Orange Peel Waste Improved the Growth Performance, Feed Utilization, Oxidative Stress and Hematological Parameters of *Labeorohita*

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ABSTRACT

Expensive and ineffective feed ingredients in fish feed are becoming a major problem for fish growth in the aquaculture industry. Therefore, the experiment was conducted to investigate the utilization of fruit processing wastes as a dietary component in the feed of rohu (*Labeorohita*). Four different feed formulations were prepared using commercial diet and supplemented with different levels of orange peel powder (CT= 0%, T1= 15%, T2= 30% and T3= 45%). The experiment was performed in aquariums of dimensions 1x1.5x2 feet, filled with approximately 79 liters of water.Fish were fed two times a day at rate of 3% of their body weight for 35 days. The results showed that the T1, T2, and T3 treatments had almost 104%, 160% and 68% higher weight gain than that of CT, respectively. The T1 (7.55%), T2 (17.78%), and T3 (4.26%) treatments had lower feed conversion ratio than that of CT. The T1, T2, T3 treatments had almost 14.18%, 15.27% and 6.11% lower mean corpuscular hemoglobin concentration than that of CT. The T1 and T2 treatments had almost 7.01% and 17.42% higher total serumproteinthan that of CT, respectively. The T1 (0.90%) and T2 (6.23%) treatments had higher A/G ratio than that of CT, respectively and T3 (0.01%) had lower A/G ratio than that of CT. Overall, the dietary supplementation of 30% orange peel improved growth performance, feed utilization, oxidative stress and hematological parameters in *Labeorohita*.

Keywords: Feed utilization; growth performance; orange waste; oxidative stress

1. INTRODUCTION

The cost effective and complete fish nutrition is one of the tremendous barriers to the development, sustainability and endurance of the aquaculture industry(Metian et al., 2020). Aquaculture has been admired most extended and ideal source of protein from animal origin consumed by humans. Globally it is providing a safety measure towards the human nutritional requirement. The rapid growth and successful marketing of fish have made aquaculture an economically significant industry(Jahan et al., 2021). The rising world population is likely to hit 9 million by 2050 and food requirements for this prospective population must be fulfilled. Whereas, aquaculture occupies substantial potential and aims to reach the remarkable demands of biological worth protein. Globally the production of freshwater aquaculture recently extended 47.9 million tons in 2016 and 59.7% had been reported to carps. To meet the proposed consumption appeal, worldwide aquaculture needs to put out an additional 47.5 million tons of fish by 2050. Universally, *Labeorohita* is significant aquaculture specie in countries like Pakistan, India, Bangladesh, Myanmar, Vietnam, Laos and Nepal. *Labeorohita* is contributing to about 3.7% of total finfish production and as a single specie come up with about 15% of worldwide freshwater aquaculture. In the Asian sub-continent *Labeorohita* is considered more classical due to its high growth rate, omnivorous feeding behavior, well adaptability to commercial feed,

excessive nutritional importance, palatability as well as high public demand. *Labeorohita* possesses polyculture ability and therefore can be reared easily with other fish species. Intensive fish culture with technological interference is the only well-adapted option to feed the ever-growing world population(Jobling, 2016). However, intensive fish culture systems are manifested to a number of external environmental constraints that affect their immune system. In this way, fish farmers face challenges related to disease and costly feed elements(Lim et al., 2021). For cost-effective fish production, the reuse of fruit by-products as feed constituents can be a feasible option(Md Nor and Ding, 2020).

The high cost of fish feed has affected the viability and yielding profit of fish farming(Glencross et al., 2020). Traditional ingredients of fish feed report high production costs. Prices of traditional aquafeed constituents such as soya beans are steadily increasing due to low availability and high demand(Naylor et al., 2021). Thus, some other ingredients such as fruit by-products are broadly used in fish feed by means of functional feed additives and as non-traditional ingredients(Felix e Silva et al., 2020). Grapes, apples, bananas and citrus fruits are extensively used up fresh and also utilized in food processing industries(Ben-Othman et al., 2020). All around the world orange (Citrus *aurantiumdulcis*) is a vital citrus fruit with a production of 49.6 million tons in 2016/17(Maharjan et al., 2017). On the whole, approximately 70% of orange production(Siles et al., 2016) is utilized in making jam and juice and about 50% of orange peel is obtained throughout the juice production which is also reputed as a by-product with low economic value(Verma et al., 2020). A very small amount of orange peel is graded in food preparations like in baking, beverages and sauces. While the rest of the peels represent serious soil pollution(Xiao et al., 2018). Noticeably, the misuse of orange peel could result in the loss of beneficial nutrients. Therefore, recycling of fruit waste is necessary to avoid environmental problems and generate profitable sources of raw products for animal feed(Lee et al., 2019). By-products of fruit processing provide favorable sources of useful compounds comprising essential vitamins, minerals, antioxidants and phytochemicals(Qiang et al., 2019). Manufacturing feed from fruit waste could be the potential to reduce production expenses and enhance aquaculture profits(Choi et al., 2016). Therefore, we hypothesized that addition of orange peel in fish could be beneficial for fish growth due to high nutritional value. Specifically, the study objectives were to; 1) analyze the feed utilization efficiency and growth performance of *Labeorohita*, 2) the effects of orange peel feed on *Labeorohita* oxidative stress and Hematological indices.

2. MATERIAL AND METHODS

2.1. Processing of Orange Peels

Orange peels were collected from the local markets of Sheikhupura and Faisalabad, Pakistan. The orange peels were washed with plain water to eliminate pollutants and further were oven dried at 60°C until a constant weight was obtained. Dried orange peels were atomized using an electric blender and were sieved through a 320µm mesh.

2.2. Diet formulation

Four experimental diets were prepared according to the nutritional requirement of *Labeorohita* by NRC(2012) and orange peels were used as a substitution of yellow corn. Orange peel powder of 0%, 15%, 30% and 45% respectively for CT, T1, T2 and T3 groups was mixed with commercial feed to make four experimental diets. Formulated diets were stored at +4°C until use. The nutritional components of the formulated diets, including crude protein, crude fat, crude fiber, moisture and ash content, were analyzed using the standard methods by AOAC (Chemists, 2009) showed in Table 1. Analysis of orange peel revealed 7.91% crude protein, 3.41% fat, 13.02% fiber, 5.15% moisture and 5.32% ash content.

Table 1. Diet ingredients and chemical composition of four formulated diets.

Ingredients (% kg^{-1} diet)	CT 0% orange peel	T1 15% orange peel	T2 30% orange peel	T3 45% orange peel
Fish meal[1]	14	14	14	14
Soybean	16	16	16	16
Rice bran	11	11	11	11
Yellow corn	49	34	19	4
Corn gluten	5	5	5	5
Vegetable oil	3	3	3	3
Mineral[2]	1	1	1	1
Vitamin[3]	1	1	1	1
Orange peel	0	15	30	45
Total	100	100	100	100
Proximal Analysis (%)				
Moisture	10.81	10.75	10.61	10.89
Ash	5.33	6.46	6.53	7.72
Crude protein	24.98	24.82	24.80	24.77
Crude fats	5.36	6.49	7.61	8.69
Crude fiber	6.23	6.54	7.66	8.21
Carbohydrates[4]	47.29	44.94	42.79	40.00
Energy (kJ/g)[5]	16.19	16.19	16.26	16.19
P/E ratio (mg/kJ)[6]	1542.57	1532.50	1525.08	1529.17

[1]Fish meal with 60% crude protein

[2]K, Al, Mg (55g), Cu (600mg), P (135g), Na (45g), Fe (1000mg) and Ca (155g).

[3]Vitamin B12 (40 mg), Vitamin C (15,000 mg), Vitamin K3 (8000 mg), Nicotinic acid (60,000mg), Calcium pantothenate (12,000 mg), Folic acid (1500 mg), Vitamin D3 (3,000,000 IU), Vitamin E (30000 IU), Vitamin A (15,000,000 IU), Vitamin B1 (3000 mg), Vitamin B2 (7000 mg) and Vitamin B6 (4000 mg).

[4]Carbohydrates (%) = 100 – (% crude protein + %crude protein + %carbohydrates + ash +moisture + fiber)(Castell and Tiews, 1980)

[5]Energy (kJ/g diet) = (% crude protein x 23.6) + (% crude lipid x 23.6) +(%carbohydrates x 23.6) (Chatzifotis et al., 2010)

[6]Protein to energy ratio (P/E) (mg/kJ) = % crude protein/energy

2.3 Fish and feeding trials

Forty *Labeorohita* fingerlings being apparently healthy and weighing 28±0.05 g were collected from Fish Seed Hatchery, Sheikhupura, Punjab, Pakistan. Fingerlings were kept under acclimation period for 10 daysduring which they were fed exclusively with commercial feed and after the acclimation period, the fish were divided into four dietarygroups (n=10). The study was conducted in 1x1.5x2 feet (width x height x length) aquariums, each filled with about 79 liters of water. Throughout the experiment, the fish were fed with experimental diet (constituting of 25% crude protein) twice daily (7:00 AM and 4:00 PM) comprising 3% of their body weight following a photoperiod of 12 hours. Daily water renewal activity was performed two times a day to eliminate feces and food leftovers.

2.4 Water Quality Parameters

Constant aeration was supplied in all the four aquaria filled with de-chlorinated tap water with optimal level of physio chemical characteristics (water temperature at 26.02±0.57°C, dissolved oxygen levels of 4.01±0.37ppm, pH levels of 5.48±0.27, ammonia levels of 0.21±0.17mg/L, and total hardness of 44.36±0.07mg/L) for 35 days.

2.5Growth Performance

Growth response of *Labeorohita* under different percentages of orange peel were recorded for a period of 35 days. After 35 days, fish were starved for 24h, weighted for calculation of growth parameters. Standard formulas were used to calculate the weight gain (WG), weight gain percentage (WG%), average daily gain (ADG), specific growth rate (SGR), and survival rate.

WG (g) = Final weight -Initial weight (1)

(WG%)= (Final weight (g) – Initial weight (g))/(Initial weight (g))
(2)

SGR (%/day)=(Ln Final weight – Ln Initial weight)/(Trial days) ×100
(3)

ADG (g/fish/day)=(Average final weight – Average initial weight)/(Trial days) ×100
(4)

Survival rate (%)=(Final fish count)/(Initial fish count) ×100
(5)

2.6. Feed Utilizing Efficiency

By using the following standard formulas, feed conversion ratio (FCR), feed efficiency (FE), and protein efficiency ratio (PER) were calculated.

FCR =(Total feed consuption (g))/(Weight gain (g))
(6)

FE =(Weight gain (g))/(Total feed consuption (g))
(7)

PER =(Weight gain (g))/(Protein fed (g))
(8)

2.7. Hematological Parameters

At the end of the experiment, triplicate from each aquarium were taken and were anesthetized with 25 mg L-1 clove oil. Blood samples were collected from the caudal vein using 1mL sterile syringe and samples were quickly transferred to a vial coated with EDTA (an anti-coagulant) and kept refrigerated at 4°C for subsequent analysis. The results for red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and hematocrit (Hct) were determined by using hematology analyzer CELLDYN Emerald 22 AL. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined using formulas as specified by Bain et al. (2012).

MCV (fL)=(Hct)/(RBC) ×100
(9)

MCH (pg)=Hb/(RBC) ×100
(10)

MCHC (g/dL)=Hb/(Hct) ×100 (11)

2.8. Protein Metabolic and Oxidative Stress Enzymes Activity

To analyze serum parameters, blood samples were obtained from three fish in each group. The blood samples were left to coagulate at room temperature and then centrifuged in sterilized vials. Following centrifugation at 3000×g for 10 minutes, the serum was carefully separated from the clotted blood and collected in sterile Eppendorf tubes. The collected serum was stored at -20°C for subsequent analysis of serum biochemical parameters. Analysis of AST and Alt activities of serum and liver was carried out by using colorimetric assay kits, ERBA SGPT KIT (Code Number – 120207), and ERBS SGOT KIT (Code Number – 120204) respectively. The total protein and albumin levels were evaluated using colorimetric methods, as described by Henry(Wootton, 1964) and Wootton(Reitman and Frankel, 1957), respectively. The globulin level was determined by subtracting the albumin level from the total protein level. Subsequently, the albumin-globulin ratio was calculated.

2.9. Statistical Analysis

Data are expressed as mean±SE. Statistix version 8.1 was used for statistical analysis. One-way-analysis of variance (ANOVA) was used to study the differences in growth parameters, feed utilization, hematological parameters, and protein metabolic and oxidative enzyme

activity of *Labeorohita*. Differences in means were determined by using Tukey's test and significant difference were set at $p<0.05$.

3. RESULTS

3.1 Growth performance

In the present study, varying levels of orange peel significantly (P<0.05) affected the growth in terms of WG, WG%, ADG, and SGR of *Labrorohita* (Table 2). The results showed that T2 had maximum WG, WG% and ADG as compared to other treatments. Specifically, the T1, T2, and T3 treatments had almost 104%, 160% and 68% higher WG than that of CT, respectively. The difference among orange peel treatments T1 and T2 was about 21.42% and 54. 76% higher WG, WG% and ADG as compared to T3. SGR was significantly higher in T2. The T1, T2, and T3 treatments had almost 82%, 120% and 55% higher SGR than that of CT, respectively. Moreover, T1 and T2 was about 17.02% and 41.33% higher SGR as compared to T3, respectively. Survival rate of *Labeorohita* was not affected by dietary supplementation of orange peel. The results showed that all treatment groups (T1, T2 and T3) and control group had same survival rate of about 100% (Table 2).

Table 2. Growth performance of *Labeorohita* fed diets supplemented with graded levels of orange peel.

Treat-ments	Orange Peel	IW (g)	FW (g)	WG (g)	WG (%)	ADG (g/fish/day)	SGR (%/day)	Survival Rate (%)
CT	0%	28±0^{a}	36.33±0.88^{c}	8.33±1.52^{c}	29.76±3.15^{c}	0.23±0.02^{c}	0.74±0.06^{c}	100±0^{a}
T1	15%	28±0^{a}	45±0.57^{b}	17±0.57^{b}	60.71±2.06^{b}	0.48±0.01^{b}	1.35±0.03^{b}	100±0^{a}
T2	30%	28±0^{a}	49.66±0.88^{a}	21±0.88^{a}	77.38±3.15^{a}	0.61±0.02^{a}	1.63±0.05^{a}	100±0^{a}
T3	45%	28±0^{a}	42.0±0.57^{b}	14.0±0.57^{b}	50±2.06^{b}	0.4±0.01^{b}	1.15±0.03^{b}	100±0^{a}

IW= Initial weight, FW= Final weight, WG= Weight gain, ADG= Average daily gain, SGR= Specific growth rate.

3.2 Feed utilizing efficiency

In the present study, varying levelsof orange peel significantly (P<0.05)affected feed utilizing efficiency of *Labeorohita* (Figure 1). The results showed that T2 had best (lowest) FCR as compared to other treatments. Specifically, the T1, T2, and T3 treatments had almost 7.55%, 17.78% and 4.26% lower FCR than that of CT, respectively. The T1 and T2 had about 17.02% and 41.33% lower FCR as compared to T3, respectively. The maximum FE and PER was recorded in T2 as compared to other treatments. The T1, T2, and T3 treatments had almost 9.17%, 22.01% and 3.66% higher FE than that of CT, respectively. The T1, T2, and T3 treatments had almost 8.69%, 21.96% and 4.34% higher PER than that of CT, respectively. The T1 and T2 were about 4.16% and 16.88% higher PER as compared to T3, respectively(Figure 1).

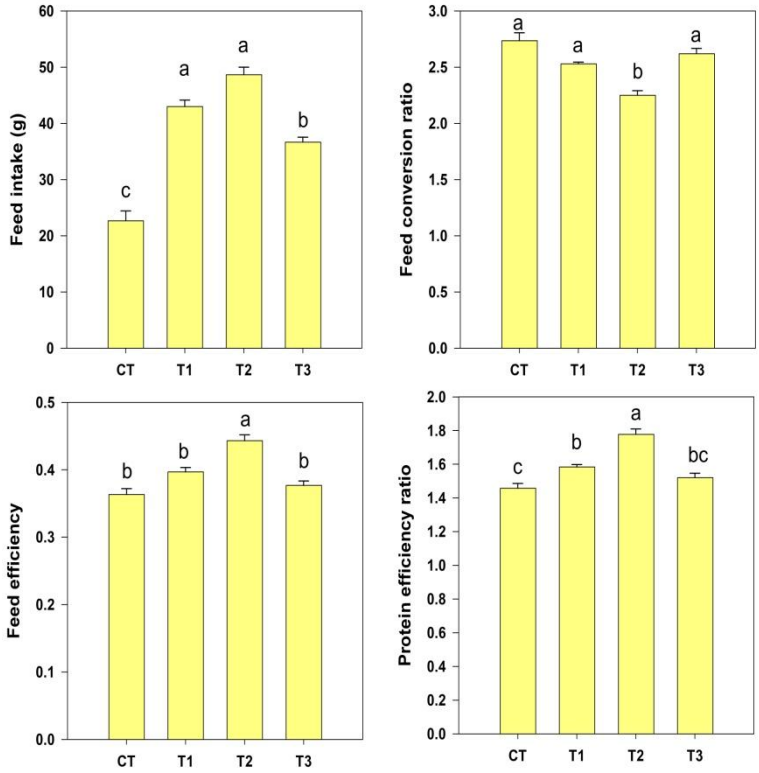

Figure 1. Feed utilizing efficiency parameters of *Labeorohita* fed with graded levels of orange peel. (FI= Feed intake, FCR= Feed conversion ratio, FE= Feed efficiency, PER= Protein efficiency ratio).

3.3 Hematological analyses

In the present study, varyinglevelsof orange peel significantly (P<0.05)affected hematological parameters of *Labeorohita*(Table 3)The results showed that T2 had maximum RBC as compared to other treatments. Specifically, the T1, T2, and T3 treatments had almost 18.95%, 29.93% and 1.69% higher RBC than that of CT, respectively. It was noticed that T2 had best (lowest) WBC as compared to other treatments. Specifically, the T1 and T2 treatments had almost 18.95%, 37.28% lower WBC count than that of CT, respectively and T3 had 12.27% higher WBC count than that of CT. The maximum values of Hb and Hct were recorded in T2 as compared to other treatments. The T1, T2, and T3 treatments had almost 1.62%, 8.07% and 0.64% higher Hb than that of CT, respectively. The T1 and T2 were about 0.98% and 7.37% higher Hb as compared to T3, respectively. The T1, T2, and T3 treatments had almost 18.44%, 27% and 7.20% higher Hct than that of CT, respectively. The results revealed that T2 had best (lowest) HCV, MCV and MCHC values as compared to other treatments. Specifically, the T1 and T2 treatments had almost 0.03%, 1.84% lower HCV than that of CT, respectively and T3 had 5.47% higher HCV than that of CT. The difference among orange peel treatments, T1 and T2 were about 5.22% and 6.94% lower MCV as compared to T3, respectively. Similarly, the T1, T2, and T3 treatments had almost 14.21%, 16.81% and 0.97% lower MCH than that of CT, respectively. The T1 and T2 were about 13.37% and 15.99% lower MCH as compared to T3, respectively. The T1, T2, and T3 treatments had almost 14.18%, 15.27% and 6.11% lower HCMC than that of CT, respectively.

Table 3.Hematological parameters of *Labeorohita* fed diets supplemented with graded levels of orange peel.

Treatments	Orange peel	RBC (10^{12}/L)	WBC (10^{9}/L)	Hb (g/dL)	Hct (%)	HCV (fL)	MCV (pg)	MCHC (g/dL)
CT	0%	1.57±0.01^{c}	24.46±0.04^{b}	9.78±0.02^{b}	25.43±0.01^{d}	162.01±1.11^{b}	62.31±0.38^{a}	38.46±0.02^{a}
T1	15%	1.86±0.05^{d}	19.82±0.06^{c}	9.94±0.00^{b}	30.12±0.00^{b}	161.95±0.45^{b}	53.45±0.11^{b}	33.00±0.01^{c}
T2	30%	2.04±0.01^{a}	15±34±0.08^{d}	10.57±0.23^{a}	32.43±0.17^{a}	159.01±1.58^{b}	51.84±1.36^{b}	32.59±0.55^{c}
T3	45%	1.59±0.02^{c}	27.46±0.26^{a}	9.84±0.00^{b}	27.26±0.00^{c}	170.88±3.14^{a}	61.71±1.16^{a}	36.11±0.02^{b}

RBC= Red blood cell, WBC= White blood cell, Hb= Hemoglobin, Hct= Hematocrit, MCV=Mean corpuscular volume, MCH= Mean corpuscular hemoglobin, MCHC= mean corpuscular hemoglobin concentration.

3.4 Protein Metabolic and Oxidative Stress Enzyme Activity

In the present study, varying levelsof orange peel significantly (P<0.05)affected protein metabolic and oxidative stress enzyme activity of *Labeorohita* (Table 4). The results showed that T2 had best (lowest) AST and ALT as compared to other treatments. Specifically, the T1, T2, and T3 treatments had almost 8.35%, 11.73% and 3.77% lower AST than that of CT, respectively. The T1 and T2 treatments were about 4.76% and 8.27% lower AST as compared to T3. The T1, T2, and T3 treatments had almost 12.58%, 19.07% and 6.61% lower ALT than that of CT, respectively. The difference among orange peel treatments, T1 and T2 were about 6.39% and 13.34% lower ALT as compared to T3, respectively. The results revealed that T2 had maximum serumtotal protein, albumin, globulin and A/G ratio as compared to other treatments. Specifically, the T1 and T2 treatments had almost 7.01% and 17.42% higher total protein than that of CT, respectively and T3 had 3.39% lower serumtotal protein than that of CT. The difference among orange peel treatments, T1 and T2 was about 10.77% and 21.54% higher serumtotal protein as compared to T3, respectively. Specifically, the T1 and T2 treatments had almost 7.48% and 20.86% higher albumin than that of CT, respectively and T3 had 3.40% lower albumin than that of CT. The T1 and T2 treatments had about 11.26% and 25.11% higher albumin as compared to T3, respectively. Similarly, the T1 and T2 treatments had almost 6.52% and 13.76% higher globulin than that of CT, respectively and T3 had 3.38% lower globulin than that of CT. The difference among orange peel treatments, T1 and T2 were about 10.25% and 17.75% higher globulin as compared to T3, respectively. The T1 and T2 treatments had almost 0.90% and 6.23% higher A/G ratio than that of CT, respectively and T3 had 0.01% lower A/G ratio than that of CT. The difference among orange peel treatments, T1 and T2 were about 0.91% and 6.25% higher A/G ratio as compared to T3, respectively.

Table 4. Protein metabolic and oxidative stress enzyme activity of *Labeorohita* fed diets supplemented with graded levels of orange peel for 35 days.

Treatments	Orange peel	AST (U/L)	ALT (U/L)	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G Ratio
CT	0%	48.33±0.01^{a}	32.27±0.02^{a}	2.85±0.01^{c}	1.47±0.00^{c}	1.38±0.00^{c}	1.06±0.00^{c}
T1	15%	44.29±0.02^{c}	28.21±0.02^{c}	3.05±0.01^{d}	1.58±0.00^{b}	1.47±0.00^{b}	1.07±0.00^{b}
T2	30%	42.66±0.01^{d}	26.11±0.02^{d}	3.34±0.02^{a}	1.77±0.00^{a}	1.57±0.00^{a}	1.13±0.00^{a}

T3	45%	46.51±0.02[b]	30.14±0.01[b]	2.75±0.01[d]	1.42±0.00[d]	1.33±0.00[d]	1.06±0.00[c]

AST= Aspartate aminotransferase, ALT= Alanine aminotransferase, A/G Ratio= Albumin/Globulin Ratio.

4. DISCUSSION

Byproducts from fruit and vegetable processing have long been used as key components in the formulation of animal feed(Altendorf, 2018). The impact of these residues on animal growth performance has been widely researched(Pfaltzgraff et al., 2013). Citrus peels, which are often discarded as waste after juice extraction, contain a variety of bioactive compounds such as vitamin C, phenols and flavonoids(Singh et al., 2020). A study revealed that diets supplemented with citrus peel under heat or low oxygen stress showed improved growth, immune system, antioxidant capacity and blood profile of Nile tilapia(Vicente et al., 2019). In addition, several studies have reported positive results of using citrus peel extracts as a nutritional additive in aquafeeds. Similarly, Shabana et al.(2019) studied the effects of orange peel and the results showed significant increase in the survival, growth, digestive enzyme activity, muscle biochemical composition and profile amino acid and fatty acid.

Fruit waste could provide nutrients for fish metabolism, as citrus by-products contain a variety of bioactive compounds, including phenolic compounds, carotenoids, vitamins, essential oils, and fibers. These compounds provide nutritional value and health benefits to aquatic lives when used in proper proportion with commercial feed(Magalhães et al., 2023). In this study significant improvement in terms of WG, ADG, and SGR of *Labeorohita* (Table 2) by the dietary inclusion of orange peel, highlighting the potential of orange peel supplementation to stimulate fish growth. This improvement was possibly due to the orange peel nutritional composition, mainly due to the presence of phenolic compounds like gallic acid and ferulic acid(Dawood et al., 2020). In particular, these compounds could enhance digestive processes by increasing enzyme activity, improving nutrient digestibility, and feed absorption, leading to enhance the growth in fish and livestock(Cai et al., 2020). Previously, Salem and Abdel-Ghany (2018) reported that dietary inclusion of orange peel had better growth in Nile tilapia. In the current study, the lowest values of growth were observed in *Labeorohita* fed with 0% followed by 15%, and 45% orange peel supplemented diet which could be attributed to varying concentrations of antioxidants under different application rates (Table 2). These concentrations may not meet the fish's optimal antioxidants requirement(Singh et al., 2020). It is worth to mention that similar results were observed by Doan et al.(2018) who investigated the impact of pectin extracted from orange peel (OPDP) on the growth performance of Nile tilapia (*Oreochromisniloticus*) and showed that lowest growth performance was observed by the dietary administration of 5 and 20g kg-1 OPDP. Statistical analysis ($p>0.05$) indicated no significant difference in survival rate among the groups, suggesting that the treatments involving varying concentrations (0.0%, 15%, 30%, and 45%) of orange peel did not impose negative effect on the survival of *Labeorohita*. Similar findings were reported by Salem et al.(2019) in their study on sea bream (*Sparusaurata*) with orange peel feed.

The feed conversion ratio (FCR) is a metric used to evaluate the effectiveness of a particular feed or feeding strategy in terms of its efficiency(United States Agency for International Development (USAID), 2011). The protein efficiency ratio (PER) is utilized to estimate the efficiency of converting feed protein into aquatic animal protein(Boyd, 2018). In the present study of 35 days, it was observed that *Labeorohita* exhibited the highest FE and the lowest FCR (Figure 1) with 30% orange peel and this dietary supplementation led to the highest protein efficiency ratio, indicating a more effective utilization of dietary protein by the fish. This could be due to the enzymes found in fruit by-products that could improve villus length,

mucosal folds, lipase and pepsin enzymatic activity, as well as improve gut tissue and intestinal absorptive cells, leading to improved nutrient absorption, and digestion in fish(Bowyer et al., 2020). It has been suggested by studies that orange peel contains a significant quantity of saponin(Oluremi et al., 2007). A study conducted on Nile tilapia fingerlings investigated the effects of diets supplemented with ginseng herb containing saponin. The results indicated that the inclusion of herbal supplements with saponin significantly enhanced the growth and dietary utilization efficiency of the Nile tilapia fingerlings (Goda, 2008). Contrarily, 0%, 15%, and 45% orange peel supplementation showed lower feed intake and higher feed conversion ratios and consequently, resulted in a lower protein efficiency ratio compared to the group with 30% orange peel supplementation, indicating reduced efficiency in nutrient utilization. This can be attributed to the presence of varying concentrations of phytochemicals in orange peel supplementation, which may act as absorbents that bind and eliminate undesirable constituents, including pathogens, in the digestive tract, while simultaneously enhancing the absorption of essential nutrients(Virgili and Marino, 2008). Similar results were obtained by Mayssara A. Abo Hassanin et al.(2019)who studied the effects of varying concentrations of orange peel on growth performance and feed utilization of red tilapia. In agreement with Salem and Abdel-Ghany, (2018) we also confirmed that dietary orange peel with an optimum level could improve the intestine nutrient absorptive ability of fish.

Fish blood, orhematology, is a valuable tool in evaluating the physiological health of fish. Particularly in the management of cultured fish, hematological indices are widely recognized as indicators of physiological characteristics(de MORAES et al., 2017). In the present study, 30% of orange peel supplemented diet had best blood profile regarding all blood components including RBC, WBC, Hb, Hct, HCV, MCH, and HCMC in comparison to other groups fed with 0%, 15% and 45% orange peel. Possibly, due to the quality of their diet and particularly optimal concentrations of feed substances(Svobodova et al., 1991) (Table 4). In addition, proper nutrition ensures that fish have the necessary components to support healthy blood composition and superior oxygen-carrying capacity(Esmaeili, 2021). The increase in RBC, Hb, and Hct in groups fed with varying concentrations of orange peel (15%, 30% and 45%) as compared to control, indicated beneficial effects of orange peel on the health of *Labeorohita*. This could be attributed to flavonoids present in orange peels that might prevented phospholipid peroxidation on the erythrocyte membrane, thereby protecting red blood cells from lysis(Mukrimaa et al., 2016). Acar et al.(2015) revealed that orange peel in the diets of tilapia significantly increased ($p<0.05$) erythrocyte count, hemoglobin and hematocrit values. The levels of leukocytes in the bloodstream can vary based on factors such as environmental quality, nutritional status and the presence of infectious agents(Humphries et al., 2021). This study revealed that fish fed with 30% orange peel had lowest values of WBC that could be due to the increasing frequency of feed intake by the fish(Tani et al., 2021). The blood parameters such as MCV, MCH, and MCHC, are sometimes collectively referred to as red blood cell indices which assist in evaluating the size, hemoglobin content and concentration of red blood cells(Zhang et al., 2022). In the present study the vales of MCV, MCH and MCHC of *Labeorohita* at varying levels of orange peel are shown in Figure 2. Fazio, (2019) demonstrated that fish fed with orange peel maintained consistent biochemical values within the typical range observed in healthy fish.

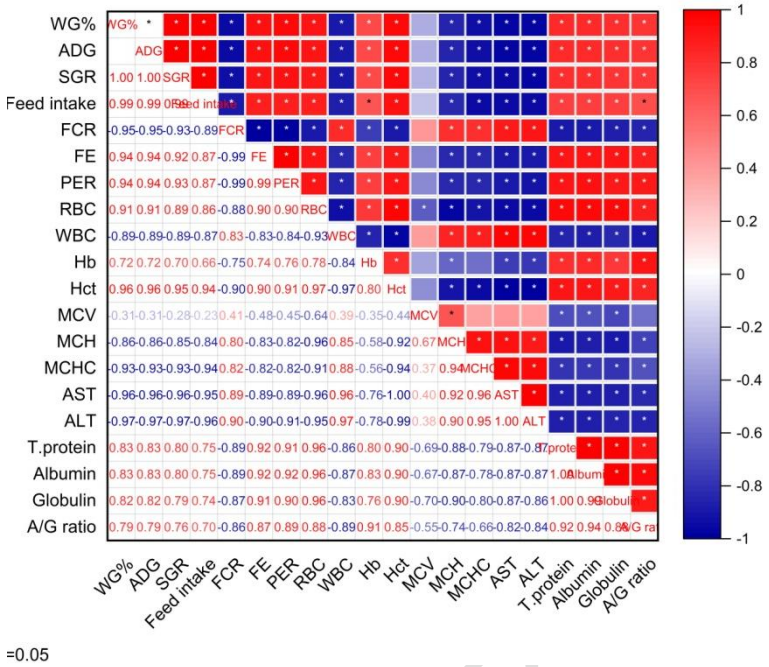

Figure 2. Relationship of growth parameters, feed utilizing efficiency, hematological profile, protein metabolic and oxidative stress enzyme activity of *Labeorohita* fed diets supplemented with graded levels of orange peel for 35 days.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes that are measured to assess liver health or detect liver damage or disease(Shahsavani et al., 2010). When liver enzymes, such as AST and ALT, release in irregular manner, it is usually an indication that the fish's liver cells have experienced damage or injury(Abdel-Daim et al., 2020). In the present study the liver of *Labeorohita* in all treatments was in good condition and had not showed any inflammation as evidenced by the decreased values of AST and ALT (Table 4). Consequently, these results indicated the potential of orange peel to safeguard the membrane integrity of liver cells as high levels of AST and ALT in fish blood may indicate liver damage or disease(Lu et al., 2013). In the current study, *Labeorohita* fed with 30% orange peel exhibited lowest AST and ALT values as compared to other groups, which could be due to the optimal diet composition (Table 1) and metabolic adaptation that could contribute to bring variations in hematological and blood biochemical variables in fish(Keri Alhadi et al., 2012). Baba et al.(2016) revealed that orange peel improved serum-biochemical activity in Nile tilapia. In fish, albumin and globulin are the primary plasma proteins, playing crucial roles in various physiological processesGunter et al.(1961) as indicated in correlation analysis. Additionally, serum total protein is a significant non-specific immune variable, providing valuable information about the fish's immune response and overall health status (Magnadóttir, 2006). In the current study, groups fed with 15% and 30% orange peel exhibited elevated levels of total serum protein, albumin, globulin, and A/G ratio compared to the control group. These findings indicated improved nutritional status, enhanced vascular system integrity and enhanced liver function in *Labeorohita*, highlighting the beneficial effects of orange peel supplemented diet consumption on the fish's overall

health(M.A.A. Metwally and A.M. El-Gellal, 2009). Similar results were found byAcar et al. (2015) for total serum protein, albumin, globulin, and A/G ratio in Mozambique tilapia (*Oreochromismossambicus*) when fed essential oil extracted from orange peel.

5. CONCLUSION

As conventional feedstuffs for fish diets have become increasingly expensive, it has become necessary to search alternative energy sources in fish diets. This study clearly showed that among treatments, T2 (30% orange peel) with conventional fish meal for 35 daysconsistently exhibited significant results in terms of growth performance, feed utilization, hematological profile and protein metabolic and oxidative stress enzyme activity. Based on the results obtained, it can be inferred that the inclusion of orange peel powder at an optimal level of 30% can serve as an effective growth promoter in fish, improve their feed efficiency, blood profile, and liver enzyme activity.

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