) is one of the important by-product of tomato processing industry for sauce or ketchup production. Thus, dried tomato pomace meal (TPM) could be an important alternative of DORB in fish feed. Keeping this view in mind, the present study was aimed to optimise the utilisation of tomato pomace meal as DORB alternative in the diet of *Labeo rohita* through exogenous fiber digesting enzyme supplementation and microbial fermentation.

In the present study, three hundred and fifteen (315) acclimated fingerlings of *Labeo rohita* (average body weight 12.08 ± 0.5 g) were randomly distributed in seven treatments *viz.* C, Control {Diet with 30% de-oiled rice bran (DORB) and 0% tomato pomace meal (TPM)}, TPM_{15} (Diet with 15% TPM in replacement of 50% DORB), TPM_{30} (Diet with 30% TPM in replacement of 100% DORB), TPM_{15}+EE

(TPM_{15} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1), TPM_{30}+EE (TPM_{30} diet supplemented with 0.1% cellulose-xylanase mixture at 1:1), $FTPM_{15}$ {Diet with 15% fermented tomato pomace meal (FTPM) in replacement of 50% DORB}, $FTPM_{30}$ (Diet with 30% FTPM in replacement of 100% DORB) in triplicate with the stocking density of 15 fish/tank. Accordingly, seven isonitrogenous (30% CP), isolipidic (6%) and isocaloric (360 kcal DE/100g) experimental diets *viz.* C, Control, TPM_{15}, TPM_{30}, TPM_{15}+EE, TPM_{30}+EE, $FTPM_{15}$ and $FTPM_{30}$ were prepared. The experiment was conducted for 60 days. Feeding was done with respective diet on satiation basis twice daily at 09:00 am and 5:00 pm. The initial and final body weight of fish were measured to assess growth. The proximate analysis of feed revealed that the nutrient contents were as per the requirement of *Labeo rohita* fingerlings.

From this experiment, it is suggested that tomato pomace meal (TPM) can successfully replace DORB in the diet of *Labeo rohita*. TPM contain 17.9% crude protein, which is higher than the DORB but contain more crude fiber (28.4%) with comparable digestible energy content. Hence, supplementation of exogenous fiber digesting enzymes and fermentation of TPM appear to be beneficial in terms of higher digestibility and reducing the stress in fish by making more digestible energy available. Results indicate that the WG, WG%, SGR and PER of control and TPM fed *Labeo rohita* fingerlings were similar. The highest ($p<0.05$) WG, WG%, SGR and PER was observed in $FTPM_{30}$ fed group. The FCR showed the opposite trend of growth in fish. The fish of all groups showed more than 86% survival. The fish of TPM_{15} and $FTPM_{30}$ groups showed similar ($p>0.05$) Intestinal somatic index (ISI) value to control, but the other groups showed significantly lower ($p<0.05$) ISI value than control group. The $FTPM_{30}$ group showed significantly higher ($p<0.05$) protease activity than control, but the activity of this enzyme of other groups was similar ($p>0.05$) to control group. Significantly the highest ($p<0.05$) amylase activity was found in $FTPM_{30}$ group, but TPM_{30} group showed the lowest ($p<0.05$) amylase activity. The control and TPM_{30} groups showed similar ($p>0.05$) hepatic AST activity, but the highest activity of hepatic and muscle AST was found in $FTPM_{15}$ and $FTPM_{30}$ groups. The muscle ALT activity of $FTPM_{15}$ & $FTPM_{30}$ was significantly higher ($p<0.05$) than control group, but the other groups showed similar ($p>0.05$) activity to control group. The fish of TPM_{15}, TPM_{30}, $FTPM_{15}$ and $FTPM_{30}$ groups showed similar ($p>0.05$) muscle LDH activity to control group. However, exogenous enzyme supplemented groups (TPM_{15}+EE & TPM_{30}+EE)

showed significantly lower ($p<0.05$) muscle LDH activity than control group. All of TPM, exogenous enzyme supplemented TPM and fermented TPM fed groups showed similar ($p>0.05$) hepatic LDH activity to control. All of TPM, exogenous enzyme supplemented TPM and fermented TPM fed groups showed similar ($p>0.05$) muscle MDH activity to control group. The $FTPM_{15}$ and $FPTM_{30}$ groups had significantly lower ($p<0.05$) hepatic MDH activity than control group, but other groups showed similar activity to control. The hepatic SOD activity of TPM fed groups (TPM_{15} & TPM_{30}) was statistically similar ($p>0.05$) to control group. However, the exogenous supplemented and fermented TPM fed groups (TPM_{15}+EE, TPM_{30}+EE, $FTPM_{15}$ & $FTPM_{30}$) showed significantly lower hepatic SOD activity than control group. The fish of TPM_{15}, TPM_{30}, $FTPM_{15}$ and $FTPM_{30}$ groups showed similar ($p>0.05$) serum glucose level to control group. However, exogenous enzyme supplemented groups (TPM_{15}+EE & TPM_{30}+EE) showed significantly lower ($p<0.05$) serum glucose level than control group. The values of serum triglycerides, cholesterol, total protein, albumins, globulin and albumin to globulin ratio did not vary significantly ($p>0.05$) among different experimental groups.

In conclusion, TPM can be incorporated in the diet of *Labeo rohita* fingerlings up to 30% with 100% replacement of DORB without compromising the growth performance and health status of fish. However, feeding of TPM with supplemented exogenous cellulose-xylanase mixture (1:1) at 0.1% level does not give any extra benefit in terms of growth performance. On the other hand, 30% FTPM in the diet of *Labeo rohita* shows the highest growth performance and health status of fish might be due to optimum nutrient balance with presence of bioactive compounds.

Future Recommendations:

- However, above 30% FTPM inclusion may give better performance but further research and studies need to be done.
- Long-term feeding trial is suggested to validate the result obtained for *Labeo rohita* through wet laboratory trial.
- Nutrigenomic aspects on the evaluation of tomato pomace meal in the diet of *Labeo rohita* fingerlings need to be addressed.

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APPENDICES

Abbreviation:

ADC : Apparent digestibility coefficients

ALT : Alanine aminotransferase

ANOVA : Analysis of variance

AST : Aspartate aminotransferase

BHT : Butylated hydroxyltoluene

CAT : Catalase

CMC : Carboxy methyl cellulose

CP : Crude protein

DM : Dry matter

DORB : De-oiled rice bran

EE : Exogenous enzyme

FCR : Feed conversion ration

g : Gram

DE : Digestible energy

DORB : De-oiled rice bran

Hr : Hour

Hrs : Hours

HSI : Hepato-somatic index

TPM : Tomato pomace meal

FTPM: Fermented tomato pomace

ISI : Intestinal-somatic index

Kg : Kilogram

L : Liter

LDH : Lactate dehydrogenase

MDH : Malate dehydrogenase

mg : Milligram

Mg/L : Milligram per liter

n mole : Nano mole

NBT : Nitro blue tetrazolium

NFE : Nitrogen free extract

PER : Protein efficiency ratio

SGR : Specific growth rate

SOD : Superoxide dismutase

TA : Total ash

WG% : Percent weight gain

µg : Microgram

µl : Microliter