International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating: 5.29
IJABR 2025; 9(6): 666-676
www.biochemjournal.com
Received: 22-03-2025
Accepted: 26-04-2025

Ajay Kumar M
Fisheries Research and Information Centre, Hesaraghatta, KVAFSU, Bidar, Karnataka, India

Manjappa N
Fisheries Research and Information Centre, Hesaraghatta, KVAFSU, Bidar, Karnataka, India

Harsha Nayak
Inland Fisheries Unit, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

Pavan Kumar P
Fisheries Research and Information Centre, Hesaraghatta, KVAFSU, Bidar, Karnataka, India

Sai Sagar
Fisheries Research and Information Centre, Hesaraghatta, KVAFSU, Bidar, Karnataka, India

Ganapathi Naik M
Department of Aquaculture, College of Fisheries, KVAFSU, Bidar, Karnataka, India

Chandrakant Lingdhal
Fisheries Research and Information Centre, KVAFSU, Bidar, Karnataka, India

Vijayakumar S
Fisheries Research and Information Centre, KVAFSU, Bidar, Karnataka, India

Ravindragouda Patil
Department of Aquaculture, College of Fisheries, KVAFSU, Bidar, Karnataka, India

Corresponding Author:
Harsha Nayak
Inland Fisheries Unit, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

Evaluation of dietary moringa (*Moringa oleifera*) supplementation on growth, haematological parameters and immune responses in Amur common carp (*Cyprinus carpio haematopterus*)

Ajay Kumar M, Manjappa N, Harsha Nayak, Pavan Kumar P, Sai Sagar, Ganapathi Naik M, Chandrakant Lingdhal, Vijayakumar S and Ravindragouda Patil

DOI: https://www.doi.org/10.33545/26174693.2025.v9.i6h.4597

Abstract
Aquaculture is one of the world's fastest growing industries. Improved disease resistance, feed efficiency and growth performance of farmed fish species are all needed. Dietary supplementation of medicinal plants has developed good defence against pathogenic bacteria and there is rising interest in them as natural antibacterial medicines. 90 days feeding trial conducted to assess the effect of moringa (*Moringa oleifera*) food supplementation on growth performance, survival, immunological response, disease resistance, haematology and gut histology in Amur common carp (*Cyprinus carpio haematopterus*). In each experiment, basal diet (30% protein) was supplemented with moringa leaf powder at rates of 5 g (T_1), 15 g (T_2) and 25 g/kg (T_3). A control diet was also provided in triplicate (T_0). According to the findings, all groups had a 100% survival rate. At the conclusion of the feeding study, T3 demonstrated a substantial improvement ($p<0.05$) over the control group in final body weight (FBW), weight gain (WG), specific growth rate (SGR) and protein retention (PR) as well as a higher feed conversion ratio. Hematological parameters viz., RBC, WBC, haemoglobin, hematocrit, MCV, MCH and MCHC revealed no significant difference between the treatments. Lysozyme activity was considerably higher in treatment (T_3) ($p<0.05$) when compared to other treatment and superoxide anion generation was also higher in T_3. There was no harm or inefficiency among the doses evaluated and they appear to be safe for use in fish meals.

Keywords: Amur, moringa, haematology, immunology

Introduction
A significant segment of the population, particularly those involved in the fishing sector, depends on the fishing industry for their livelihood, thereby playing a crucial role in the nation's nutritional security. India ranks as the fourth-largest exporter of fish products globally, contributing roughly 7.7% of the total global production. However, fish reared in intensive culture systems are often susceptible to viral and bacterial infections due to immunosuppression caused by stressful environmental conditions. The concept of functional feeds designed to provide benefits that extend beyond basic nutritional requirements—remains relatively novel in the aquaculture industry, and further exploration of this approach could yield considerable benefits (Li P & Gatlin, 2004) [36]. In many cultured species, including African catfish, carp, and tilapia, growth promoters are routinely employed to enhance feed-to-muscle conversion rates as well as optimize fat and protein deposition (Nowosad *et al.*, 2023) [44]. Research indicates that these additives can significantly improve feed efficiency, production performance, immunological responses, and overall health. To maintain a sustainable aquaculture, both environmentally and financially, disease prevention is crucial. For this reason, preventative measures based on the stimulation of the fish immune system have been successfully applied, and they are now an integral component of the management of contemporary aquaculture processes. Since the discovery of antibiotics with growth-promoting and disease-fighting properties, the industry regularly uses considerable doses of antibiotics to prevent and/or control infectious diseases.

The possibility of detrimental effects on animal and human health, as well as the aquatic environment, has increased as a result of antibiotics' extensive use as therapeutic and growth-promoting medications. It has been extensively documented that many bacterial illnesses connected to fish disease exhibit antibiotic resistance (Richter *et al.*, 2003) [54]. Phytobiotics and plant derivatives, also known as "phytogenics" (Dongmeza *et al.*, 2006) [19] and "phytobiotics" which are primarily derived from aromatic plants and their essential oils, have been extensively used to improve the growth performance of fish (Hoseinifar *et al.*, 2011) [30]. Globally, India is the largest producer and supplier of *Moringa oleifera* (CBI, MoFA, 2018) [11] and it is categorized as "Novel Food" on the European market. The leaves and seed pods of moringa are rich in minerals, vitamins and essential amino acids (Kasolo *et al.*, 2010) [33]. Moringa leaf contains crude protein (CP) with about 260 g/kg of leaf, of which about 87% is true protein (Makkar and Becker, 1996) [40]. Essential amino acids found in moringa leaf are methionine, cysteine, tryptophan and lysine. They are rich source of calcium, potassium, vitamin A, vitamin C and choline (Ramachandran *et al.*, 1980; CBI, MoFA, 2018) [50, 11]. Moringa based products could provide enhanced health benefits due to their high mono, polyunsaturated fatty acid content and low saturated fatty acids (Saini *et al.*, 2014) [56]. Siddhuraju *et al.* (2003) [54] found that moringa natural antioxidants are functionally similar to widely used synthetic antioxidants such as butylated hydroxyl toluene and butylated hydroxyl anisole. *M. oleifera* is used as potential antioxidant, anticancer, anti-inflammatory, anti-diabetic and antimicrobial agent and it contains a large composition of polyphenolic compounds such as hydroxycinnamic acid derivatives and flavonoids known to have antioxidant activities towards oxidative stress (Morimitsu *et al.*, 2000) [41].

Amur carp is a popular fish species worldwide, known for its exquisite taste and high nutritional value. As the demand for Amur carp increases globally, there has been an increased focus on improving their growth rate and overall health through innovative feeding strategies. In recent years, the use of inulin-based diets has gained popularity as a promising approach to promoting the growth and health of Amur carp (Wang *et al.*, 2022) [67]. Inulin is a natural prebiotic fiber that has been found to enhance the growth and health of Amur carp. Studies have shown that inulin-based diets can improve the quality of gut microflora, leading to better nutrient absorption and immune function in Amur carp (Radhakrishnan *et al.*, 2020) [49]. In addition to its phytobiotic properties, Moringa also has low caloric value and can serve as a substitute for other high-calorie feed ingredients (White & Hekmat, 2018) [69], thereby potentially reducing the cost of feed and promoting sustainable aquaculture practices. Overall, the moringa diet can be a promising strategy for promoting the growth and health of Amur carp while also contributing to sustainable aquaculture practices (Verdegem *et al.*, 2021) [15].

The study of hematologic factors can be used to evaluate the state of aquatic animals in reaction to stress, pollutants, nutrients and biological and metabolic conditions. The assessment of blood cells, blood biochemistry and hormones could be helpful for illness detection and monitoring the physiological state of fish (Stoskopf, 1993) [63]. The use of moringa based diets has been found to enhance the growth and health of Amur carp, making it popular as a promising approach for promoting aquaculture. Studies have demonstrated that moringa is a natural supplementation improved growth, immunity against harmful microorganisms (Omitoyin *et al.*, 2019) [45]. This study comprised a quantitative analysis of growth, immune response, and blood parameters in amur common carp fed diets supplemented with moringa.

Methodology

Collection of Fish

A total of 180 healthy Amur carp fingerlings were procured from the Fisheries Research and Information Centre, Hesaraghatta, Bangalore (mean individual weight 4.0±0.5 g). They were reared in FRP Tanks and fed with a balanced diet at 5% body weight. Twice a week, the water was partially renewed and it was routinely checked. 25ºC was maintained as the temperature. For a few weeks, fishes were kept in the aquariums for adoption.

Preparation of Experimental Diet

For protein optimization, three test diets were formulated with a protein content of 30% by using the square technique (Hardy, 1980) [27]. Three experiment diets were prepared by incorporating moringa 5g, 15g and 25g per kg of feed and after that, it is referred to as T_1, T_2 and T_3. The control diet was prepared without incorporation of Moringa and is referred to as T_0 (Tables 1).

Experimental Design

The experiment was conducted in 250-liter FRP tanks at the Fisheries Research and Information Centre, Hesaraghatta, Bengaluru. Uniform in size Amur common carp fingerlings, with an average length and weight of 6.0±0.5 cm and 4.0±0.5 g respectively, were introduced into tanks at a stocking density of 15 individuals per tank. The experiment was conducted over a period of 90 days. The water exchange was done twice every week to remove the left-over feed and fecal matter. The fish were sampled once every 15 days to record their growth and survival.

Table 1. Proportion of various ingredients in different experimental diets.

Ingredients (%)	Treatment			
	T_0	T_1	T_2	T_3
Fish-meal	36.07	36.3	36.07	36.07
Groundnut oil cake	36.07	36.07	36.07	36.07
Rice bran	12.93	12.43	11.43	10.43
Tapioca	13.93	13.93	13.93	13.93
Vitamin-mineral mixture	1	1	1	1
Moringa	0	0.5	1.5	2.5
Total (100g)	100	100	100	100

Growth and Survival performance

Fishes of all replicates were weighed after 90 days of feeding trials, and body gain was measured. Total weight gain (WG), Specific growth rate (SGR), Protein efficiency ratio (PER), Feed conversion ratio (FCR) and mortality were observed throughout the experimental period.

$$\mathrm{SGR}(\%) = \frac{[\mathrm{Ln(Final\ weight)} - \mathrm{Ln(Initial\ weight)}]}{\mathrm{No.\ of\ days}} \times 100$$

$$\mathrm{Weight\ gain}(\%) = \frac{[\mathrm{Final\ weight(g)} - \mathrm{Initial\ weight(g)}]}{\mathrm{Initial\ weight(g)}} \times 100$$

$$\text{FCR} = \frac{\text{Dry weight of the feed given}}{\text{Gain in wet weight of fish}}$$

$$\text{PER} = \frac{\text{Gain in body}}{\text{Protein intake}}$$

Blood sampling
The fish from each replication were subjected to anaesthesia and blood samples were obtained by breaking the caudal vein using a 2 ml syringe that had been washed with an anticoagulant (Heparin). The blood was immediately transferred to heparin-coated vials. Blood was collected in 2ml centrifuge tubes without any anticoagulant and centrifuged for serum collection after centrifugation supernatant is pooled and stored at-20 °C.

Hematological parameters
Hematology parameters such as hematocrit, hemoglobin count, mean corpuscular hemoglobin (MCH), mean Leucocyte count, cell volume, erythrocyte count and mean corpuscular haemoglobin concentration and values were analyzed. An automatic hematology analyzer was used for the analysis of blood parameters. The MCV, MCH and MCHC values acquired from the hematology analyzer were further verified using standard formulae.

$$\text{MCHC} = \frac{\text{Hemoglobin (g/dl)}}{\text{Hematocit (\%)}} \times 100$$

$$\text{MCH} = \frac{\text{Hemoglobin (g/dl)}}{\text{RBC count (million mm}^{-3}\text{)}} \times 10$$

$$\text{MCV} = \frac{\text{Hematocrit (\%)}}{\text{RBC count (million mm}^{-3}\text{)}} \times 10$$

Immunological parameters
The immunological parameters, including serum albumin, serum globulin and total serum protein, were assessed using a serum analyzer (Clinical Chemical Analyzer, AGDTM 2020) using kits provided by AGDTM Biomedicals Pvt. Ltd., located in Mumbai, India. The Nitroblue tetrazolium (NBT) assay was performed using Anderson and Siwicki (1995) [3] method (Anderson and Siwicki, 1995) [3]. The turbidimetric technique proposed by Parry *et al.* (1965) [48] was used to quantify lysozyme activity (Parry *et al.*, 1965) [48].

Statistical analysis
The experiment was conducted in triplicate, with each tank containing 15 fish as the experimental unit. The statistical methodology used in this research was the application of one-way analysis of variance (ANOVA) to ascertain the existence of a statistically significant disparity in the average growth, survival and immunological response in relation to the treatment variable. The researchers proceeded to perform the Duncan Multiple Range Test using the SPSS software (version 20.0) in order to further examine these observed variations.

Results
The Growth performances and survival rate in Amur carp fingerlings were determined (Table 2). During the experimental period, the dietary supplemented Moringa showed improved feed conversion ratio, specific growth rate, weight gain, protein conversion ratio, and survival, with significant differences in these parameters within the diets. Survivability in the experimental period is almost similar in all the treatments. For the Amur fingerlings, after a 90-day feeding trial, the diets fed with 5 g, 15 g and 25 g showed better growth performances, including SGR, weight gain, and PER with high significance ($p<0.05$) and with that, the lowest FCR were seen in the treatments fed with moringa incorporated diets compared to control diets ($p<0.05$). The survival rates in Amur carp fingerlings have shown significant improvements in Moringa incorporated diets compared to control diets ($p<0.05$).

Table 2: Under various treatments and in the control group, FCR, SGR, PER, WG and fish survival were measured.

Parameters	Treatments			
	T_0	T_1	T_2	T_3
WG (%)	168.05±1.342	224.09±1.365	260.42±3.627	315.73±4.097
SGR (% per day)	1.09±0.007	1.30±0.003	1.42±0.012	1.58±0.012
FCR	1.52±0.006	1.36±0.006	1.27±0.003	1.13±0.006
PER	3.28±0.017	3.92±0.012	4.27±0.033	4.75±0.033
Survival (%)	100	100	100	100

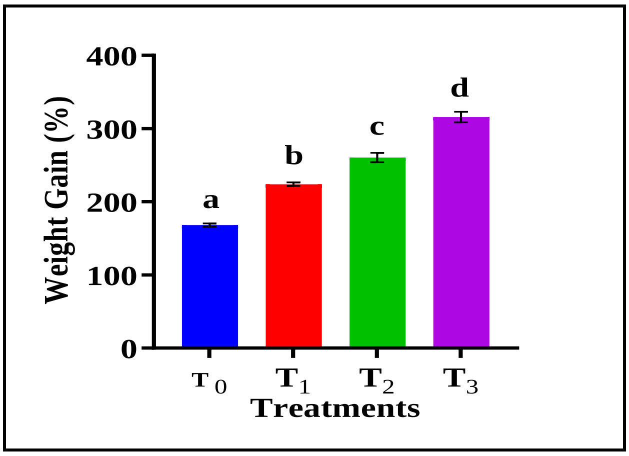

Fig 1: Influence of Moringa on carp weight increase

Figure 1 depicts the influence of Moringa on carp weight increase. T_0 has the least amount of Moringa influence on fish weight. Compared to the control group T_0, T_3 had the largest effect of Moringa on fish weight gain. T_1 showed the Moringa effect on fish weight to be the least significant.

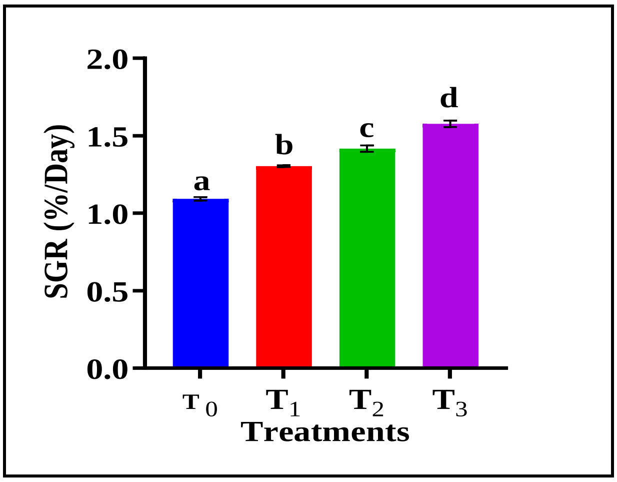

Fig 2: Influence of Inulin on carp specific growth rate

Figure 2 shows the impact of Moringa on fish (carp) specific growth rate. T_3 had the highest influence of Moringa on fish-specific growth rate compared to control T_0, followed by T_2 and T_1.

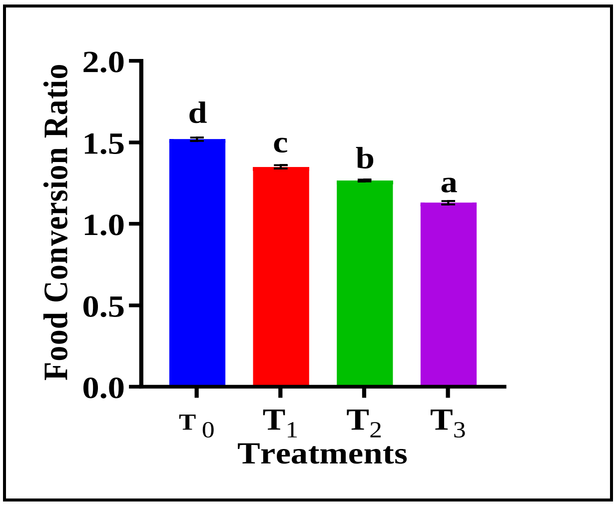

Fig 3: Food conversion ratio in fish carp after administration Moringa

Figure 3 illustrates the food conversion ratio in fish carp after administering the dietary supplement Moringa. The influence of the food supplement on Treatments is less to that of the control group T_0. The dietary supplement was shown to have minimal impact on T_3.

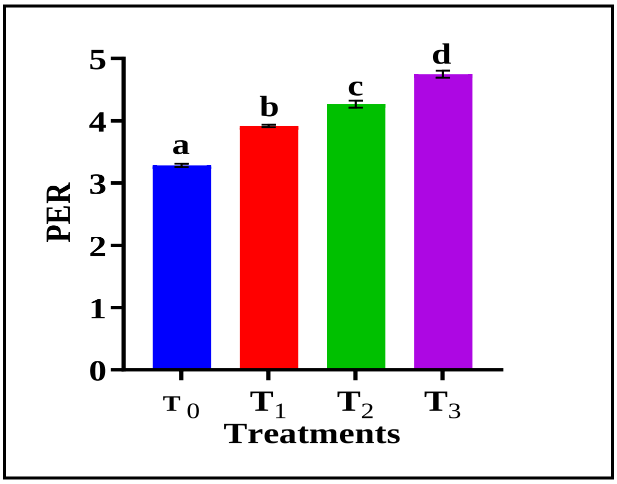

Fig 4: Protein conversion ratio in fish after administration of Moringa

Figure 4. shows the protein conversion ratio in fish after administration of the dietary supplement Moringa. The effect of the dietary supplement Moringa was observed maximum in T_3 compared to control T_0. The minimal effect of the dietary supplement Moringa was observed in T_1.

The hematological parameters were analyzed (Table 3). The dietary supplementation of Inulin has shown improved hemoglobin levels, RBCs, WBCs, Haematocrit, MCV, MCH and MCHC concentrations with high significance compared to the control diet ($p<0.05$).

Table 3. Hematological parameters of Amur common carp (*Cyprinus carpio haematopterus*) were recorded in different treatments and control groups.

Parameters	Treatments			
	T_0	T_1	T_2	T_3
Hemoglobin (g/dl)	17.0±0.107	17.8±0.244	18.2±0.195	19.4±0.173
RBC (10^6/mm^3)	7.11±0.223	8.01±0.115	8.11±0.031	8.41±0.147
WBC (10^3/mm^3)	14.9±0.311	15.0±0.157	15.6±0.090	16.6±0.040
Hematocrit (%)	51±0.906	53.4±0.085	54.6±0.294	58.2±0.120
MCV (fl)	70.6±0.643	70.8±0.415	72±0.182	72±0.150
MCH (pg)	23.6±0.238	23±0.170	24.2±0.458	24.4±0.303
MCHC (%)	34.8±0.254	35.4±0.408	36.0±0.164	36.8±0.196

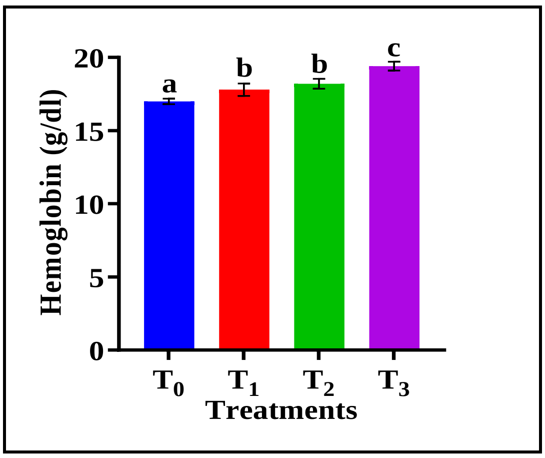

Fig 5: Impact of Moringa on haemoglobin

The impact of Moringa on hemoglobin is seen in Figure 5. T_1 has an equivalent impact on hemoglobin in the presence of Moringa as compared to T_2. T_3 has the highest degree of influence of Inulin on hemoglobin.

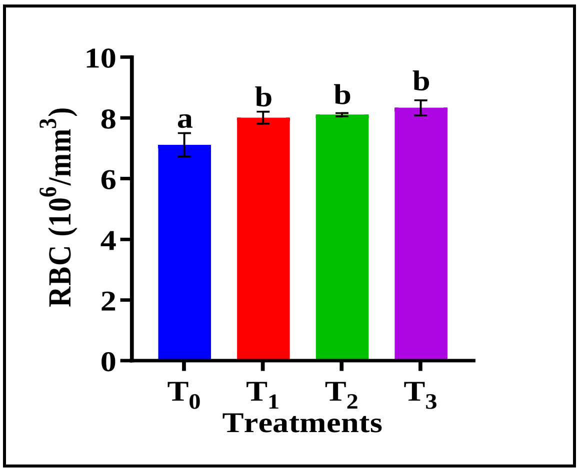

Fig 6: Influence of dietary supplementation of Moringa on the RBC

Figure 6 presents the influence of dietary supplementation of Moringa on the RBC levels. Treatment with moringa has the most significant observable influence of Moringa when compared to the control treatment T_0, whereas treatment T_1 displays a comparatively weaker effect. The effect of Moringa in the treatment group labelled as T_3 was determined to be maximum.

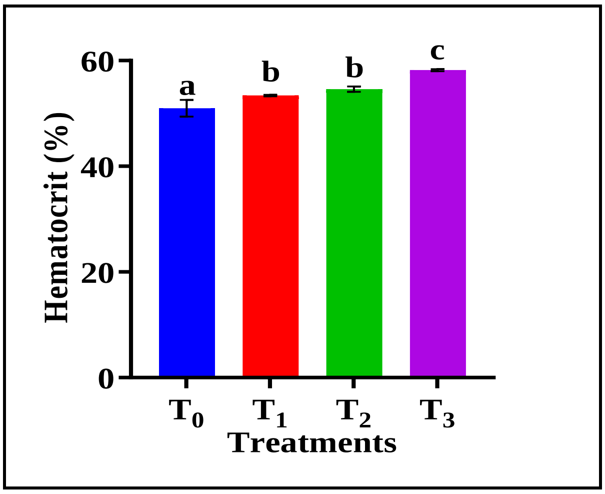

Fig 7: Impact of dietary supplementation of Moringa on the hematocrit levels of fish

The influence of dietary supplementation of Moringa on the hematocrit levels of fish is shown in Figure 7. Treatment T_3 has the highest observed impact of Moringa compared to the control treatment T_0, with treatment T_1 exhibiting a lesser effect.

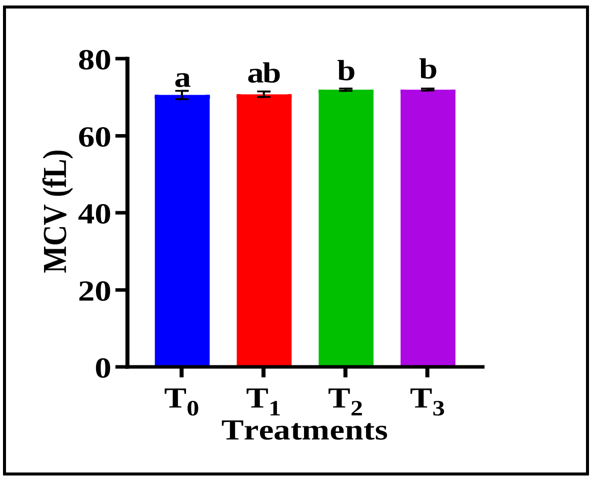

Fig 8: Influence of dietary supplementation of Moringa on fish's MCV (ft) levels

Figure 8 presents the influence of dietary supplementation of Moringa on fish's MCV (ft) levels. Treatments of Moringa were shown higher values when compared to the control treatment T_0, whereas treatment T_1 displays a comparatively weaker effect.

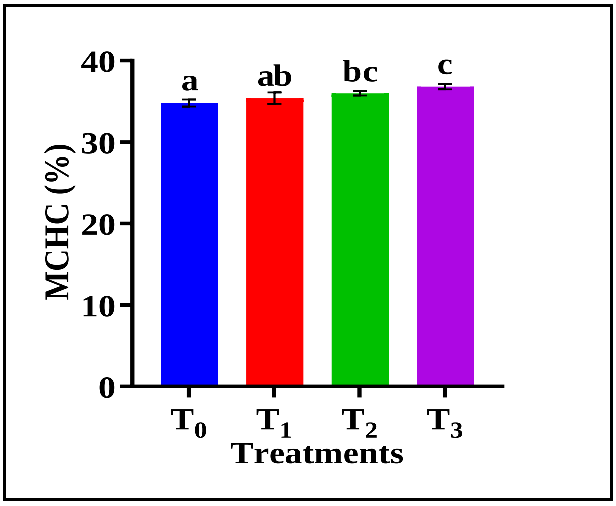

Fig 9: Impact of dietary supplementation of Moringa on fish's MCH concentration levels

The impact of dietary supplementation of Moringa on fish's mean corpuscular hemoglobin concentration levels is shown in Figure 9. Treatment T_3 has the most pronounced and statistically significant impact on Moringa compared to the control treatment T_0, followed by T_2 and T_1.

The outcome of the dietary Moringa supplementation on immune parameters is shown in Table 4. The Moringa-supplemented fishes have significantly improved values of NBT, Lysozyme activity compared to control-fed fishes (p<0.05). The study revealed that fishes fed control diets exhibited higher blood protein, globulin, and albumin globulin ratio levels than those fed Moringa. This difference was statistically significant across the various treatments ($p < 0.05$). Following the administration of various doses of an Moringa-based dietary supplement, the observed Lysozyme activity (10^3/l) in fish is shown in Table 4. When compared to the control treatment T_0, the T_3 therapy shows greater levels of Lysozyme activity (10^3/l) in fish. Following the administration of dietary Moringa supplementation, the lowest measured NBT activity in fish was seen in T_1.

The total serum protein (g/dl) in fish after the administration of different doses of an Moringa-based dietary supplement is shown in Table 4. The treatment groups (T_1, T_2 and T_3) demonstrates elevated levels of NBT activity in fish compared to the T_0 (control) treatment. The albumin (g/dl) level in fish after the administration of different doses of a Moringa-based dietary supplement is shown in Table 4. The treatment groups (T_1, T_2 and T_3) demonstrates elevated levels of albumin (g/dl) level in fish in comparison to the T_0 (control) treatment

Similarly, table 4 presents the observed globulin (g/dl) level in fish after the administration of varying dosages of a moringa based dietary supplement. The T_0 treatment exhibits higher levels of globulin (g/dl) in fish compared to the treatment, T_3. The lowest measured globulin (g/dl) level in fish was seen in T_1 following the administration of dietary moringa supplementation.

Table 4: Serum immunological parameters of Amur common carp (*Cyprinus carpio haematopterus*) recorded in different treatments and control group.

Parameters	Treatments			
	T_O	T_1	T_2	T_3
Total serum protein (g/dl)	7.6±0.078	7.8±0.090	8.0±0.093	8.1±0.015
Albumin (g/dl)	4.2±0.035	4.8±0.046	4.8±0.046	5.0±0.074
Globulin (g/dl)	3.4±0.212	3.0±0.046	3.2±0.078	3.1±0.025
Albumin-globulin ratio	1.24±0.067	1.60±0.012	1.50±0.030	1.61±0.015
NBT activity (OD at 620 nm)	0.489±0.047	1.25±0.067	1.28±0.015	1.37±0.049
Lysozyme activity (10^3/l)	240±1.732	550±12.583	700±7.638	980±13.204

Discussion

Aquaculture has rapidly emerged as the fastest-growing segment in food production, playing a critical role in sustaining global food security. Optimal nutrition in aquatic animal production is essential for cultivating high-quality, resilient products while also controlling costs, as feed expenses account for 40%-50% of overall production expenditures. Given that traditional antibiotics and chemotherapeutics can suppress immune responses and encourage the development of resistant pathogens (Chinabut & Puttinaowarat, 2005) [12], there is a pressing need for alternative strategies that ensure sustainable growth and effective health management. Although alternative therapies such as vaccination are under investigation, solid evidence of their effectiveness for many farmed fish species remains elusive (Mahious *et al.*, 2006) [39]. Consequently, the aquaculture industry has increasingly turned to feed additives including vitamins, minerals, and phytobiotics to enhance the overall welfare of aquatic animals and reduce disease risk. The growing popularity of these functional additives is primarily driven by persistent challenges in managing disease outbreaks in fish intended for human consumption and the need to stimulate the host's non-specific defense mechanisms against pathogens (Chinabut & Puttinaowarat, 2005) [12].

The use of medicinal plants as a natural feed addition in fish diets is advantageous as an alternative for phytobiotics and antibiotics, both of which are necessary to support fish health. Fish can benefit from the growth-promoting, antibacterial and immunostimulant effects of these plants (Reverter *et al.*, 2014) [54]. Moringa leaves powder is considered as one of the most potential dietary medicines for the prevention and treatment of a variety of fish diseases. In the current study, the supplementation of moringa leaves powder in the diet increased weight gain, specific growth rate and feed conversion ratio in Amur common carp. The fish fed moringa leaves powder incorporated diet at a rate of 25 g/kg feed showed a substantial increase (p<0.05) in weight gain (23.99±0.304 g), specific growth rate (1.55±0.009), protein retention (4.66±0.021) and a significant decrease in FCR (1.11±0.031).

The moringa leaf powder incorporated in the commercial feed resulted in distinct impact on the Amur common carps specific growth rate, daily length growth rate, feed conversion ratio and protein retention over the period of a 90 days feeding trial. This may be due to the result from the digestive tract optimal absorption process or from the feeds absorption of nutrients. Moringa has been shown in other research to considerably improve fish growth indices and feed utilisation (Ozovehe, 2013; Sherif *et al.*, 2014) [46, 59].

Fish fed 200 mg MOAE/kg food showed considerable growth performance measures (FW, WG and SGR) in comparison to other groups (Emam *et al.*, 2021) [22]. Shourbela *et al.* (2020) [60] also clarified that *O. niloticus* fingerlings fed on MOAE containing diet showed an enhanced growth performance. Heidarieh *et al.* (2013) [29] indicated that *M. oleifera* had improved the food digestibility and absorption capacity of *Oncorhynchus mykiss*.

The sea bass fed *Cissus quadrangularis* powder supplemented diets at 0.5 g, 1.0 g, and 1.5 g/kg inclusion levels increased their body weight significantly ($p<0.05$) more than the basal diet fed fishes (Devakumar and Chinnasamy, 2017) [16]. Ribeiro *et al.* (2016) [53] discovered a high feed conversion ratio in tambaqui fish fed with a peppermint-infused diet at a rate of 1.5%/kg feed. The ginger diet served as an appetiser enhancer and its supplementation at the rates of 5 g and 10 g/kg feed was most beneficial for Asian sea bass growth and survival (Talpur and Ikhwanuddin, 2013) [65].

Similarly, clove at 0.5% improved digestion, which resulted in faster development in *Labeo rohita* (Asimi *et al.*, 2015) [8]. *Tinospora cordifolia* dietary inclusion in all concentrations increased weight gain, feed conversion ratio, gross conversion efficiency, and specific growth rate in Amur carp fingerlings (Anita *et al.*, 2016) [4]. Dietary inclusion of *Ficus carica* polysaccharides (0.4%) improved final weight, feed conversion ratio and survival rate of crucian carp (Wang *et al.*, 2022) [67]. Aloe vera diets at 1% and 2%/kg feed significantly ($p<0.05$) increased weight gain, absolute growth rate and specific growth rate in GIFT tilapia (Gabriel *et al.*, 2015) [25]. Feeding with *Mentha pipperita* diet at 5g/kg feed to *Lates calcarifer* stimulated hunger and resulted in considerably enhanced weight gain, specific growth rate and feed conversion ratio (Talpur, 2014) [64]. Dietary inclusion of 2% Peppermint (*Mentha piperita*) measured weight gain, specific growth rate, and food conversion ratio in *Labeo rohita* (Padala *et al.*, 2018) [47].

Dietary supplementation with moringa leaf powder has less effect on the survival of Amur common carp. Treatment T_3 showed a higher survival rate followed by T_2 and T_1. Although there are a few studies that demonstrate moringa leaf powder has a deleterious influence on some species at higher dosages of absorption. The survival rate of Nile tilapia on a diet consisting 20% moringa leaves was high (Doctolero and Bartolome, 2019) [18]. Nile tilapia diets supplemented with ethanolic extract of *M. oleifera* flower, specifically at 0.25% inclusion level, can be helpful to modulating the immune status of the fish and hence improve the disease resistance (Tekle *et al.*, 2015) [66]. After eight weeks of the dietary trial, fish receiving *O. vulgare* in their diets showed an improved survival rate when compared to that of the control treatment after 10 days post-infection with *A. hydrophila* (Rashidian *et al.*, 2021) [51]. In the current experiment, there were no evident deviations in the fish development patterns or behaviour.

In aquaculture, haematological parameters have been established as key indicators of fish nutritional and physiological health (Fazio, 2019) [24]. Red blood cells, white blood cells, haemoglobin, packed cell volume and related indices, such as mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration, have all been tested to determine erythrocyte status and oxygen carrying ability in fish (Houston, 1997) [31]. Dietary supplementation with *M. oleifera* leaves increased African catfish haematological indices such as packed cell volume (PCV), haemoglobin (Hb), red blood cell count (RBCs) and white blood cell count (WBCs) compared to the control group. Ayoola *et al.* (2012) [9] discovered a significant drop in mean cell haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) values when compared to controls and stated that these could be the effects of the hemolysis caused by *A. hydrophila*.

The maximum red blood cell (RBC) value ($2.95\times106/mm^3$) was obtained in *Labeo rohita* fed with 10% MOLM based food, while the lowest value ($1.50\times106/mm^3$) was observed in fish fed 40% MOLM based diet. The best value was observed in fish fed a 10% MOLM diet, which was substantially different ($p>0.05$) from fish fed 0%, 20%, 30% and 40% MOLM diets (Arsalan *et al.*, 2016) [7]. *Oreochromis mossambicus* fed moringa-based diets showed an increase in haematological indices such PCV, RBCs, WBCs, Hb and haematocrit (HTC) levels. Common carp fed moringa leaf meal showed better haematological indices including PCV, Hb, RBCs and WBCs.

Moringa incorporation in feed significantly increased all hematological parameters at pre and post starvation exposure (Elabd *et al.*, 2019) [20]. Nile tilapia fed diets with 10% and 15% *M. oleifera* leaves for six weeks significantly increased blood indices (Sherif *et al.*, 2014) [59]. In the present study, significantly higher RBCs, haemoglobin and WBCs levels observed in treatment (T_3) fish fed with moringa leaf powder incorporated diets compare to control group. Fish fed a diet that contained 50% *M. olifera* leaf meal had the highest value of WBC, $7.90 \times 10^3/mm^3$. The fish fed diets with 40 and 50 percent *M. olifera* leaf meal had lymphocyte counts with the highest value of 70.00%, according to the results of the test. The body's immune system is composed of lymphocytes and white blood cells (WBC) (Dienye and Olumuji, 2014) [17].

O. niloticus supplemented with different concentrations of moringa showed considerably improved haemograms (RBC, Hb, PCV, MCV, MCH and MCHC), showing that the integration of moringa improved health status (Sherif *et al.*, 2014; Nascimento *et al.*, 2016) [59, 42]. Sherif *et al.* (2014) [59] reported that hemogram including RBC, Hb, PCV, MCV, MCH and MCHC of *O. niloticus* supplemented with different concentrations of *M. oleifera* is significantly improved. The fish fed a 200 mg MOAE/kg diet showed significant enhancement in RBCs counts, Hb concentration and PCV values compared to the control group (Mahamoud *et al.*, 2021) [38]. The fish fed 400 mg MOAE/kg diet expressed significant decreases in RBC, Hb, PCV and MCHC with an increase in MCV resulting in macrocytic hypochromic anemia. This owed to *M. oleifera* causes enhancement in health status (Nascimento *et al.*, 2016) [42] as it contains saponin (Khalil and Eladawy, 1994) [34], alkaloids and flavonoids which elevates RBCs, HB, and PCV (Anwar *et al.*, 2007) [6]. The elevated WBCs count may be owed to the immunomodulatory capacity of *M. oleifera* in fish (Stadtlander *et al.*, 2013) [62]. Elabd *et al.* (2019) [20] discovered that food supplementation with MOLM increased all haematological parameters of treated Nile tilapia, including PCV, Hb, RBCs and WBCs, when compared to controls. Reduced RBC numbers and haemoglobin concentrations show that leucocytosis activity is destroying RBCs in erythrocytic anaemia, leading to erythroblastosis (Haney *et al.*, 1992) [26]. Infected fish fed diets with varying quantities of button mushroom (*Agaricus bisporus*) had higher RBC count and Hb content than infected fish fed the control diet (Harikrishnan *et al.*, 2018) [28]. Leucocyte cells are one of the primary components of body defence; an increase in the amount of WBC in blood may indicate the presence of inflammation in the organism (Czech *et al.*, 2009) [14]. Total and differential leucocytes were found to be considerably higher in fish fed moringa powder-infused meals in the present study. Total leucocytes increased significantly both before and after the challenge, with greater leucocyte counts reported in fish fed a moringa powder included diet at a rate of 2.5 g/kg feed. The large rise in WBC suggests that the immune system in fish has been stimulated. WBC is vital in immunological responses and improves the animal's ability to fight infection (Jane and Jane, 2010) [32].

Fish serum immunological markers are important because they provide data on metabolism and general health. The degree of stress and presence of pathogenetic agents in the environment clearly represent several blood metabolites such as total serum protein, albumin, and globulin. Moringa supplemented meals exhibited no negative effects on blood immunological markers in the current study, and in fact, several of them improved. The NBT assay primarily measures the formation of free oxidative radicals by leukocytes in response to fish infections (Cook *et al.*, 2003, Sahoo *et al.*, 2005) [13, 55]. Nitroblue Tetrazolium Assay (NBT) activity is regarded as one of the most important defensive mechanisms in fishes and is an essential biomarker of oxygen-dependent bactericidal activity (Secombes, 1990) [58]. It is widely assumed that after activation, fish phagocytes generate superoxide anion and its reactive derivatives such as hydrogen peroxide and hydroxyl radicals during high oxygen consumption (Secombes, 1990) [58].

In the present study, the NBT assay level was high in treatment T_3 (1.34±0.118) followed by T_2 (1.28±0.075) and T_1 (1.25±0.087) treatments. The control group showed low (0.54±0.050) NBT assay level in compared to other treatment groups. Antony *et al.* (1999) [5] reported that Curcuminoids have good inhibitory effects on reactive oxygen species (ROS) production. Dietary devil pepper (*Rauvolfia tetraphylla*) extract significantly improved ($p<0.05$) respiratory burst activity in rohu against *Aphanomyces invadans* infection (Yogeshwari *et al.*, 2015) [72]. Ngugi *et al.* (2015) [43] discovered that a 5% stinging nettle (*Urtica dioica*) supplemented meal boosted respiratory burst activity in Victoria labeo (*Labeo victorianus*). Lysozyme is a blood enzyme that actively lyses bacteria; a higher concentration in fish has been thought to represent a natural protective mechanism. Lysozyme is thought to be produced by neutrophils, and the enzyme appears to be more bactericidal than lysozyme from higher vertebrates (Ellis, 2001) [21]. The lysozyme is one of the most significant components of fish defensive mechanisms, acting via complement activation and phagocytosis (Magnadottir, 2006) [37]. Lysozyme is an essential component of fish immune systems. Bacteriolysis occurs as a result of hydrolyzing bacterial cell wall peptidoglycans and it also acts as an opsonin (Magnadottir, 2006) [37].

In the present study, enhanced serum lysozyme activity was recorded in all the treatments when compared to control. There was significant difference ($p>0.05$) observed between various dietary treatments. The highest lysozyme activity was recorded in treatment T_3 (1203±0.579) followed by T_2 (991±1.154), T_1 (982±1.155) and T_0 (214±1.152). The role of the bioactive compounds in the extract is to boost the nonspecific defence system including the lysozyme activity, phagocytic activity and the respiratory burst activity of the immune cells Phytobiotics, medicinal herbs possess immune-stimulating abilities with proven bactericidal activities against numbers of pathogenic bacteria affecting man, animal and fishes and are possible alternatives to antibiotics (Khan *et al.*, 2009) [35]. Lysozyme is a leukocytic innate immunity mediator that has antibacterial activity and stimulates phagocytosis; it is a good indicator of fish nonspecific immune functions (Saurabh and Sahoo, 2008) [57].

Feeding *M. oleifera* leaf powder increased the Nile tilapia's 100% relative percent survival (RPS), respiratory burst, phagocytic, and LYZ activities, as well as IgM levels (El-Gawad *et al.*, 2019) [20]. Tekle and Sahu (2015) [66] discovered that consuming *M. oleifera* flower extract in the diet significantly raised serum LYZ, phagocytic and respiratory burst activities in addition to improving resistance to *A. hydrophila*. In *M. oleifera* supplemented groups, respiratory burst, phagocytic and lysozyme activity increased significantly ($p<0.05$) compared to controls.. The innate immune response of common carp fed 10 g MSLE/kg diet enhanced significantly ($p<0.05$) when compared to fish fed a control diet (Adeshina *et al.*, 2019) [1]. The bioactive components in *Mitracarpusscaber* leaves extract may be responsible for increased respiratory boost activity, lysozyme, SOD and catalase activities (MSLE). Tilapia given 0.1 and 0.5% *Astragalus radix* for one week increased lysozyme activity and stimulated phagocytosis by phagocytic blood cells for three weeks, but did not increase phagocytic cells respiratory burst activity (Yin *et al.*, 2006) [71]. Rainbow trout fed with 1.5 dill (*Anethum graveolens* L.) has significantly higher levels of lysozyme, alternative complement pathway haemolytic activity, superoxide dismutase and catalase activity in their serum than the others (Zeilab *et al.*, 2020) [73].

The total serum protein concentration is often regarded as a key measure of fish health. The main serum proteins are albumin and globulin, which both play significant roles in the immune response. Fish with higher blood protein, albumin and globulin levels are thought to have a stronger immune system (Wiegertjes *et al.*, 1996) [70]. Serum contains two types of proteins: albumin and globulin. Globulin is made up of fragments of 1, 2 and globulins that were previously utilised to generate Ig. Proteins are the most important components in serum and their concentration is utilised to assess fish health (Alexander *et al.*, 2011) [2]. Higher serum protein levels are thought to be associated with a stronger innate immune response (Magnadottir, 2006)

[37]. The most essential serum protein is globulin, which is involved in the immunological response (Alexander *et al.*, 2011) [2].

Conclusion

The results obtained in this study showed that dietary phytobiotic inclusion at various doses of feed has improved growth performance and survivability in Amur carp. In addition, the dietary inclusion of phytobiotics increased the hematological and immunological parameters. Regarding growth metrics, hematological parameters, survival rate and biochemical parameters, the therapy T_3 produced the best results. All fish survived after being given to Amur carp (*Cyprinus carpio haematopterus*), indicating no effect on the fish's survival rate. Between treatments and the control group, all measures have significant associations ($P < 0.05$). In every therapy, the survival rate was 100%. Hence, the incorporation of dietary Moringa as a supplement in animal feed is a viable option that offers advantages in terms of improving growth parameters and strengthening the immune response in amur carp.

References

1. Adeshina SA, Adedigba AP, Adeniyi AA, Aibinu AM. Breast cancer histopathology image classification with deep convolutional neural networks. In: 2018 14th International Conference on Electronics Computer and Computation (ICECCO). 2018:206-212.
2. Alexander C, Sahu NP, Pal AK, Akhtar MS. Haemato-immunological and stress responses of *Labeo rohita* (Hamilton) fingerlings: effect of rearing temperature and dietary gelatinized carbohydrate. Journal of Animal Physiology and Animal Nutrition. 2011;95(5):653-663.
3. Anderson DP, Siwicki AK. Basic hematology and serology for fish health programmes. Diseases in Asian Aquaculture. 1995;2:185-202.
4. Anita, Dubey MK, Khati A, Chauhan RS. Immunostimulatory and growth promoting potential of *Tinospora cordifolia* Miers on fingerlings of amur carp. Indian Journal of Experimental Biology. 2016;54:659-663.
5. Antony S, Kuttan R, Kuttan G. Immunomodulatory activity of curcumin. Immunological Investigations. 1999;28:291-303.
6. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: a food plant with multiple medicinal uses. Phytotherapy Research. 2007;21:17-25.
7. Arsalan MZH, Hussain SM, Asrar M, Anwar H, Rehan MMH, Shahzad MM, Wahab N. Effects of *Moringa oleifera* leaf meal (MOLM) based diets on carcass composition and hematology of *Labeo rohita* fingerlings. Journal of Biodiversity and Environmental Sciences. 2016;9:214-223.
8. Asimi OA, Sahu NP, Pal AK. Immunomodulatory and growth response of *Labeo rohita* to dietary fortification of clove and/or cardamom extract. Global Journal of Fisheries and Aquaculture. 2015;3(5):230-237.
9. Ayoola SO, Bassey BO, Alimba CG, Ajani EK. Textile effluent induced genotoxic effects and oxidative stress in *Clarias gariepinus*. Pakistan Journal of Biological Sciences. 2012;15:804-812.
10. Ayotunde EO, Ada FB, Udeh GN. Effect of partial replacement of fishmeal with *Moringa oleifera* leaf meal on the haematology, carcass composition and growth performance of *Clarias gariepinus* (Burchell 1822) fingerlings. International Journal of Fisheries and Aquatic Studies. 2016;4:307-311.
11. CBI, Ministry of Foreign Affairs. Centre for the Promotion of Imports from Developing Countries. 2018.
12. Chinabut S, Puttinaowarat S. The choice of disease control strategies to secure international market access for aquaculture products. Developments in Biologicals. 2005;121:255-261.
13. Cook MT, Hayball PJ, Hutchinson W, Nowak BF, Hayball JD. Administration of a commercial immunostimulant preparation, Eco Activa™ as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (*Pagrus auratus*, Sparidae (Bloch and Schneider)) in winter. Fish & Shellfish Immunology. 2003;14(4):333-345.
14. Czech A, Kowalczuk E, Grela ER. The effect of an herbal extract used in pig fattening on the animals' performance and blood components. Annales Universitatis Mariae Curie-Skłodowska. 2009;27:25-33.
15. Deng Y, Kokou F, Eding EH, Verdegem MCJ. Impact of early-life rearing history on gut microbiome succession and performance of Nile tilapia. Animal Microbiome. 2021;3(1):81-92.
16. Devakumar C, Chinnasamy A. Dietary administration of natural immunostimulants on growth performance, haematological, biochemical parameters and disease resistance of Asian sea bass *Lates calcarifer* (Bloch, 1790). Aquaculture Research. 2017;48(3):1131-1145.
17. Dienye HE, Olumuji OK. Growth performance and haematological responses of African mud catfish *Clarias gariepinus* fed dietary levels of *Moringa oleifera* leaf meal. Net Journal of Agricultural Science. 2014;2(2):79-88.
18. Doctolero JS, Bartolome RM. Utilization of horseradish (*Moringa oleifera*) as an alternative protein-source feed ingredient in the diet of red Nile tilapia (*Oreochromis niloticus*). International Journal of Fisheries and Aquatic Studies. 2019;7(5):94-97.
19. Dongmeza E, Siddhuraju P, Francis G, Becker K. Effects of dehydrated methanol extracts of *Moringa oleifera* leaves and three of its fractions on growth performance and feed nutrient assimilation in Nile tilapia (*Oreochromis niloticus* L.). Aquaculture. 2006;261:407-422.
20. Elabd H, Soror E, El-Asely A, Abdel-Gawad AEA. Dietary supplementation of Moringa leaf meal for Nile tilapia *Oreochromis niloticus*: effect on growth and stress indices. Egyptian Journal of Aquatic Research. 2019;45(3):265-271.
21. Ellis AE. Innate host defense mechanisms of fish against viruses and bacteria. Developmental & Comparative Immunology. 2001;25(8-9):827-839.
22. Emam MA, Shourbela RM, El-Hawarry WN, Abo-Kora SY, Gad FAM, Abd El-latif AM, Dawood MA. Effects of *Moringa oleifera* aqueous extract on the growth performance, blood characteristics, and histological features of gills and livers in Nile tilapia. Aquaculture and Fisheries. 2021;6(6):662-671.
23. Abdel-Gawad EA, El-Asely AM, Soror EA, Abbass AA, Austin B. Effect of dietary *Moringa oleifera* leaf on the immune response and control of *Aeromonas*

hydrophila infection in Nile tilapia (*Oreochromis niloticus*) fry. Aquaculture International. 2020;28:389-402.
24. Fazio F. Fish hematology analysis as an important tool of aquaculture: a review. Aquaculture. 2019;500:237-242.
25. Gabriel NN, Qiang J, He J, Ma XY, Kpundeh MD, Xu P. Dietary *Aloe vera* supplementation on growth performance, some haematobiochemical parameters and disease resistance against *Streptococcus iniae* in tilapia (GIFT). Fish & Shellfish Immunol. 2015;44(2):504-514.
26. Haney DC, Hursh DA, Mix MC, Winton JR. Physiological and hematological changes in chum salmon artificially infected with erythrocytic necrosis virus. J Aquat Anim Health. 1992;4:48-57.
27. Hardy R. Fish feed formulation. In: Fish feed technology. FAO, Rome; 1980. p. 111-170.
28. Harikrishnan R, Naafar A, Musthafa MS, Ahamed A, Arif IA, Balasundaram C. Effect of *Agaricus bisporus* enriched diet on growth, hematology and immune protection in *Clarias gariepinus* against *Flavobacterium columnare*. Fish Shellfish Immunol. 2018;73:245-251.
29. Heidarieh M, Mirvaghefi AR, Sepahi A, Sheikhzadeh N, Alishahbazfar A, Akbari M. Effects of dietary *Aloe vera* on growth performance, skin and gastrointestine morphology in rainbow trout (*Oncorhynchus mykiss*). Turk J Fish Aquat Sci. 2013;13(2):387-393.
30. Hoseinifar SH, Mirvaghefi A, Merrifield DL, Amiri BM, Yelghi S, Bastami KD. The study of some haematological and serum biochemical parameters of juvenile beluga (*Huso huso*) fed oligofructose. Fish Physiol Biochem. 2011;37(1):91-96.
31. Houston AH. Are the classical haematological variables acceptable indicators of fish health? Trans Am Fish Soc. 1997;126:879-894.
32. Jane DW, Jane KW. Schalm's Veterinary Hematology. Oxford: John Wiley & Sons, Blackwell Publishing Ltd.; 2010. p. 1232.
33. Kasolo JN, Bimenya GS, Ojok L, Ochieng J, Ogwal-Okeng JW. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. J Med Plants Res. 2010;4(9):753-757.
34. Khalil A, Eladawy T. Isolation, identification and toxicity of saponins from different legumes. Food Chem. 1994;50(2):197-201.
35. Khan A, Zaman G, Anderson RA. Bay leaves improve glucose and lipid profile of people with type 2 diabetes. J Clin Biochem Nutr. 2009;44(1):52-56.
36. Li P, Gatlin III DM. Dietary brewer's yeast and the prebiotic Grobiotic-AE influence growth performance, immune response and resistance of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) to *Streptococcus iniae* infection. Aquaculture. 2004;231:445-456.
37. Magnadottir B. Innate immunity of fish (overview). Fish Shellfish Immunol. 2006;20(2):137-151.
38. Mahamoud E, Shourbela RM, El-Hawarry WN, Abo-Kora SY, Gad FAM, Abd El-latif AM, Dawood MA. Effects of *Moringa oleifera* aqueous extract on the growth performance, blood characteristics, and histological features of gills and livers in Nile tilapia. Aquac Fish. 2021;6(1):72-79.
39. Mahious AS, Gatesoupe FJ, Hervi M, Metailler R, Ollevier F. Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, 1758). Aquac Int. 2006;14(3):219-229.
40. Makkar HA, Becker K. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Anim Feed Sci Technol. 1996;63(1-4):211-228.
41. Morimitsu Y, Hayashi K, Nakagawa Y, Fujii H, Horio F, Uchida K, Osawa T. Antiplatelet and anticancer isothiocyanates in Japanese domestic horseradish, wasabi. Mech Ageing Dev. 2000;116(2-3):125-134.
42. Nascimento JA, Magnani M, Sousa JM, Araujo KL, Epaminondas PS, Souza AS, Souza AL, Silva MCD, Souza AG. Assessment of the antioxidant effects of *Moringa oleifera* Lam. extracts in fish oil during storage. J Food Process Preserv. 2016;40(1):29-36.
43. Ngugi CC, Oyoo-Okoth E, Mugo-Bundi J, Orina PS, Chemoiwa EJ, Aloo PA. Effects of dietary administration of stinging nettle (*Urtica dioica*) on the growth performance, biochemical, hematological and immunological parameters in juvenile and adult *Victoria Labeo* (*Labeo victorianus*) challenged with *Aeromonas hydrophila*. Fish Shellfish Immunol. 2015;44(2):533-541.
44. Nowosad J, Abdel-Latif HMR, Łuczyński MJ, Boroń A, Kapusta A, Kujawa R, Przybył A, Kucharczyk D. Artificial reproduction of vimba bream (*Vimba vimba*) cultured generation F2 and eggs management under controlled conditions. Aquaculture. 2023;575:739741.
45. Omitoyin BO, Ajani EK, Orisasona O, Bassey HE, Kareem KO, Osho FE. Effect of guava *Psidium guajava* (L.) aqueous extract diet on growth performance, intestinal morphology, immune response and survival of *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. Aquac Res. 2019;50(7):1851-1861.
46. Ozovehe BN. Growth performance, haematological indices and some biochemical enzymes of juveniles *Clarias gariepinus* (Burchell 1822) fed varying levels of *Moringa oleifera* leaf meal diet. J Aquac Res Dev. 2013;4(2):1-7.
47. Padala D, Ganapathi Naik M, Anjusha KV, Ramesh KS, Abhiman PB, Rakesh K. Growth promoter effect of peppermint (*Mentha piperita*) on rohu (*Labeo rohita*). Int J Fish Aquat Stud. 2018;6:537-539.
48. Parry JR, Chandan RC, Shahani KM. A rapid and sensitive assay of muramidase. Proc Soc Exp Biol Med. 1965;119(2):384-386.
49. Radhakrishnan DM, Velayudhannair K, Schmidt BV, Radhakrishnan DK. Effects of bio-flocculated algae on the growth, digestive enzyme activity and microflora of freshwater fish *Catla catla* (Hamilton 1822). Aquac Res. 2020;51(11):4533-4540.
50. Ramachandran C, Peter KV, Gopalakrishnan PK. Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. Economic Botany. 1980;34(3):276-283.
51. Rashidian G, Boldaji JT, Rainis S, Prokic MD, Faggio C. Oregano (*Origanum vulgare*) extract enhances zebrafish (*Danio rerio*) growth performance, serum and mucus innate immune responses and resistance against *Aeromonas hydrophila* challenge. Animals. 2021;11(2):299.

52. Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture. 2014;433:50-61.
53. Ribeiro SC, Castelo AS, Silva BMPD, Cunha ADS, Proietti Junior AA, Oba-Yoshioka ET. Hematological responses of tambaqui *Colossoma macropomum* (Serrassalmidae) fed with diets supplemented with essential oil from *Mentha piperita* (Lamiaceae) and challenged with *Aeromonas hydrophila*. Acta Amazonica. 2016;46(1):99-106.
54. Richter N, Siddhuraju P, Becker K. Evaluation of nutritional quality of moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture. 2003;217(1-4):599-611.
55. Sahoo PK, Kumari J, Mishra BK. Non-specific immune responses in juveniles of Indian major carps. Journal of Applied Ichthyology. 2005;21(2):151-155.
56. Saini RK, Shetty NP, Giridhar P. GC-FID/MS analysis of fatty acids in Indian cultivars of *Moringa oleifera*: potential sources of PUFA. Journal of the American Oil Chemists' Society. 2014;91(6):1029-1034.
57. Saurabh S, Sahoo PK. Lysozyme: an important defence molecule of fish innate immune system. Aquaculture Research. 2008;39(3):223-239.
58. Secombes CJ. Isolation of salmonid macrophages and analysis of their killing activity. Techniques in Fish Immunology. 1990;1:137-163.
59. Sherif AH, El-Gamal AM, Tolan AE. Incorporation of *Moringa oleifera* leaf in Nile tilapia (*Oreochromis niloticus*) diet and its effect on growth performance and immune status. Journal of Veterinary Science. 2014;1:806-814.
60. Shourbela RM, El-Hawarry WN, AM AEL, Abo-Kora SY. Potentiality of *Moringa oleifera* aqueous extract as a growth modulator and antistress in acute hypoxic Nile tilapia *Oreochromis niloticus*. Iranian Journal of Fisheries Science. 2020;19(1):67-84.
61. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. Journal of Agricultural and Food Chemistry. 2003;51(8):2144-2155.
62. Stadtlander T, Sander C, Kumar V, Makkar H, Becker K. Effects of *Moringa oleifera* Lam. dietary seed protein extracts on growth, nutrient utilization and blood parameters in common carp (*Cyprinus carpio* L.) and Nile tilapia (*Oreochromis niloticus* L.). Planta Medica. 2013;79(13):21.
63. Stoskoph M. Fish medicine. Philadelphia: W. B. Saunders Company; 1993. p. 67-125.
64. Talpur AD. *Mentha piperita* (Peppermint) as feed additive enhanced growth performance, survival, immune response and disease resistance of Asian seabass, *Lates calcarifer* (Bloch) against *Vibrio harveyi* infection. Aquaculture. 2014;420:71-78.
65. Talpur AD, Ikhwanuddin M. *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. Fish & Shellfish Immunology. 2013;34(1):254-264.
66. Tekle EW, Sahu NP. Immunomodulatory and growth responses of Nile tilapia fingerlings to dietary extract of *Moringa* flower. International Journal of Scientific Research. 2015;5:285-296.
67. Wang E, Chen X, Liu T, Wang K. Effect of dietary *Ficus carica* polysaccharides on the growth performance, innate immune response and survival of crucian carp against *Aeromonas hydrophila* infection. Fish & Shellfish Immunology. 2022;120:434-440.
68. Wang WS, Wang DH. Enhancement of the resistance of tilapia and grass carp to experimental *Aeromonas hydrophila* and *Edwardsiella tarda* infections by several polysaccharides. Comparative Immunology, Microbiology and Infectious Diseases. 1997;20(3):261-270.
69. White J, Hekmat S. Development of probiotic fruit juices using *Lactobacillus rhamnosus* GR-1 fortified with short chain and long chain inulin fiber. Fermentation. 2018;4(2):27.
70. Wiegertjes GF, Stet RM, Parmentier HK, Van WB. Immunogenetics of disease resistance in fish: a comparative approach. Developmental & Comparative Immunology. 1996;20(6):365-381.
71. Yin G, Jeney G, Racz T, Xu P, Jun X, Jeney Z. Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non-specific immune response of tilapia, *Oreochromis niloticus*. Aquaculture. 2006;253(1-4):39-47.
72. Yogeshwari G, Jagruthi C, Anbazahan SM, Mari LSS, Selvanathan J, Arockiaraj J, *et al.* Herbal supplementation diet on immune response in *Labeo rohita* against *Aphanomyces invadans*. Aquaculture. 2015;437:351-359.
73. Zeilab Sendijani R, Abedian Kenari A, Smiley AH, Esmaeili M. The effect of extract from dill (*Anethum graveolens*) on the growth performance, body composition, immune system, and antioxidant system of rainbow trout. North American Journal of Aquaculture. 2020;82(2):119-131.