

Effect of Muskmelon, *Cucumis melo* and Pears, *Pyrus communis* extract on growth, haematology, immune response and disease resistance in *Labeo rohita* against *Pseudomonas aeruginosa*

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Abstract

The 45 days feeding trial was carried out to assess the effects on the *Labeo rohita* fish growth, biochemical analysis, hematological parameters, disease resistance to against the fish pathogen *Pseudomonas aeruginosa*. The Immune response of *L. rohita* when fed with using fruits methanol extraction of *Cucumis melo* (CM) and *Pyrus communis* (PC) compared to a control diets. The fishes were randomly stocked into five experimental groups, and diets containing different concentrations for control 0% (basal diets), 1% (CM and PC), 2% (CM and PC), were fed to triplicate groups of fish (average weight; 2.60 ± 0.02) for 45 days. At the end of the feeding trail, test fish were intraperitoneal injected with a pathogen, *Pseudomonas aeruginosa*. Resulted in significant ($P < 0.05$) higher weight gain and feed intake were noticed in groups of fish fed 2% CM-treated diets compared to PM treated diets. At the same time, none of the biochemical and hematological parameters was significantly affected. After 45 days bacterial challenge, *labeo rohita* fed 2% *Cucumis melo* had significantly higher survival rate compare to the PM and control groups. Moreover, Immune parameters such as, phagocytic activities were most significantly enhanced in 2% CM especially after a bacterial challenge. This study revealed that dietary *Cucumis melo* at 2% could promote the growth, Immune system, Cost effective, eco friendly alternative antibiotics and improved survival of *labeo rohita* compared to PC.

Keywords: *labeo rohita*; *Cucumis melo*; *Pyrus communis*; Bacterial pathogen

Introduction

Aquaculture is currently fast-growing food-producing trade worldwide. Large-scale aquaculture production is conquered by freshwater fish species (56.4%), especially carp species (71.9%) (FAO, 2012). *Labeo rohita* (rohu) is most important Indian major fish carps. *L. rohita* one of the most popular and delicious freshwater fish grow developed in India and other near closest countries. Moreover, it has been higher enlargement growth potential and important nutritional source of protein and giant human preference (Memon *et al.*, 2010). Escalation

of aquaculture operations, which is now universal trend for commercial aquaculture (Msangi *et al.*, 2013), is generally accompanied by stress leading fish growth fall, immune suppression, susceptibility to contagious diseases and causing major monetary loss to the farmers (Abdelsalam *et al.*, 2017; Food and agriculture organization, 2016; Mona *et al.*, 2015; Syahidah *et al.*, 2015).

In addition, Fishes are always vulnerable to a wide range of pathogenic bacterial strains (Banerjee *et al.*, 2016). Bacterial diseases in fish are the major challenges that obstruct the production and affect the country financial system. *Pseudomonas aeruginosa* is a gram-negative, rod shaped bacterium belonging to the family Pseudomonadaceae. This species very important modifiable and environmental opportunistic pathogen, capacity of surviving in environmental sphere, including aquatic resources (Abdullahi *et al.*, 2013). (Thomas *et al.*, 2014) which causes skin darkness, abdominal ascites, exophthalmia, ulcerative syndrome, dropsy, gill rot, destruction of the primary and secondary lamella in fishes. Therefore, many problems have been most part addressed with the use of antibiotics that leads to the drug-resistant to pathogens, the impact of released antibiotics on the atmosphere and food safety concerns (Upadhaya *et al.*, 2017).

Recently, the use of immunostimulants in the fish culture developed industry has been important in dropping antibiotic use and profitable losses (Citarasu, 2010; Hoseinifar *et al.*, 2016). There is upward in the substitution of chemical drugs with herbal medicine and algal extracts, due to their possible health benefits with inadequate side effects (Peddie, Zou & Secombes 2002; Najafian & Babji 2012; Faggio *et al.*, 2015). Furthermore, Plants with traditional medicinal usage have been identified as potential feed additives for increase resistance to pathogens, improved feed efficiency and growth performance subsequently (Setufe *et al.*, 2018). Some products of herbal plants have been play vital role in host innate immunity and activation of macrophages, neutrophils, and lymphocytes at infection sites for pathogen elimination, promotes hematological and biochemical performance, enhance the fish growth and also protects from the diseases (Johnson and Banerji, 2007).

Now days, fruits also have been studied due to their numerous and important health benefits such as free radical scavengers, anti-oxidants, immunostimulants, anti-inflammatory, anti-cancer and anti-microbial activity (Dragsted, Strube and Larsen, 1993). *Cucumis melo* fruits are a luscious, delicious fruit of the Cucurbitaceae family. It is civilized in all the temperature regions of the worldwide due to its good adaptation to soil and climate. A study previously done by Vouldoukis *et al.* (2004) found that the muskmelon pulp extract contained anti-inflammatory, high antioxidant properties posses' useful medicinal properties activities of anti-platelet, anti ulcer, anti-cancer, anti-microbial, hepatoprotective, anti-diurectic, anti-diabetic, anti-helminthic and anti-fertility activity.

Another fruits, *Pyrus communis* belongs to Rosaceae family. *P. communis* fruits used as a conventional folk therapy for relieving alcoholism, eliminating constipation, relieving cough, and moistening the lung for more than 2000 years in Asian countries, particularly in China, Japan, and Korea. Further, bioactive compounds such as polyphenols are an important group of secondary metabolites distributed in the plant kingdom and these active compounds are usually more rigorous in the peel than in the fruit flesh (Andreotti, Costa & Treutter, 2006; Kolniak-Ostek, 2016). Pear fruits properties of antioxidant and anti-inflammatory affects their products (Li *et al.*, 2014).

Therefore, the fruits compounds properties against the fish pathogen and did not affect the fish growth. This study is investigated for assessing the effect of *C. melo* and *P. communis* extract on growth performance, biochemical analysis, hematological parameters, immune response of *labeo rohita* fingerlings and its Susceptibility to pathogenic *Pseudomonas aeruginosa*.

Materials and Methods

2.1 Sample collection (fruits) and preparation

The Musk melon and Pears were collected from the local Super Market Vadavalli Coimbatore, Tamilnadu. The fruits were washed to remove the any dirt adhering to the surface and peeled the outer layer of the fruits. The separation of the pulp with seed was done manually. After the separation the pulp is pounded into small pieces and then dried. The pieces were shade dried. They were Powdered by mixer grinder and stored at -20°C for further use.

2.2 Extraction of Muskmelon (*Cucumis melo*) and Pears (*Pyrus communis*)

The fruit powder (100g) for each fruit was packed into the extraction chamber of the soxhelt extractor. While a solvent (methanol 99%) 250 ml was poured into the round bottom flask of the extractor. The whole set up was mounted on a heating mantle at 65°C and allowed to reflux for about 16 hours. The extract was filtered (to remove impurities) and evaporated using a rotary evaporator to isolate the free flow lipid from the solvent. The residues obtained after evaporation of methanol was kept in sterilized screw cap glass container for further use.

2.3 Collection and Maintenance of Experimental Animal

In the present study *Labeo rohita* fingerlings (2.60±0.02) were used as a experimental organism. Healthy and diseases free fingerlings of *Labeo rohita* fishes were purchased from Pandian fish seed farm Dindigul, Tamilnadu, India. Specimens were acclimatization fish 2 weeks. The investigational animals were fed with rice bran for maintenance. Thereafter, specimens were randomly distributed into five poly vinyl circulating troughs (75L) at a density of 20 fish per tank. Fish were hand-fed twice a day (09:00 and 15:00) as described elsewhere (Roosta, *et al.* 2014). Treatment was carried out under static aerated water conditions with 50% water change every day. For this purpose an 45-days feeding trail was also conducted under the same experimental setup in order to determine the appropriate ration size of the fish by feeding fish at the rate of 0%, 1%, 2% BW/day. The uneaten feed was collected after active feeding approximately for 40 min with the help of siphoning pipe and collection tubes. The collected feed was then oven-dried at 100°C to calculate the final feed conversion ratio (FCR). No feed was offered to the fish on the day of weekly measurement. At the end of the experimental trail, desired numbers of fish were randomly sacrificed for the assessment of whole body composition.

2.4 Diets analysis

Diets were analysed for proximate composition using method described by the Association of Official Analytical Chemists (AOAC, 2005) Table 1.

Table 1 Proximate and chemical composition of the experimental diets (g/kg)

Ingredient	Control (g)	T1 (CM-1%)g	T2 (CM-2%)g	T3 (PC-1%)g	T4 (PC-2%)g
Fish meal	30	30	30	30	30
Soybean meal	20	20	20	20	20
Rice bran	20	20	20	20	20
Groundnut oil cake	10	10	10	10	10
Wheat flour	5	5	5	5	5
Tapioca flour	5	5	3	5	3
Egg albumin	3	3	3	3	3
Cod liver oil	3	3	3	3	3
Vitamins & mineral mixture	4	2	2	2	2
Fruit extract	0	2	4	2	4
Total	100	100	100	100	100
Proximate Composition %					
Moisture Content	9.70	9.25	8.75	9.50	9.10
Crude fibre	3.60	3.85	4.22	3.90	4.42
Ash	4.88	5.20	5.93	5.18	5.46

Note; Diets were prepared with locally available feed ingredients.

Fishmeal (150 g kg⁻¹) and soybean meal (100 g kg⁻¹) were used as protein sources; rice bran (100 g kg⁻¹); groundnut oil cake (50 g kg⁻¹) wheat flour (25 g kg⁻¹) Tapioca flour (25g) were used as carbohydrate sources; cod liver oil (15 g kg⁻¹) was used as lipid source; Binding agent egg albumin (15 g kg⁻¹) was also added; composition of vitamins mineral mixture tablets sadamindia Ltd, Mumbai, India (vitamin A, cholecalciferol, thiamine mononitrate, riboflavin, pyridoxine hydrochloride, cyanocobalamin, nicotinamide, calcium pantothenate,

ascorbic acid, α -tocophenyl acetate, biotin (20 g); mineral mixture (cu,zn free) sodium molybdate dehydrate, sodium borate, black oxide of iron, red oxide of iron, magnesium sulphate, manganese sulphate, cellulose (20 g kg⁻¹) was also added.

2.5 Growth performance

Before measuring the growth performance of *L. rohita* were starved for 24 hrs. Eight fish were randomly selected from each experimental groups and transfer on the tray. Total weight gain, Specific growth rate, and feed conversion ratio were calculated (castell J & Tiewks K, 1980).

Various parameters as follows:

Total gain (g fish⁻¹) = WT-WI, where as WT is a final weight, WI is a initial weight

The specific growth rate (SGR-% day⁻¹) = 100× (In WT-In WI)/duration/day

The feed conversion ratio (FCR) = total feed intake (g)/total gain (g).

Survival percentage = Number of fish in each group remaining after the 45- days period/ initial number of fish × 100

2.6. Biochemical and Haematological analyses of *Labio rohita* fed experimental diets

At the end of the 45 –day feeding trail, 10 fish per treatment were randomly caught using hand net and blood was drawn from the caudal vein region of fishes by using a hypodermic syringe that was previously rinsed with EDTA an anti-coagulant. The collected blood was diluted with EDTA (2mg/ml). The hemoglobin concentration of blood was estimated by the method using Sahli's haemoglobinometer (Put, 1923).

White blood cells were determined using a Neubauer haemocytometer as described by Kaplow (1955). Erythrocytes indices in the blood were determined according to Jain (1986). The protein content in different samples was estimated by employing the Folin-Ciocalteu method of (Lowry *et al.*, 1951). Finally, colour developed was read at 620 nm and with UV spectrophotometer. Standards were also run simultaneously with OD value the total protein content of the sample was calculated. The method of, Rob (1955) was employed to 500 mg of the sample was homogenized with 100ml of distilled water and centrifuged for the 5 minutes at 4000 rpm. The supernatant was used for the estimation of carbohydrate. The amounts of carbohydrate present in the sample were calculated. The method of Folch *et al.* (1957) was employed to extract the 500mg sample was homogenized in 2ml of chloroform: methanol, which is centrifuged at 3000 rpm for 10 minutes. The process is repeated thrice; the supernatant was pooled and concentrated at 40- 45°C in flask vacuum evaporator. The total lipid concentration of the sample was calculated from the standard graph and expressed as mg/ml wet tissue.

2.7 Challenging study

Bacterial strain of *P. aeruginosa* was purchased from PSG Medical College, Coimbatore. The bacteria cultured in nutrient broth at 37°C for 24 hours. The bacteria culture was adjusted to 1 × 10⁶ CFU/ ml by serial dilution. Twenty fish randomly selected from each experimental group and divided into two groups each of 6 fish. The first group was intraperitoneally injected with 0.2 ml PBS containing 1 × 10⁶ CFU/ ml virulent of *P. aeruginosa* (Kocour *et al.*, 2005). The second group was intraperitoneally injected with 0.2ml of saline solution as a control. Before infection, fish were starved for 24 hrs. All fish were kept under observation for 15 days to record the daily mortalities and abnormal clinical signs. Relative percentage survivals (RPS) among the challenge fish were determined using following equation (Misra *et al.*, 2006).

$$\text{RPS\%} = \text{Number of surviving fish after challenge/ Number of fish injected with bacteria} \times 100$$

2.8 Phagocytic activity

Fishes were immobilized by a sharp blow to the cranium, and about 2 ml of blood collected from the caudal vein using a heparin-treated, 150 units ml⁻¹ syringe, 0.1 ml of *Staphylococcus aureus*, 1 × 10⁸ ml⁻¹ suspended in PBS was added to 0.1 ml of blood in the microtiter well. The amalgamation was incubated at room

temperature for 20 min and mixed thoroughly using a pipette to ensure contact of the bacteria and leukocytes. Fifty microliters were placed on a glass slide to make a smear similar to the method of making a blood smear. The slide was air dried, fixed with 95% ethanol, re-dried, and then stained with 0.15% safranin solution for 10 min. The phagocytic cells were observed and engulfed bacteria were counted for calculation of phagocytic index. The percent phagocytosis was calculated according to the following after counting at least 100 phagocytic cells either phagocytizing or not (Mamnur rashid, 2002).

$$\text{PA\%} = \text{Number of phagocytizing cells} \times 100 / \text{Total number of phagocyte cells counted}$$

2.9 Statistic analysis

Results for each parameter were expressed as the mean \pm SD. Data were analyzed using one-way, ANOVA and the comparison of the mean values were done by using Duncan multiple range test using software program SPSS version 16 for windows. Differences were considered statically significant when, $P < 0.05$.

III. Results

3.1 Growth performance

Growth parameters of *L. rohita* fed on CM and PC supplemented diet are presented in Table 2. There was a steady increase in the weight of all experimental groups during the whole experimental period. After feeding trail, weight gain (WG %) and Specific growth rate (SGR %) of experimental fish showed significant higher ($P < 0.05$) values in 2 % CM compared to the control. Fish fed with 2 % CM supplementation had the optimum fish growth producing the lowest FCR value ($P < 0.05$) and another parameter of fish survival rate (%) attained the two fruits using *cucumis melo* and *pyrus communis* for 45 days. In this results analysed 100% survival rate were observed by using CM (2%) treated when compared other treated groups.

Table 2 Growth parameters and feed utilization of *Labeo rohita* fed on diets containing different *cucumis melo* and *pyrus communis* fruits extract for 45 days

S. No	Control	T1 (CM-1%)	T2 (CM-2%)	T3 (PC-1%)	T4 (PC-2%)
Initial weight (g)	2.6 \pm 0.08	2.76 \pm 0.18	2.99 \pm 0.08	2.83 \pm 0.26	2.73 \pm 0.16
Final weight (g)	6.93 \pm 0.04	8.57 \pm 0.01	9.71 \pm 0.02	8.89 \pm 0.004	6.29 \pm 0.01
WG	4.33 \pm 0.23 ^d	5.86 \pm 0.12 ^c	7.26 \pm 0.20 ^a	6.066 \pm 0.49 ^{bc}	6.63 \pm 0.30 ^{ab}
FCR	2.73 \pm 0.12 ^a	2.3 \pm 0.21 ^b	1.68 \pm 0.08 ^d	2.06 \pm 0.16 ^{bc}	1.8 \pm 0.08 ^{cd}
SGR	1.42 \pm 0.05 ^d	2.13 \pm 0.26 ^c	3.2 \pm 0.35 ^a	1.98 \pm 0.23 ^{bc}	2.58 \pm 0.18 ^b
Survival Rate %	83.5 \pm 1.22 ^d	92 \pm 1.63 ^c	100 \pm 0.0 ^a	95.6 \pm 0.94 ^b	98 \pm 0.81 ^{ab}

Note; Regressions were measured significant at $p < 0.05$. WG, Weight Gain; SGR, Specific growth rate; FCR, Feed conversion ratio; Data were expressed as Mean \pm SD, n = 3 replicates; ^{a,b,c}, Mean values in the same row with different superscripts differ significantly ($p < 0.05$).

3.2 Hematological Parameters

The hematological profiles of *labeo rohita* fed *C. melo* and *P. communis* extract-based diets are presented in Table 3 (Fig.1). There was a significant increase ($P < 0.05$) in the recorded values for Hb, RBC in the 2 % *cucumis melo* treated groups when compared with the control. Highest values of Hb concentration (10.09), RBC (2.56) were recorded for T2 while control had the least values for these parameters. The *C.melo* and *P communis*

fruits extract using 1% and 2% separately treated groups over entire feeding period as compared to the control. The WBC level was observed in all treated groups in particular *C.melo*- 2% treated was 14.25 significantly decreased when compared to control.

3.3 Biochemical parameters

Protein content in muscle of the fish was significantly increased, $P < 0.05$ in all treated groups with muskmelon and pears on days 45 compared to the control group. Table 3 shown highest protein level 17.3 in *C. melo* (CM) 2 % feed when compared to *P. communis* (PC) 2% protein level 16.8 and control 14.6 (Fig.1). The carbohydrate level of 2% muskmelon, 2% pears treated group and control. This result shows the carbohydrate level increased 2.56 which treated muskmelon 2% when compared to the pears and control. Similarly biochemical parameters the lipid level was decreased by treated *Cucumis melo* when compared to other treated group.

Table 3 Haematological serum biochemical parameters of *Labeo rohita* fed diet containing different *cucumis melo* and *pyrus communis* fruits extract for 45 days

S. No	Control	T1 (CM-1%)	T2 (CM-2%)	T3 (PC-1%)	T4 (PC-2%)
HB	8.23±0.12 ^d	9.23±0.20 ^{bc}	10.09±0.285 ^a	8.69±0.42 ^{cd}	9.61±0.10 ^{ab}
RBC	1.33±0.12 ^d	1.63±0.04 ^c	2.56±0.16 ^a	1.53±0.28 ^c	2.1±0.008 ^b
WBC	18.1±0.14 ^a	15.2±0.69 ^b	14.25±0.23 ^c	13.1±0.23 ^d	13.5±0.28 ^{cd}
Protein	14.6±0.12 ^e	16.06±0.16 ^c	17.3±0.20 ^a	15.23±0.12 ^d	16.8±0.21 ^b
Carbohydrate	1.33±0.12 ^d	1.63±0.04 ^c	2.56±0.16 ^a	1.53±0.28 ^c	2.1±0.008 ^b
Lipid	18.1±0.14 ^a	15.2±0.69 ^b	13.1±0.23 ^c	14.5±0.23 ^d	13.5±0.28 ^{cd}

Note; Regressions were measured significant at $p < 0.05$. HB, Haemoglobin; RBC, Red Blood Cells; WBC, White Blood Cells. Data were expressed as Mean ±SD, n = 3 replicates; ^{a,b,c} Mean values in the same row with different superscripts differ significantly ($p < 0.05$).

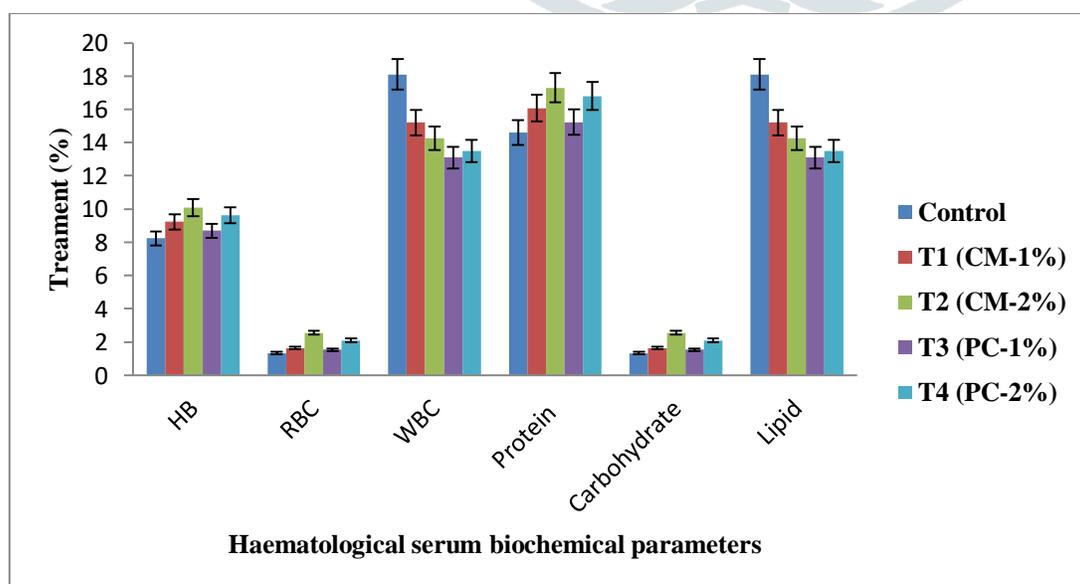


Figure 1: Haematological serum biochemical parameters of *Labeo rohita* fed diet containing Treatments

3.4 Phagocytic activity

Phagocytic activity was significantly, $P < 0.05$ different in the experimental groups over the control. Higher phagocytic activity percentage, 34.4% in T2 muskmelon 2% feed and 25.5% in T4 pears 2% feed as compared to control. After challenge study, there was a significant increase in all treated groups (Table 4; Fig.2).

Table 4 Phagocytic activity of *L.rohita* fingerlings fed with different fruits

Muskmelon			Pears		
S.No	Pre-challenge	Post-challenge	S.No	Pre-challenge	Post-challenge
Control	18.39±0.02 ^e	18.48±0.02 ^d	Control	18.39±0.02 ^e	18.48±0.02 ^d
T1 (CM-1%)	20.53±0.23 ^c	20.6±0.22 ^c	T3 (PC-1%)	19.85±0.1 ^d	19.97±0.1 ^c
T2 (CM-2%)	32.7±0.36 ^a	34.4±0.37 ^a	T4 (PC-2%)	23.1±0.2 ^b	25.5±0.22 ^b

Note; Data were expressed as Mean ±SD, n = 3replicates; ^{a,b,c} Mean values in the same row with different superscripts differ significantly ($p < 0.05$).

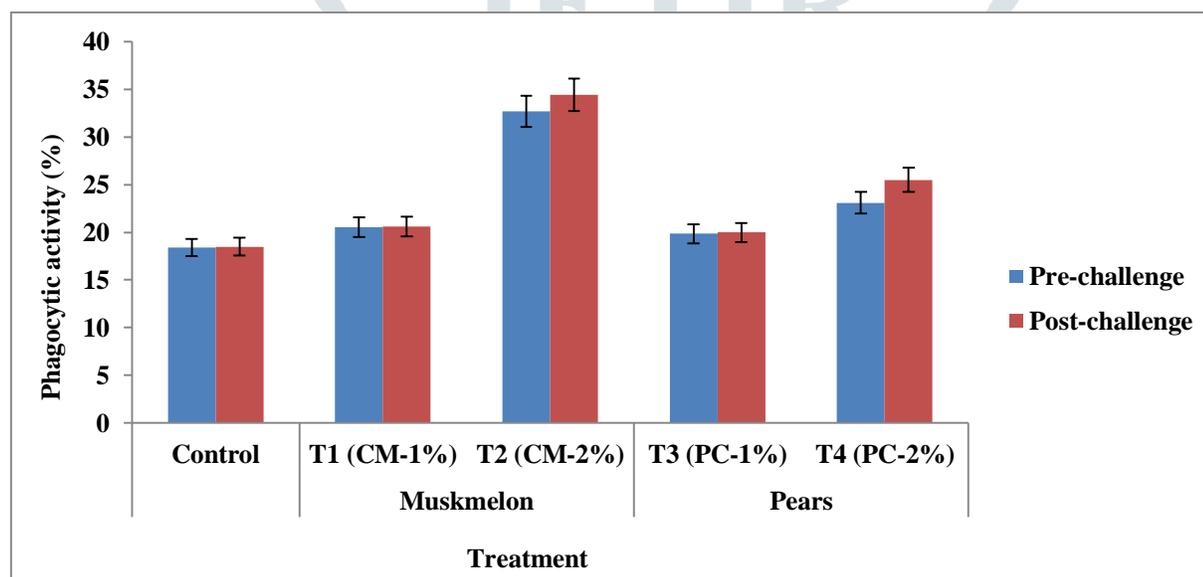


Figure. 2 Fruits extract using Phagocytic activity of *L.rohita*

3.5 Challenge test

The survival rate of fish challenged with *P. aeruginosa* (1×10^6 CFU/ ml) for 15 days after the 45-days feeding experiment showed the highest mortality in control (100%), while T2 recorded the least mortality (20%). A reduced mortality of *P. aeruginosa* was observed in groups fed with *C.melo* extract compared to PC and control (Table 5). The survival rates of the mortality were used to calculate Relative Survival Percentage (RPS).

Table 5 Challenge study of *labeo rohita* fed diets prepared with fruits extraction

S. No	No of fish infected	Total No of mortality in 15 days	% of mortality	RPS
Control	10	10	100	0
T1 (CM)	10	5	50	50
T2 (CM)	10	2	20	80
T3 (PC)	10	8	80	20
T4 (PC)	10	4	40	60

Note: Relative Percentage Survival (RPS)

IV. Discussion

According to our knowledge, there is a little information about fruits uses given in the literature. Hence, we have selected natural promoting immune stimulant using fruits while developing the fish growth, reduced mortality those were helpful to minimizing the use of antibiotics. This study is the foremost attempt to investigate the end result of *C. melo* and *P. communis* fruit extract as dietary immuno-stimulant on the growth performance, hematological performance, bio-chemical analysis and disease resistance of *L. rohita*. At the final stages of feeding trail, significant difference were noticed, $P < 0.05$ increases in weight gain have been recorded at 2% *C. melo* on various treatment groups compared to *P. communis* and control. However, significant difference noticed SGR, FCR was observed between control and experimental groups. Furthermore, growth profiles has been measured and found to be superior weight gain percentage in T4, LE diet fed fish. Similarly, significant, $P < 0.05$ SGR found in treated diet and highest SGR found in T4, 0.4% LE diet fed fish. Falling trends were noticed in FCR as the quantity of lapsi extract increased. Lapsi powder supplementation diets groups T7 and T8 while compared to T2 and T3 incorporated with *Choerospondias axillaris*, lapsi extracts found to be performing better growth than the lapsi powder supplemented diet fed (Shyam *et al.*, 2017). On other hand, dietary supplementation with 5% BPF, i.e., B5 for 60 days significantly increased WG and SGR in *L. rohita* (Sib Sankar Giri *et al.*, 2016).

Assessment of nutritional status, wellbeing status, and the capability for fish adaptation to the external environment study using that Hematological and biochemical investigations are important key tools (Abdel-Tawwab *et al.*, 2016; Fazio *et al.*, 2013). Hemoglobin content was increased in 2% *C. melo* fruit extract. Hematological parameters provide an index of the morphological and physiological condition of fish (Fadefared *et al.*, 2018). Likewise, flavonoids give out an fundamental role in obstacle oxidation of hemoglobin by diverse factors, such as hypochlorous acid (Gebicka *et al.*, 2012). The hemoglobin rate was good quality indicators for oxygen distribution capacity of fish, thus manufacture it achievable to establish relationship with the oxygen concentration obtainable in the habitat and the health condition of these fishes (Barros *et al.*, 2002).

We revealed that *L. rohita* fed with 2% *C. melo* fruit extract supplemented diet showed significantly higher RBC level, $P < 0.05$ then the *P. communis* and other experimental period over the feeding trails. In addition, it constitutes the transportation organization of oxygen from the lungs to tissues. It have been verified that a huge concentration of polyphenols might boost the resistance of RBC to oxidative stress, (Youdim *et al.*, 2000). Whereas, prospective elucidation for the outcome was showed a better content of polyphenols, erythropoiesis in Pomegranate juice may have been prevented RBC destruction due to reduced oxidative stress (Eirini manthou *et*

al., 2017). WBC counts were significantly, $P < 0.05$ diverse in experimental groups over entire feeding period as while comparing the control in T0. The higher WBC counts were noticed T2, muskmelon among other experimental groups while lower WBC counts were seen in, T0. Adewolu *et al.* (2008) reported that the significant raise in the values of WBC concentration of tobacco dirt raised could be attributed to elevate in leucocyte synthesis as a defense mechanism against the destruction of erythrocytes. Leucocytes are most plentiful cells comprising the lymphocytes, which may function in the production of antibodies and chemical substances serve as a defense against contagion. Moreover, that they noted the raised in MCHC, WBC, MCV and MCH. Many studies took down the medical plants could act as immuno-stimulants increase the WBC count, Kumar *et al.*, 2014; Baba *et al.*, 2016. On the contrary, Ibrahim Adeshina *et al.* (2019) the hematological profile of African catfish fed ECBE-enriched diets was significantly different, $P < 0.05$.

Fish species are the excellent sources of superior quality protein and possess high amount of essential and functional amino acids which regulate and take part in various metabolic pathways and with beneficial health effects on growth, survival, development, lactation and development of an organism (Wu, 2010). Proteins have more essential functions in all the biological activity due to enzymatic activity, transport, storage, mechanical conservancy, growth and cellular differentiation control (Lowry *et al.* 1951). Protein contented in muscle tissue of the fish was significantly increased, $P < 0.05$ in all treated groups with muskmelon and pears on day 45 compared to the control group. Furthermore, amino acids bring crucial functions in cell signaling and act as regulators of gene expression and protein phosphorylation cascade (Wu, 2010), similar trend as their values recorded in the study follow as (Abdel-Tawwab *et al.*, 2010; Ulukoy *et al.*, 2017). Ibrahim Adeshina *et al.* (2019) have reported that total protein, albumin was increased after the ECBE feeding when compared to the control diet.

Carbohydrate is one of the very important components of metabolism and it supplies the energy needed for respiration and other most important processes (Hedge *et al.*, 1962). The Lower carbohydrate level were noted in 2% *C. melo* fruit extract, T4 and 2% *P. communis* fruit extract died fed fish and the higher carbohydrate level were noted in control. Whereas, the study confirmed that the growth of *M. montanus* was more influenced by the animal source of fishes along with the nutritional source of wheat flour. The omnivorous fish *M. montanus* need a low amount, 9.48% of dietary carbohydrate for its maximum growth (Raj *et al.*, 2008). Similarly the outcome showed that lipid level was lower at 2% *C. melo* fruit extract, T4 than 2% *P. communis* fruit extract died fed fish and control. Total lipid content in body parts varied in different fresh water fishes studied (Swapna *et al.*, 2010). Earlier studies have reported that pomegranate may reduce blood lipid levels (Basu *et al.*, 2009).

Post-challenge mortality was significantly reduced in fish fed *C. melo* and *P. communis*-containing diets. The highest death rate was recorded in fish fed control diet while the low mortality was obtained in fish fed at 2 % *C. melo* diet. Moreover, *L. acidissima* fruits supplementation activated the innate immunity and reduced mortality of the fish, (ponnuraj srinivasan *et al.*, 2015). A pathogen challenge test evaluated the effects of ECBE as an immune stimulant have been tolerated to pathogenic bacteria of *A. hydrophila* augmented significantly as dietary ECBE levels increased, shown higher fish survival other than the control fish group. These results coincide with the results of Abdel-Tawwab (2012); Abdel-Tawwab and Abbass (2017); Abdel-Tawwab *et al.* (2010); Abdel-Tawwab *et al.* (2018) and Adeshina *et al.* (2017) who noticed an improved tolerance of Nile tilapia and African cat fish against *A. hydrophila* infection.

Phagocytosis activities have been documented as an very important cellular progression in the nonspecific immune system of fish (Bergljot Magnadottir *et al.*, 2006). Phagocytic activity was significantly, $P < 0.05$ different in the experimental groups over the control. Higher phagocytic activity were found to be 34.4% in T2, muskmelon 4% feed and 25.5% in T2, pears 2% feed as compared to the control. Furthermore, as the primary line of defence various peptides such as lysozyme, antibodies, and complement factors inhibit the adhesion and colonization of microorganisms, leading to the prevention of infection and disease (Esteban *et al.*, 2012).

CONCLUSION

Therefore, our results concludes that the *C. melo* and *P. communis* fruits methanol extract improves the digestibility of protein in fish diets as well as to increase fish growth of Indian major carps (*labeo rohita*) and also *C. melo* fruits extracts act as disease resistance of fish bacterial diseases and were proved to be maintain the

sufficient health, prevent the high risk of diseases and fish mortality when compared to *P. communis* fruits methanol extract.

VI. ACKNOWLEDGEMENT

In this manuscript. K. Pavithra is grateful to the Department of Science and Technology promotion of University Research and Scientific Excellence, India (DST Purse -II) providing financial support.

VII. REFERENCES

- [1] Abdelsalam, M., Elgendy, M.Y. Shaalan, M., Moustafa, M. and Fujino, M. 2017. Rapid identification of pathogenic streptococci isolated from moribund red tilapia (*Oreochromis* spp.). *Acta Veterinaria Hungarica*, 65(1), 50-59.
- [2] Abdel-Tawwab, M. and Abbass, F.E. 2017. Turmeric powder, *Curcuma longa* L., in common carp, *Cyprinus carpio* L., diets: growth performance, innate immunity, and challenge against pathogenic *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*, 48(2):303-12.
- [3] Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A. and Shalaby, AM. 2010. Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, 298(3-4):267-74.
- [4] Abdel-Tawwab, M., Ahmad, M.H., Seden, M.E. and Sakr, SF. 2010. Use of green tea, *Camellia sinensis* L., in practical diet for growth and protection of Nile tilapia, *Oreochromis niloticus* (L.), against *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*, 41:203-13.
- [5] Abdel-Tawwab, M., Samir, F., Abd El-Naby, A.S. and Monier, M.N. 2018. Antioxidative and immunostimulatory effect of dietary cinnamon nanoparticles on the performance of Nile tilapia, *Oreochromis niloticus* (L.) and its susceptibility to hypoxia stress and *Aeromonas hydrophila* infection. *Fish & shellfish immunology*, 74:19-25.
- [6] Abdel-Tawwab, M. 2016. Effect of feed availability on susceptibility of Nile tilapia, *Oreochromis niloticus* (L.) to environmental zinc toxicity: Growth performance, biochemical response, and zinc bioaccumulation. *Aquaculture*, 464:309-15.
- [7] Abdel-Tawwab, M. 2012. Effects of dietary protein levels and rearing density on growth performance and stress response of Nile tilapia, *Oreochromis niloticus* (L.). *International Aquatic Research*, 4(1):3.
- [8] Abdullahi, R., Lihan, S., Carlos, B.S., Bilung, M.L., Mikal, M.K. and Collick, F. 2013. Detection of oprL gene and antibiotic resistance of *Pseudomonas aeruginosa* from aquaculture environment. *European Journal of Experimental Biology*.3(6):148-52.
- [9] Adeshina, I., Adewale, Y.A. and Tihamiyu, LO. 2017. Growth performance and innate immune response of *Clarias gariepinus* infected with *Aeromonas hydrophila* fed diets fortified with *Curcuma longa* leaf. *West African Journal of Applied Ecology*, 25(2):87-99.
- [10] Adeshina, I., Jenyo-Oni, A., Emikpe, B.O. Ajani, E.K. and Abdel-Tawwab, M. 2019. Stimulatory effect of dietary clove, *Eugenia caryophyllata*, bud extract on growth performance, nutrient utilization, antioxidant capacity, and tolerance of African catfish, *Clarias gariepinus* (B.), to *Aeromonas hydrophila* infection. *Journal of the World Aquaculture Society*, 50(2):390-405.

- [11] Adewolu, M.A., Adeniji, C.A., and Adejobi, A.B., 2008. Feed utilization, growth and survival of *Clarias gariepinus* (Burchell, 1822) fingerlings cultured under different photoperiods. *Aquaculture*, 283, 64–67
- [12] Andreotti, C., Costa, G. and Treutter, D. 2006. Composition of phenolic compounds in pear leaves as affected by genetics, ontogenesis and the environment. *Scientia horticulturae*. 109(2):130-7.
- [13] Ángeles Esteban, M. 2012. An overview of the immunological defenses in fish skin. *ISRN immunology*.
- [14] AOAC. 2005. International. Official methods of analysis of AOAC International. AOAC International.
- [15] Aydin, F. and Harmantepe, F.B. 2018. Effects of Sage oil (*Salvia officinalis L.*) on haematological and growth parameters in Nile Tilapia (*Oreochromis niloticus*). *Pakistan J. Zool*, 50(3):921-8.
- [16] Baba, E., Acar, Ü., Öntaş, C., Kesbiç, O.S. and Yılmaz, S. 2016. Evaluation of *Citrus limon* peels essential oil on growth performance, immune response of Mozambique tilapia *Oreochromis mossambicus* challenged with *Edwardsiella tarda*. *Aquaculture*, 465:13-8.
- [17] Banerjee, G., Nandi, A. and Ray, A.K. 2017. Assessment of hemolytic activity, enzyme production and bacteriocin characterization of *Bacillus subtilis* LR1 isolated from the gastrointestinal tract of fish. *Archives of microbiology*, 199(1):115-24.
- [18] Barros, M.M., Lim, C. and Klesius, P.H. 2002. Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of Channel Catfish (*Ictalurus punctatus*) to *Edwardsiella ictaluri* challenge. *Aquaculture*, 207(3-4):263-79.
- [19] Basu, A. and Penugonda, K. 2009. Pomegranate juice: a heart-healthy fruit juice. *Nutrition reviews*, 67(1):49-56.
- [20] Buettner, A. and Schieberle, P. 2001. Evaluation of key aroma compounds in hand-squeezed grapefruit juice (*Citrus paradisi Macfayden*) by quantitation and flavor reconstitution experiments. *Journal of Agricultural and Food Chemistry*. 49(3):1358-63.
- [21] Castell, J.D. and Tiews, K. 1979. Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research, Hamburg, Federal Republic of Germany, 21-23 March 1979. Documents Techniques de la CECPI (FAO).
- [22] Citarasu, T. 2010. Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, 18, 403-414.
- [23] Dragsted, L.O., Strube, M. and Larsen, J.C. 1993. Cancer-Protective Factors in Fruits and Vegetables: Biochemical and Biological Background. *Pharmacology & toxicology*, 72:116-35.
- [24] Fadeifard, F., Raissy, M., Jafarian, M., Boroujeni H.R., Rahimi, M., and Faghani, M. 2018 Effects of black seed (*Nigella sativa*), ginger (*Zingiber officinale*) and cone flower (*Echinacea angustifolia*) on the immune system of rainbow trout, *Oncorhynchus mykiss*. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 70(1), 199-204.
- [25] Faggio, C., Fazio, F., Marafioti, S., Arfuso, F. and Piccione, G. 2015. Oral administration of Gum Arabic: effects on haematological parameters and oxidative stress markers in *Mugil cephalus*. *Iranian Journal of Fisheries Sciences*, 14(1):60-72.
- [26] FAO. 2012. The State of World Fisheries and Aquaculture, FAO, Rome, Italy.

- [27] Fazio, F., Marafioti, S., Torre, A., Sanfilippo, M., Panzera, M. and Faggio, C. 2013. Haematological and serum protein profiles of *Mugil cephalus*: effect of two different habitats. *Ichthyological Research*, 60(1):36-42.
- [28] Fisheries, F.A. 2012. Aquaculture Department. The state of world fisheries and aquaculture, 1-53.
- [29] Folch, J., Lees, M. and Stanley, G.S. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *Journal of biological chemistry*. 226(1):497-509.
- [30] Food and Agriculture Organisation. 2016. The state of World Fisheries and Aquaculture: Contributing to food security and nutrition for all (p.24) Rome, Italy: FAO.
- [31] Gebicka, L. and Banasiak, E. 2012. Hypochlorous acid-induced heme damage of hemoglobin and its inhibition by flavonoids. *Toxicology in vitro*, 26(6):924-9.
- [32] Giri, S.S., Jun, J.W., Sukumaran, V. and Park, S.C. 2016. Dietary administration of banana (*Musa acuminata*) peel flour affects the growth, antioxidant status, cytokine responses, and disease susceptibility of rohu, *Labeo rohita*. *Journal of immunology research*. 16; 2016.
- [33] Hedge, I.E. and Hofreiter, B.T. Carbohydrate Chemistry i7 (Eds Whistler RL and Be Miller, JN) Academic Press New York.
- [34] Hoseinifar, S.H., Ringo, E., Shenavar Masouleh, A. and Esteban, M.Á. 2016. Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. *Reviews in Aquaculture*, 8(1):89-102.
- [35] Jain, N.C. 1986. Schalm's veterinary hematology. Lea & Febiger; 1986.
- [36] Johnson, C. and Banerji, A. Influence of extract isolated from the plant *Sesuvium portulacastrum* on growth and metabolism in freshwater teleost, *Labeo rohita* (Rohu).
- [37] Kaplow, L.S. 1955. A histochemical procedure for localizing and valuating leukocyte alkaline phosphatase activity in smears of blood and marrow. *Blood* 10, 1023.
- [38] Kocour, M., Gela, D., Rodina, M. and Linhart, O. 2005. Testing of performance in common carp *Cyprinus carpio* L. under pond husbandry conditions I: top-crossing with Northern mirror carp. *Aquaculture Research*, 36(12):1207-15.
- [39] Kolniak-Ostek, J. 2016. Chemical composition and antioxidant capacity of different anatomical parts of pear (*Pyrus communis* L.). *Food chemistry*, 203:491-7.
- [40] Kumar, V., Kumar, S., Pandey, P.K., Raman, R.P., Prasad, K.P., Roy, S., Kumar, A. and Kumar, K. 2014. Growth and hemato-immunological response to dietary i-carrageenan in *Labeo rohita* (Hamilton, 1822) juveniles. *The Israeli Journal of Aquaculture-Bamidgeh*.
- [41] Labh, S.N., Shakya, S.R., Gupta, S.K., Kumar, N. and Kayastha, B.L. 2017. Effects of lapsi fruits (*Choerospondias axillaris* Roxburgh, 1832) on immunity and survival of juvenile tilapia (*Oreochromis niloticus* Linnaeus, 1758) infected with *Aeromonas hydrophila*. *International Journal of Fauna and Biological Studies*, 5:571-7.

- [42] Li, Z., Geng, Y.N., Jiang, J.D. and Kong, W.J. 2014. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. Evidence-Based Complementary and Alternative Medicine, Jan 1;2014.
- [43] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. Journal of biological chemistry, 193:265-75.
- [44] Magnadóttir, B. 2006. Innate immunity of fish (overview). Fish & shellfish immunology, 20(2):137-51.
- [45] Mamnur Rashid, M., Sardar, M. and Islam, M.R. 2002. In vitro phagocytic study of blood leucocytes and peritoneal macrophages of walking catfish *Clarias batrachus* (Ham.) against *Aeromonas hydrophila* and *Escherichia coli*. Bangladesh Journal of Fisheries Research, 6(1):35-41.
- [46] Manthou, E., Georgakouli, K., Deli, C.K., Sotiropoulos, A., Fatouros, I.G., Kouretas, D., Haroutounian, S., Matthaiou, C., Koutedakis, Y. and Jamurtas, A.Z. 2017. Effect of pomegranate juice consumption on biochemical parameters and complete blood count. Experimental and therapeutic medicine, 14(2):1756-62.
- [47] Memon, N.N., Talpur, F.N. and Bhangar, M.I. 2010. Nutritional aspects and seasonal influence on fatty acid composition of carp (*Labeo rohita*) from the Indus River. Polish Journal of Food and Nutritional Sciences, 60(3), 217-223.
- [48] Misra, C.K., Das, B.K., Mukherjee, S.C. and Meher, P.K. 2006. The immunomodulatory effects of tuftsin on the non-specific immune system of Indian Major carp, *Labeo rohita*. Fish & shellfish immunology, 20(5):728-38.
- [49] Mona, H.M., El-Sayed, T.R., Salama, W.M. and Younis, M.L. 2015. Efficacy of probiotics, prebiotics, and immunostimulant on growth performance and immunological parameters of *Procambarus clarkia* juveniles. The journal of Basic and Applied Zoology, 69,17-25.
- [50] Msangi, S., Kobayashi, M., Batka, M., Vannuccini, S., Dey, M. and Anderson, J. 2013. Fish to 2030: Prospects for fisheries and aquaculture, World Bank Report.
- [51] Najafian, L. and Babji, A.S. 2012. A review of fish-derived antioxidant and antimicrobial peptides: their production, assessment, and applications. Peptides, 33(1):178-85.
- [52] Peddie, S., Zou, J. and Secombes, C.J. 2002. Immunostimulation in the rainbow trout (*Oncorhynchus mykiss*) following intraperitoneal administration of Ergosan. Veterinary immunology and immunopathology, 86(1-2):101-13.
- [53] Ponnuraj, S., Jaganathan, D., Kanagarajan, M., Castro, J. and Deivamarudachalam, T.P. 2015. Influence of *Limonia acidissima* L. against the biofilm forming *Aeromonas hydrophila* isolated from fresh water fishes. Journal of Biochemical Technology, 6(1):910-21.
- [54] Put, T. 1923. 112 Standard for Sahli's Hemoglobinometer. The Journal of Biological Chemistry. 57:111.
- [55] Raj, A.J., Haniffa, M.A., Seetharaman, S. and Appelbaum, S. 2008. Utilization of various dietary carbohydrate levels by the freshwater catfish *Mystus montanus* (Jerdon). Turkish Journal of fisheries and aquatic sciences, 8(1):31-5.
- [56] Rob, J.R. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. Biol. Chem, 20, 335-343.

- [57] Roosta, Z., Hajimoradloo, A., Ghorbani, R. and Hoseinifar, S.H. 2014. The effects of dietary vitamin C on mucosal immune responses and growth performance in Caspian roach (*Rutilus rutilus caspicus*) fry. *Fish physiology and biochemistry*, 40(5):1601-7.
- [58] Setufe, S.B., Ajani, E.K., Emikpe, B.O. and Ogunbanwo, S.T. 2018. Growth Performance of *Clarias gariepinus* on Diets Fortified with *Lactobacillus plantarum* and *Psidium guajava* Leaf. *Journal of the Faculty of Veterinary Medicine*, 44(3):105-12.
- [59] Swapna, H.C., Rai, A.K., Bhaskar, N. and Sachindra, N.M. 2010. Lipid classes and fatty acid profile of selected Indian fresh water fishes. *Journal of Food Science and Technology*, 47(4):394-400.
- [60] Syahidah, A., Saad, C.R., Hassan, M.D. and Abdehad, Y.M. 2015. Status and potentials of herbal application in aquaculture: A review. *Iranian Journal of Fisheries Sciences*, 14(1), 27-44.
- [61] Thomas, J., Thanigaivel, S., Vijayakumar, S., Acharya, K., Shinge, D., Seelan, T.S., Mukherjee, A. and Chandrasekaran, N. 2014. Pathogenicity of *Pseudomonas aeruginosa* in *Oreochromis mossambicus* and treatment using lime oil nanoemulsion. *Colloids and Surfaces B: Biointerfaces*, 116:372-7.
- [62] Uluköy, G., Metin, S., Kubilay, A., Güney, Ş., Yıldırım, P., Güzel-Seydim, Z., Kok-Tas, T. and Gümüş, E. 2017. The Effect of kefir as a dietary supplement on nonspecific immune response and disease resistance in juvenile rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792). *Journal of the World Aquaculture Society*, 48(2):248-56.
- [63] Upadhaya, S.D. and Kim, I.H. 2017. Efficacy of phytogenic feed additive on performance, production and health status of monogastric animals—a review. *Annals of Animal Science*, 17(4):929-48.
- [64] Vouldoukis, I., Lacan, D., Kamate, C., Coste, P., Calenda, A., Mazier, D., Conti, M. and Dugas, B. 2004. Antioxidant and anti-inflammatory properties of a *Cucumis melo* LC. Extract rich in superoxide dismutase activity. *Journal of Ethnopharmacology*, 94(1):67-75.
- [65] Wu, G. 2010. Functional amino acids in growth, reproduction, and health. *Advances in Nutrition*, (1):31-7.
- [66] Youdim, K.A., Shukitt-Hale, B., MacKinnon, S., Kalt, W. and Joseph, J.A. 2000. Polyphenolics enhance red blood cell resistance to oxidative stress: in vitro and in vivo. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1523(1):117-22.