



Comparison and Evaluation of the Performance of the Mineral Mix Supplemented Diet for Asian Seabass (*Lates calcarifer*) Culture in Fresh Water-based Recirculating Aquaculture System

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ABSTRACT

Background: The major minerals required by fish are calcium, phosphorus, manganese, sodium, chloride, potassium, chlorine, sulfur, selenium and magnesium. Even though we know that fish requires minerals, the quantity required is not studied in combination. In this study, an experiment was carried out to ascertain the concentration of 8 minerals mentioned above in the feed in a fixed concentration of calcium and phosphorus at a 1% level of supplementation. This experiment provides valuable information based on growth data, biochemical parameters and hematological parameters.

Methods: The experiment was carried out for 60 days in a 500-liter PE tank fitted with a Recirculating Aquaculture System and fed with a mineral mix of eight macro and micro minerals from 0.1% to 1% in triplicates. The mineral analysis was carried out using AAS, biochemistry analyzer was used for biochemical and the hematology analysis was used for blood parameter analysis.

Result: The results show that mineral mix with 8 macro and micro minerals such as manganese, sodium, chloride, potassium, chlorine, sulfur, selenium and magnesium is required at 0.8%. The growth parameters, biochemical analysis and blood parameters confirm the results.

Key words: Asian seabass, Mineral mix, Mineral nutrition, Recirculating aquaculture system.

INTRODUCTION

Common dietary macro and micro minerals are calcium, phosphorus, manganese, sodium, chloride, potassium, chlorine, sulfur, selenium and magnesium. These minerals regulate osmotic balance and aid in bone formation and integrity. Common microminerals are iron, copper, chromium, iodine, manganese, zinc and selenium. These trace minerals are required in small amounts as components in enzyme and hormone systems. The experiment was carried out in a Recirculating Aquaculture System with a flow rate of 20,000 LPH.

MATERIALS AND METHODS

The experimental duration was 60 days. The experiment was carried out in 36 Nos. of rectangular polyethylene (PE) tanks with 30 nos of seabass fry each. All the tanks were connected to a recirculating aquaculture system with a drum filter (20,000 LPH), sand filter (20,000 LPH), carbon filter (20,000 LPH), biofilter (5 tonnes with biofilter media of 400 m²/m³), cartridge filter (20,000 LPH), bag filter (20,000 LPH) and UV filter (20,000 LPH). Approximately 2000 nos. of Asian Seabass seeds of 2 g size were procured from Canares Aquaculture, Kumta, Karnataka. The experiment included 2 controls and 10 treatments. The 10 treatments consisted of are formulated experimental diets prepared with 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1.0% of major mineral premix supplemented diets in triplicates. The experiment was conducted in a recirculating aquaculture system with flow rate of 20,000 LPH.

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Experimental diets

The experimental diets were prepared with 45% protein and 7% lipid along with 1% supplemented calcium and phosphorus minerals based on previous studies. The experimental diet was prepared with a mineral mix containing major minerals namely magnesium, sodium,

potassium, iron, copper, zinc, manganese and selenium (Table 1) in addition to calcium and phosphorus in the same concentrations in all treatments, control 1, control 2 diets were prepared without any supplemented minerals. The percentage composition of wheat flour was adjusted to increase the mineral premix inclusion without affecting the proximate composition (Table 2). Samplings were done once every 15 days and the feeding rates were revised according to the body weight. The fish were fed @ 5% of their body weight during the experimental period.

Water quality parameters

Temperature, dissolved oxygen (DO) and pH were measured in the experimental site itself using a YSI meter. Water pH was measured using a portable pH meter (Hanna Instruments, Mumbai) and salinity was measured using a hand-held refractometer (RHS 10 ATC). The total alkalinity and total hardness of the water were estimated titrimetrically using phenolphthalein, methyl orange and Eriochrome Black-T indicators following the standard methods (APHA, 2005). Total Ammonia-N, Nitrite-Nitrogen and Nitrate-Nitrogen of the water samples were estimated using standard protocols (APHA, 2005). During the experimental period, the temperature ($26.6 \pm 2^\circ\text{C}$), dissolved oxygen (4.8 ± 1.5 mg/L), pH (7.8 ± 0.75), total alkalinity (56 ± 15 mg CaCO_3), hardness (246 ± 28 mg CaCO_3), ammonia (0.05 ± 0.01 mg/L), nitrite (0.01 ± 0.01 mg/L) and nitrate (0.1 ± 0.06 mg/L) were maintained within the optimum level.

Growth parameters

The growth parameters, such as percentage weight gain, specific growth rate, feed conversion ratio, feed efficiency ratio, protein efficiency ratio, mean growth rate, thermal growth coefficient and condition factor, were estimated according to the standard formulas.

Mineral analysis

Calcium

The standards prepared were measured for absorbance. An unknown sample was loaded and run in AAS to determine known concentration of calcium.

Phosphorus

Phosphorus was estimated using the spectroscopic method described by Baird *et al.* (2017).

Other minerals

Sample for the analysis of magnesium, iron, copper, selenium, manganese and chromium measured by Instrument Atomic Absorption Spectroscopy (Systronics, India). Sodium and potassium of the samples were estimated using a Flame Photometer (Systronics, India).

Proximate composition

The protein was estimated using the Kjeldhal method Latimer (2016).

The fat composition was estimated using an Automated Soxhlet Apparatus (Pelican equipment).

The total moisture content of the whole fish body was calculated according to Latimer (2016).

The determination of ash content was carried out in a muffle furnace.

The crude fiber was estimated using Fibra Plus (Pelican Equipment).

Nitrogen free extraction (NFE)

The calculation for nitrogen free extract is:

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

Biochemical parameter

All the biochemical parameters mentioned below were estimated using Alpha chem biochemistry analyzer model number 100. In the software of the Alpha chem100 selection of appropriate tests was done and the desired biochemical parameters were displayed. For all the parameters except ALP, the standard reagent was fed to the machine which aspirates the samples and measures the OD value and the factor value was calculated automatically. This was followed by washing with distilled water. Then the sample was fed to the machine which aspirated it measured the OD value and provided the direct results.

- Glucose
- Cholesterol
- Triglycerides
- Lipase
- Urea
- Uric acid
- Albumin
- High density lipid
- Calcium
- Antistreptolysin O
- Alkaline phosphatase
- Micro albumin
- Total protein
- C- reactive protein

Hematological parameters

The sample is collected in a 2 ml K2 EDTA tube. It was mixed to avoid microclots. The sample was analysed in a complete blood cell analyzer. Before analyzing, the sample

Table 1: Mineral composition of major minerals for premix preparation.

Inorganic form	Percentage in premix
Magnesium chloride (MgCl_2)	7.51
Sodium chloride (NaCl)	9.86
Potassium hydroxide (KOH)	12.90
Ferrous sulphate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	5.27
Copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	0.98
Zinc sulfate heptahydrate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	3.35
Manganese sulfate monohydrate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$)	0.45
Sodium selenite (Na_2SeO_3)	0.04

was mixed and aspirated within 1 minute, the result will be displayed on the analyzer.

The following Hematological parameters were analyzed using cell Tech Model No.380

- White blood cell.
- Lymphocytes.
- Mid-range absolute count.
- Granulocyte.
- Haemoglobin.
- Haematocrit.
- Means corpuscular volume.
- Means corpuscular haemoglobin.
- Red cells distribution width.
- Platelet count.
- Mean platelet count.
- Platelet distribution width.

Digestive enzymes

The amylase activity was estimated using the Rick and Stegbauer (1974) method.

Protease

The protease activity was determined using the Moore and Stein (1948) method.

Lipase

The lipase activity was assayed by following the Cherry and Crandal (1932) method.

Total protein

The total protein content in the liver was determined using the Bradford (1976) method.

SOD (Superoxide dismutase)

Superoxide's dismutase activity was determined by means of inhibition of Pyrogallol auto-oxidation by the enzymes.

NBT (Nitro blue tetrazolium)

It was carried out using nitro blue tetrazolium (NBT) assay by following the modified Anderson and Siwicki (1995) method.

Serum protein and glutathione activity

The serum protein test and glutathione activity was determined by spectrophotometer.

Peroxides

The samples were subjected to enzyme assay as described by Civello *et al.* (1995) for the determination of peroxidase activity.

Glutathione peroxidase

GPx reaction was performed as per the protocol of Wendel, (1981).

Alkaline phosphatase

The Alkaline Phosphates activity (ALP) was determined using the Garen and Levinthal (1960) method.

Statistical analysis

The IBM SPSS software was used to calculate the one-way analysis of variance at a 95% level of significance for all the treatments. The mean and standard deviation were also calculated using the same software.

The experiments were carried out in the wet laboratory of Dr. M.G.R. Fisheries College and Research Institute, Ponneri during the year 2021-2022 in freshwater and water exchange was performed at 10% once in two days to maintain optimum TDS. All water quality parameters were monitored regularly and maintained in optimum condition. The experiment was conducted in accordance with the rules and regulations of the Animal Welfare Board of India.

RESULTS AND DISCUSSION

Even though there are studies on Vitamin C supplementation in European seabass KOP Aysun (2019) studies on mineral incorporation are limited in fish even though they are well-studied in broiler chicken (Boussouar, 2024) and hence this study was taken up. The maximum mean growth rate, percentage weight gain and specific growth rate of asian seabass with an extreme of as 50 ± 2.00 g, $1566.22 \pm 24\%$ and 78.10 ± 4.004 g, were obtained in T8

Table 2: Proximate composition of prepared experimental diets.

	Protein (%)	Fat (%)	Moisture (%)	Ash (%)	Fibre (%)	NFE (%)
Control 1	45.27	7.33	6.99	6.24	1.25	32.92
Control 2	45.25	7.33	7	6.24	1.26	32.92
T ₁ (0.1)	45.28	7.33	6.99	6.35	1.28	32.77
T ₂ (0.2)	45.26	7.25	7	6.25	1.28	33.96
T ₃ (0.3)	45.25	7.33	7.01	6.26	1.28	32.87
T ₄ (0.4)	45.24	7.33	7.01	6.27	1.28	32.87
T ₅ (0.5)	45.23	7.27	7.01	7.33	1.29	32.87
T ₆ (0.6)	45.23	7.33	7.02	6.28	1.31	32.83
T ₇ (0.7)	45.23	7.34	7.03	6.28	1.31	32.81
T ₈ (0.8)	45.24	7.34	7.03	6.29	1.31	32.79
T ₉ (0.9)	45.24	7.34	7.03	6.29	1.31	32.79
T ₁₀ (1.0)	45.24	7.35	7.04	6.30	1.32	32.75

and the least mean growth value of 36.96 ± 2.05 g, $1133.11 \pm 15\%$, 56.20 ± 4.015 g were obtained in C1 respectively. The feed conversion ratio of asian seabass with a maximum of 3.1 ± 0.25 was obtained in T10 and the minimum mean growth value of 2.8 ± 0.5 was obtained in C2. The Feed efficiency ratio, protein efficiency ratio and mean growth rate of asian seabass with the highest of 0.166 ± 0.01 , 1.11 ± 0.04 and 29 ± 3 g were obtained in T8 and the value of 0.150 ± 0.05 was obtained in T10, 0.80 ± 0.04 was obtained in C1 and 28 ± 1 g was obtained in C1, C2 and T10 respectively. The thermal unit growth coefficient and condition factor of asian seabass with a maximum of 0.87 ± 0.01 , 949.64 ± 13 were obtained in T8 and T1 and the minimum of 0.62 ± 0.05 , 862.96 ± 20 were obtained in C1 and T7 respectively.

Blood parameter

The White blood cell, lymphocyte and mid-range absolute count values with the extreme of $40 \pm 5 \times 10^9/l$, $46.5 \pm 3 \times 10^9/l$ and $4.5 \pm 0.5 \times 10^9/l$ were observed in T8 and moderate of $30.22 \pm 2 \times 10^9/l$, $20.25 \pm 2 \times 10^9/l$ and $2.05 \pm 2 \times 10^9/l$ in T1. The granulocyte, red blood cell and haemoglobin values with the highest of $5.3 \pm 0.17 \times 10^9/l$, $2.8 \pm 0.2 \times 10^9/l$ and 4 ± 0.5 g/dl were observed in T8 and the lowest of $2.2 \pm 0.3 \times 10^9/l$, $0.87 \pm 0.13 \times 10^9/l$ and 3.1 ± 0.1 g/dl were observed in T1. The hematocrit, Means corpuscular volume and Mean corpuscular haemoglobin with a maximum of $23 \pm 0.5\%$, 100 ± 5 fl and 44 ± 4 pg were observed in T8 and a minimum of $10.05 \pm 1.0\%$, 60 ± 5 fl and 32 ± 2 pg were observed in T1. The red cells distribution width, with greater $52 \pm 3\%$, $69 \pm 1 \times 10^9/l$ and $5 \pm 2\%$ were observed in T10, T4 and T10 respectively and the lowest of $15.2 \pm 0.2\%$, $12 \pm 3 \times 10^9/l$ and $0.01 \pm 0.01\%$ were observed in T7, C1 and C1, C2 and T7 respectively. The mean platelet count, platelet distribution width and platelet large cell count with a maximum of 12.5 ± 0.5 fl, $40 \pm 2.3\%$ and 52 ± 3 mg/dl were observed in T1, T4, T10 and a minimum of 8.3 ± 1 fl, $9.7 \pm 1.1\%$, 1 ± 0.6 mg/dl and 14.38 ± 3 mg/dl were observed in T9, T1, T7 and T10 respectively. Similar research work on hematological parameters was also performed in RAS culture under different stocking densities by Ezhilmathi *et al.* (2022).

Biochemical parameters

The glucose, cholesterol level, triglycerides level and Lipase were at the extreme of 75.56 ± 2.1 mg/dl, 238.31 ± 3.1 mg/dl, 133.65 ± 3 mg/dl and 186.78 ± 3 mg/dl were observed in T8, T1, T1 and T1 respectively. A moderate of 45.85 ± 0.9 mg/dl, 134.85 ± 2.6 mg/dl, 125.23 ± 2.8 mg/dl and 125.23 ± 2.8 mg/dl were observed in T1, T8, T8 and T8 respectively. The urea, uric acids, albumin and high-density lipoprotein with a maximum of 240.76 ± 3.1 mg/dl, 12.02 ± 2.1 mg/dl, 8.76 ± 3.1 mg/dl and 133.05 ± 1 mg/dl were observed in T8 and a minimum of 120.87 ± 1.1 mg/dl, 4.22 ± 0.4 mg/dl, 4.33 ± 2.9 mg/dl and 60.34 ± 3 mg/dl were observed in T1. The calcium, A-Streptolysin-O and alkaline phosphatase with the top most 6.40 ± 2 mg/dl, 120.44 ± 3.1 mg/dl and 87.76 ± 3.06 mg/dl were observed in T8 and the

bottom least of 2.38 ± 2 mg/dl, 40.25 ± 3.01 mg/dl and 51.72 ± 1.52 mg/dl were observed in T1, T1 and C1 respectively. The microalbumin, total protein and C-reactive protein with greater of 9.20 ± 0.5 mg/dl, 4.20 ± 1 mg/dl and 6.33 ± 2 mg/dl were observed in T8 and a lower of 3.45 ± 0.4 mg/dl, 1.80 ± 0.7 mg/dl and 2.00 ± 2 mg/dl were observed in T1 respectively.

The amylase, protease and lipase in the liver with greater than 90.06 ± 2.1 Umg⁻¹ protein⁻¹ min⁻¹, 84.07 ± 0.8 Umg⁻¹ protein⁻¹ min⁻¹ and 84.07 ± 0.8 Umg⁻¹ protein⁻¹ min⁻¹, were observed in T8 and a lower of 48.06 ± 0.4 Umg⁻¹ protein min⁻¹, 23.09 ± 0.9 Umg⁻¹ protein min⁻¹ and 49.03 ± 0.4 Umg⁻¹ protein min⁻¹ were observed in C1, T1 and C1 respectively. The amylase, protease and lipase in the Intestine with a maximum of 92.06 ± 2.1 Umg⁻¹ protein min⁻¹, 88.07 ± 0.8 Umg⁻¹ protein min⁻¹ and 92.58 ± 2.1 Umg⁻¹ protein min⁻¹ were observed in T8 and a minimum of 52.06 ± 0.4 Umg⁻¹ protein min⁻¹, 42.06 ± 0.9 Umg⁻¹ protein min⁻¹ and 52.03 ± 0.4 Umg⁻¹ protein min⁻¹ were observed in C1.

The amylase, protease and lipase in kidney were 86.06 ± 2.1 Umg⁻¹ protein min⁻¹, 88.07 ± 0.8 Umg⁻¹ protein min⁻¹ and 91.58 ± 2.1 Umg⁻¹ protein min⁻¹ in T8, while the level 58.06 ± 0.4 Umg⁻¹ protein min⁻¹, 91.58 ± 2.1 Umg⁻¹ protein min⁻¹ and 56.03 ± 0.4 Umg⁻¹ protein min⁻¹ in C1. In the stomach Amylase, protease and lipase with the highest 86.78 ± 2.1 Umg⁻¹ protein min⁻¹, 80.27 ± 0.9 Umg⁻¹ protein min⁻¹ and 86.63 ± 2.1 Umg⁻¹ protein min⁻¹ were observed in T8 and the lowest of 62.08 ± 0.4 Umg⁻¹ protein min⁻¹, 52.16 ± 0.9 Umg⁻¹ protein min⁻¹ and 58.13 ± 0.4 Umg⁻¹ protein min⁻¹ were observed in C1.

The Superoxide Dismutase, Nitro blue tetrazolium and serum protein levels were at a maximum of 94.68 ± 1.5 mg/dl, 69.46 ± 1.9 mg/dl and 92.08 ± 4.3 mg/dl in T8 while the minimum level 49.98 ± 1.55 mg/dl, 32.46 ± 1.5 mg/dl and 36.08 ± 1.7 mg/dl were observed in C1. The glutathione and peroxidase values were extreme levels at 66.47 ± 5.1 mg/dl and 76.22 ± 2.9 mg/dl in T8, while moderate levels of 25.18 ± 1.87 mg/dl and 46.00 ± 2.5 mg/dl were observed in C1.

Mineral composition

The overall supplementation of calcium and phosphorus was studied by Moses *et al.* (2024) which is the first study in the Asian Seabass on mineral supplementation of both calcium and phosphorus at a 1% level of supplementation. In the present study, 0.2% supplemented magnesium gives better results. Similar research was carried out by Shiau and Hsieh (2001) in tilapia and found that 0.002% gives optimum growth. Further studies by Liang *et al.* (2012) in grass carp also found that 0.08% is required for optimum growth. However, Wang *et al.* (2011) reported that 0.07% of magnesium is required for grass carp. 0.2% of supplemented sodium is giving better results similar research work was carried out by Fernandes *et al.* (2001) in tilapia and found that 10% is required. Further studies by Duncan *et al.* (2011) in red drum also proved that 0.92% is required. However, Shiau *et al.* (2007), reported that 0.2% of sodium is required for tilapia in fresh water. Mzengereza

and Kang (2015) also report that 1.7% of Sodium is required for tilapia. In the present study, 0.28% supplemented potassium gave the best result. In the present study, 0.21% of supplemented Iron has given better results. Similar research work was performed by Rigos *et al.* (2010) in gilthead seabream and found that 0.02% is required for optimum growth. Further studies by Shiau and Su (2003) on tilapia also proved that 0.016% of dietary supplementation is needed for optimum growth. 0.01% of supplemented manganese has given better results. Similar research work was carried out by Ishac and Dollar (1968) for dietary supplementation in tilapia and found that 0.0007% is required for optimum growth. 0.12% of supplemented zinc has given good results. Studies by Liang *et al.* (2012) in Grass carp also proved that 0.0055% is the requirement. 0.0007% of supplemented Selenium has given good results. Similar research work was carried out by Zhu *et al.* (2011) in large mouth bass and found that 0.0001% has given optimum results. Further studies by Wang *et al.* (1997) in Catfish also proved that 0.0005% is the requirement. 0.03% of supplemented copper is the requirement found by this study. Similar research work was carried out by Lin *et al.* (2008) in *Epinephelus malabaricus* and found that 20.05 mg Cu/Kg is giving optimum results. Further studies by Ling *et al.* (2010) in Common carp also proved that 4.37 mg Cu/kg is required. The biochemical parameters were also found to be in the optimum range in treatment 8 containing a 0.8% level of mineral mix in the diet. The haematological parameters also proved the results.

CONCLUSION

The inclusion of calcium and phosphorus at the rate of 1% each in the diet of Asian seabass along with the mineral mix of 0.8% containing minerals such as manganese, sodium, chloride, potassium, chlorine, sulfur, selenium and magnesium for culture in a freshwater-based RAS system significantly increased the growth rate and survival rate compared to other percentages of inclusions. Therefore, it is concluded that mineral nutrition is one the vital components for fish nutrition, especially in a recirculating aquaculture system.

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Conflict of interest

We declare that there is no conflict of interest.

REFERENCES

- Anderson, P. and Siwicki, A.K. (1995). Basic hematology and serology for fish health programs. In: Proceeding second symposium on diseases in asia aquaculture aquatic animal health and the environment. Phuket, Thailand, pp.185-202.
- APHA A, W.P.C.F., (2005). Standard methods for water and wastewater examination, twenty-first. American Public Health Association, American Water Works Association, Water Environment Federation Washington, DC, USA. pp. 1288.
- Baird, R.B., Andrew, D.E., and Eugene, W.R. (2017). Standard methods for examination of water and wastewater. American Public Health Association, Washington, DC, USA. pp. 1545.
- Boussouar H., Khenenou T., Bennoune O., Lamraoui R., Fares M.A., Rahmoun D.E., Kadrine N., Benrezak S., Kahoul M.A. (2024). Effects of a diet low in vitamin and mineral complex and darkness on the growth performance, mineralization and femur histological structure of broiler chickens. *Agricultural Science Digest*. 44(2): 355-361. doi: 10.18805/ag.DF-577.
- Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 72: 248-254.
- Cherry, L.S., Crandal, L.A. (1932). The specificity of pancreatic lipase; its appearance in the blood after pancreatic injury. *American Journal of Physiology Legacy Content*. 100(2): 266-273.
- Civello, P., Martinez, G., Chaves, A. and Anon, M. (1995). Peroxidase from strawberry fruit (*Fragaria ananassa* Duch). Partial purification and determination of some properties. *Journal of Agricultural and Food Chemistry*. 43: 2596-2601.
- Duncan, S., Gatlin D.M., MacKenzie, S.R., Craig, William H., Neill., (2011). Effects of dietary sodium chloride on red drum juveniles in waters of various salinities. *Progressive Fish Culturist*. 54: 220-227.
- Ezhilmathi, S., Ahilan, B., Arumugam, U. and Felix, N., Antony, C., Lingam, S. and Nallaiah, H. (2022). Physiological response of asian seabass reared in recirculating aquaculture system under different stocking densities. *Indian Journal of Animal Research*. doi: 10.18805/IJAR.B-4955.
- Fernandes, F.A, Russell-Pinto, F., Gomes, E., Reis-Henriques, M.A., Coimbra, J. (2001). The effect of dietary sodium chloride on some osmoregulatory parameters of the teleost, *Oreochromis niloticus*. *Fish Physiology and Biochemistry*. 23: 307-316.
- Garen, A. and Levinthal, C. (1960). A fine structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli* purification and characterization of alkaline phosphatase. *Biochimica et Biophysica Acta*. 38: 470-483.
- Ishac, M.M. and Dollar, A.M. (1968). Studies on Mn uptake in Tilapia mossambica and *Salmo gairdneri*. I-Growth and survival of Tilapia mosambica in response to Mn. *Acta Hydrobiologica Sinica*. 31: 572-584.
- KOP Aysun (2019). The effects of dietary vitamin c levels on tissue ascorbic acid concentration and growth in european sea bass (*Dicentrarchus labrax*, L. 1758). *Indian Journal of Animal Research*. 53(12): 1629-1634. doi: 10.18805/ijar.B-1134.
- Latimer, G.W. (2016). Official Methods of Analysis of AOAC International 20th edition. AOAC International; Gaithersburg, MD, USA.

- Liang, J.J., Tian, L.X., Liu, Y.J., Yang, H.J., Liang, G.Y. (2012). Dietary magnesium requirement and effects on growth and tissue magnesium content of juvenile grass carp (*Ctenopharyngodon idella*). *Aqua Nutrition*. 18: 56-64.
- Ling, J., Feng, L., Liu, Y., Jiang, J., Jiang, W.D., Hu, K., Li, S.H., Zhou, X.Q. (2010). Effect of dietary iron levels on growth, body composition and intestinal enzyme activities of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Nutrition*. 16: 616-624.
- Lin, Y.H., Lin, S.M., Shiau, S.Y. (2008). Dietary manganese requirements of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquaculture*. 284: 207-210.
- Moore, S. and Stein, W.H. (1948). Photometric ninhydrin method for use in the chromatography of amino acids. *Journal of Biological Chemistry*. 176: 367-388.
- Moses, T.L.S.S., Ahilan, B., Chidambaram, P., Felix, N. and Jayakumar, N. (2024). Optimizing the supplementation of calcium and phosphorus in the diets of asian seabass (*Lates calcarifer*) in freshwater based recirculating aquaculture system. *Indian Journal of Animal Research*. doi:10.18805/IJAR.B-5409.
- Mzengereza, K. and Kang, J.O. (2015). Effect of dietary salt (Sodium Chloride) supplementation on growth, survival and feed utilization of *Oreochromis shiranus*. *Aquaculture Research Development*. 6: 388.
- Rick, W. and Stegbauer, A.P. (1947). Measurement of Reducing Groups. In *Methods of Enzymatic Analysis*. 2: 885.
- Rigos, G., Fountoulaki, E., Morgane, H., Antigoni, V., Mente, E., Sweetman, J., Nengas, I. (2010). Evaluation of zinc supplementation in European sea bass (*Dicentrarchus labrax*) juvenile diets. *Aquaculture Research*. 41: e208-e216.
- Shiau, S.Y. and Hsieh, J.F. (2001). Quantifying the dietary potassium requirement of juvenile hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). *British Journal of Nutrition*. 85: 213-218.
- Shiau, S.Y., Su, L.W. (2003). Ferric citrate is half as effective as ferrous sulfate in meeting the iron requirement of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. *Journal of Nutrition*. 133: 483-488.
- Shiau, S.Y. and Tseng, H.C. (2007). Dietary calcium requirements of juvenile tilapia, *Oreochromis niloticus* × *O. aureus*, reared in fresh water. *Aquaculture Nutrition*. 13: 298-303.
- Wang, C., Lovell, R.T., Klesius, P.H. (1997). Response to edwardsiella ictaluri challenge by channel catfish fed organic and inorganic sources of selenium. *Journal of Aquatic Animal Health*. 9: 172-179.
- Wang, F.B., Luo, L., Lin, S.M., Li, Y., Chen, S. Wang, Y.G., Wen, H., Hu, C.J. (2011). Dietary magnesium requirements of juvenile grass carp, *Ctenopharyngodon idella*. *Aquaculture Nutrition*. 17: 691-700.
- Wendel, A. (1981) Glutathione peroxidase. *Methods Enzymology*. 77: 325-33.
- Zhu, Y., Chen, Y., Liu, Y., Yang, H., Liang, G., Tian, L. (2011). Effect of dietary selenium level on growth performance, body composition and hepatic glutathione peroxidase activities of largemouth bass *Micropterus salmoides*. *Aquaculture Research*. 1365-2109.