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Optimizing the Supplementation of Calcium and Phosphorus in the Diets of Asian Seabass (*Lates calcarifer*) in Freshwater based Recirculating Aquaculture System

T.L.S. Samuel Moses[1], B. Ahilan[2], P. Chidambaram[3], N. Felix[3], N. Jayakumar[4]

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ABSTRACT

Background: Calcium and phosphorus are important mineral nutrients for all energy-producing cellular and metabolic pathways. Fish obtain Ca and P from their feed because of the low level of calcium and phosphorus in the freshwater especially in the RAS system. This study was investigated to evaluate the growth characteristics, survival rate, calcium and phosphorus composition and haematological and biochemical parameters of Asian Seabass (*Lates calcarifer*) under the influence of varied combinations of calcium and phosphorus in the formulated sinking feed.

Methods: The experiment was carried out with Asian Seabass fingerlings, each with an initial average weight of 2.00±0.15 g which were randomly distributed in 54 Nos. of PE tanks with a volume of 500 Litres @ 30 Nos./tank. The experiment duration was 60 days and was carried out in a 6×3 factorial design. The fishes were fed with a sinking pellet diet containing calcium Ca 0.0, Ca 0.5% and Ca 1.0% and phosphorus P 0.0, P 0.5%, P 1.0%, P 1.5%, P 2.0% and P 2.5% and all the possible combinations of above percentages of calcium and phosphorus in the experimental diets.

Result: The growth characteristics and survival rates were significantly higher in 1% of calcium and phosphorus-contained feed, the calcium and phosphorus composition and haematological and biochemical parameters also confirmed the experimental results. Our study outcome was useful for formulating a calcium and phosphorus-incorporated sinking feed to Asian Seabass for better growth in the recirculating aquaculture system.

Key words: Asian seabass, Calcium, Minerals, Phosphorus, Recirculating aquaculture system, Sinking pellet.

INTRODUCTION

Aquaculture is the fastest-growing food production sector worldwide. India ranks second in the world in fish production by aquaculture with a production of about 8.6 million tons per annum (FAO, 2022). There is a huge requirement for the diversification of fish species and also in high-investment systems such as RAS. *Lates calcarifer*, commonly known as the Asian sea bass or giant perch, is barramundi in Australia. Asian Seabass is one of the excellent candidate species for Aquaculture. It is an economically important food fish in Indo-Pacific countries and It is a high-value fish cultured in our country. It belongs to the Family Centropomidae. Being a high-value species, it can be culture in advanced culture systems such as Recirculating Aquaculture Systems. Further, Asian Seabass can be acclimatized to freshwater leading to its expansion in farming. Recirculating Aquaculture Systems are increasingly being used nowadays due to the rising land cost and shrinking water resources. Since RAS is a fully clear water system, the importance of mineral nutrients is more prominent.

Among various minerals, Calcium (Ca) and Phosphorus (P) are required in higher quantities and are paramount to be included in the fish feed. Even though, we know that fish require Calcium and Phosphorus, studies on the percentage inclusion of these minerals are not there even though they are well-studied in broiler chicken (Boussouar, 2024). Moreover, freshwater has lower mineral concentration when compared to marine/brackish water, Asian Seabass being an euryhaline fish requires mineral supplements when reared in freshwater and in RAS around 5-10% of water is only exchanged and about 90% of water

[1]Department of Aquaculture, Dr. M.G.R. Fisheries College and Research Institute, Ponneri-601 204, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India.
[2]Fisheries College and Research Institute, Thoothukudi-628 008, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India.
[3]Tamil Nadu Dr. J.Jayalalithaa Fisheries University, Nagapattinam-611 002, Tamil Nadu, India.
[4]Department of Fisheries Biology, Fisheries College and Research Institute, Thoothukudi-628 008, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India.

Corresponding Author: T.L.S. Samuel Moses, Department of Aquaculture, Dr. M.G.R. Fisheries College and Research Institute, Ponneri-601 204, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Tamil Nadu, India. Email: samuelmoses@tnfu.ac.in

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will be recirculated continuously within the system Hence, this study is taken up to optimize the requirement of Calcium and Phosphorus in the diet of Asian Seabass in a freshwater-based RAS System.

MATERIALS AND METHODS

The experiments were carried out in 54 Nos. of Polyethylene (PE) tanks of 500 liters capacity in triplicates. There were a total of 18 treatments including all the possible combinations of calcium (Ca) in three percentages *viz*., 0%, 0.5% and 1.0% and phosphorus (P) in five percentages *viz*., 0%, 0.5%, 1.0%, 1.5%, 2.0% and 2.5%. All the tanks were connected with a Recirculating Aquaculture System with a Drum filter (20,000 LPH), Sand filter (20,000 LPH), Carbon filter (20,000 LPH), Bio-filter (5 tonnes with bio-filter media of 400 m^2/m^3), Cartridge filter (20,000 LPH), Bag filter (20,000 LPH) and UV filter (20,000 LPH). The experimental slow-sinking feed (2 mm) was formulated using fish meal, fish oil, wheat flour, cassava flour, binder and mineral mix. The experimental diets were prepared with 45% protein and 7% lipid along with Calcium and phosphorus-supplemented minerals. The concentration of wheat flour was adjusted against the inclusion percentage of mineral mix containing Calcium and Phosphorus without affecting the proximate composition of the diet. The calcium and phosphorous sources were calcium carbonate, calcium phosphate and disodium hydrogen phosphate. The experiment was carried out for 60 days. The fry having weight 2±0.15 g were stocked @ 30 Nos./tank. Sampling of weight data was carried out once in 15 days and the feeding rates were revised accordingly.

Water quality parameters

Water samples were collected from the experimental unit during morning hours between 8.00 am - 8.30 am for the whole experimental period. 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). Total alkalinity and total hardness of the water were estimated titrimetric ally using phenolphthalein, methyl orange and Eriochrome Black-T indicators following the standard methods (APHA, 2005). Total Ammonia-N, Nitrite-Nitrogen Nitrate-Nitrogen of the water samples were estimated using standard protocols (APHA, 2005). During the experimental period the temperature (26.6±2°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 for Asian Seabass.

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 as per the standard formulas.

Mineral analysis

Calcium

The standards prepared were measured for absorbance unknown sample was loaded was run in AAS to know the concentration of the calcium.

Phosphorus

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

Proximate composition

The protein was estimated as per the Kjedhal method (Latimer, 2016). The Fat composition was estimated in the automated Soxhlet apparatus (Pelican equipment). The total moisture content of the whole body of fish was calculated as per Latimer (2016). Determination of Ash content was carried out in the muffle furnace. The crude fibre was estimated using Fibra Plus (Pelican Equipment):

Nitrogen free extraction (NFE)

The calculation for nitrogen free extract is:

% NFE = 100 % - (% EE + % CP + % Ash + % 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 aspires the samples and measures the OD value and factor value was calculated automatically. Followed by washing by distilled water. Then the sample was fed to the machine which aspirates it and measures the OD value and gives the direct results.

- Glucose
- Cholesterol
- Triglycerides
- Lipase
- Urea
- Uric acid
- Albumin
- HDL
- Calcium
- ASO
- ALP
- Micro Albumin
- Total protein
- CRP

Haematological parameters

The sample was collected in a 2 ml K2 EDTA tube. It was a mixing of tubes to avoid microclots. The sample was performed in a complete blood cell analyzer. Before

analysing, the sample was mixed and aspirated within 1 minute to display the result on the analyzer.

The following Hematology 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 (1947) method.

Protease

The Protease activity was determined using Moore and Stein's (1948) method.

Lipase

The lipase activity was assayed by following Cherry and Cherry's (1932) method.

Total protein

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

SOD (Superoxide dismutase)

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

RBT

It was carried out using nitro blue tetrazolium (NBT) assay by following the modified Anderson and Siwiki (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 two-way analysis of variance (Two way ANOVA) at 95% level of significance for all the treatments. The mean and standard deviation were also been 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 the water quality parameters were monitored regularly and maintained in optimum condition. The experiment was carried out as per 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. The maximum weight gain of 47.756±0.0305 g was obtained in T15 with 1% each of calcium and phosphorus, the percentage weight gain, the specific growth rate, feed conversion ratio, the feed efficiency ratio, the protein efficiency ratio, the mean growth rate, the thermal unit growth coefficient and the condition factor are shown in Table 1-9. It was observed that treatment 15 with 1% each of supplemented Calcium and phosphorus had better values during the culture period of 60 days in a freshwater-based Recirculating Aquaculture System. Similar results were obtained by Shim *et al*. (1989) for the dietary supplementation of Calcium in the diet of Guppies. Studies by Liang *et al*. (2012) in grass carp also proved that 1.04% of dietary supplementation is required for optimum growth. However, Sanchez *et al*. (2000) reported that 0.18%of Calcium is required for American cichlids. Further Shiau and Tseng (2007) reported that only 0.47% of calcium is required for optimum growth of tilapia. However, there were no studies on the calcium requirement in Asian Seabass. The difference in the result obtained in other species may be due to the species variation and Asian Seabass may comparatively require a higher percentage of calcium in its diet for optimum growth performance, especially in fresh water-based Recirculating Aquaculture System. In the present study, it was observed that the maximum weight gain was obtained in treatment 15 with 1% each of supplemented phosphorous during the culture period of 60 days in a fresh water-based Recirculating Aquaculture System. Similar research was carried out by Nwanna *et al*. (2009) for the dietary supplementation of African catfish further studies by Furuya, *et al*. (2008) in Nile Tilapia also proved that 0.71% of dietary supplementation is required for optimum growth. However, Oliva *et al*. (2004) reported that 0.65% of phosphorous is required for European Seabass in seawater. Further Shim and Ho (1989) reported that only 0.53% of phosphorous is required for optimum growth of Guppy. Rodehutscord

et al. (2000) reported that 0.67% of phosphorous is required for red drum. However, there were no studies on the phosphorus requirement in Asian Seabass. The difference in results obtained in other species may be due to the species variation and Asian Seabass may comparatively require a higher percentage of phosphorous in its diet for optimum growth performance, especially in fresh water-based Recirculating Aquaculture System. No bacterial infection as reported by Devadason Chandravathany (2023) was reported during the culture period.

Glucose level of Asian Seabass ranged between 46.2800±2.01814 mg/dl and 74.6500±1.97335 mg/dl with maximum in T18 and minimum in T3. The cholesterol level of Asian Seabass ranged between 179.3343±6.33043 mg/dl

Table 1: Growth rate (g) of Asian Seabass.

	Ca 0%	Ca 0.5%	Ca1.0%
P0	40.005^{o}±0.02082	42.2533^{l}±0.02082	44.25^{i}±0.02082
P0.5	41.35^{k}±0.02082	42.7567^{h}±0.02082	44.573^{g}±0.0208
P01.0	42.152^{e}±0.01528	42.8567^{b}±0.02309	47.756^{c}±0.0305
P01.5	42.5667^{n}±0.02082	42.55^{j}±0.04583	47.20^{m}±0.01
P02.0	42.7533^{i}±0.03512	43.74^{j}±0.01528	47.34^{f}±0.0264
P02.5	41.7556^{d}±0.02082	44.15^{a}±0.04583	47.1567^{a}±0.035

Table 2: Percentage weight gain (%) of Asian Seabass.

	Ca 0%	Ca 0.5	Ca 1.0%
P0	1491.45^{o}±2.00	1809.517^{e}±9.45	1814.59^{h} ±5.00
P0.5	1642.662^{l}±6.00	1946.53^{b}±4.00	1932.89^{i}±3.00
P01.0	1814.65^{h}±4.00	1945.96^{c}±5.00	2262.58^{f}±3.00
P01.5	1692.09^{k}±2.00	1924.055^{h}±4.00	2115.95^{d}±1.00
P02.0	1925.55^{g}±1.00	1892.05^{j}±2.00	2082.46^{a}±3.00
P02.5	1814.65^{h}±1.00	1894.99^{m}±3.00	2162.72^{a}±3.00

Table 3: Specific growth rate (G) of Asian Seabass.

	Ca 0%	Ca0.5%	Ca1.0%
P0	49.729^{h}±1.00	58.42^{fg}±1.00	66.7687^{ab}±1.05
P0.5	51.461^{hg}±1.00	56.33^{cde}±3.00	65.4677^{ab}±2.09
P01.0	53.55^{hgf}±2.00	61.376^{cd}±2.00	77.5613^{a}±3.08
P01.5	51.42^{gf}±5.00	63.122^{bc}±2.00	74.9993^{a}±3.19
P02.0	56.322^{ef}±1.00	65.75^{cd}±2.00	76.0413^{a}±.35
P02.5	57.312^{def}±1.00	62.4773^{ab}±6.18	75.2787^{ab}±1.77

Table 4: Feed conversion ratio of Asian Seabass.

	Ca 0%	Ca 0.5%	Ca 1.0%
P0	3.826^{de}±0.2	3.125^{gh}±0.1	3.1538^{gh}±0.2
P0.5	3.725^{e}±0.1	3.1363^{gh}±0.2	3.1865^{gh}±0.2
P01.0	3.652^{ef}±0.2	3.1255^{gh}±0.1	2.836^{cd}±0.2
P01.5	3.163^{gh}±0.2	3.1227^{gh}±0.35	3.074^{a}±0.2
P02.0	3.1459^{gh}±0.1	3.148^{gh}±0.2	3.028^{a}±0.1
P02.5	3.623^{ef}±0.2	3.117^{gh}±0.3	3.075^{a}±0.1

Table 5: Feed efficiency ratio of Asian Seabass.

	Ca 0%	Ca 0.5%	Ca 1.0%
P0	0.085^{a}±0.02	0.073^{b}±0.01	0.071^{b}±0.02
P0.5	0.085^{a}±0.01	0.073^{b}±0.02	0.071^{b}±0.02
P01.0	0.086^{a}±0.02	0.072^{b}±0.01	0.061^{c}±0.02
P01.5	0.074^{a}±0.02	0.073^{b}±0.03	0.065^{c}±0.02
P02.0	0.073^{b}±0.01	0.073^{b}±0.02	0.064^{c}±0.01
P02.5	0.086^{a}±0.02	0.070^{b}±0.03	0.064^{c}±0.01

and 194.9387±3.52433 mg/dl with higher in T15 and lower in T1. The triglycerides ranged between 328.0387±8.97386 mg/dl and 462.5943±3.21376 mg/dl with better of T15 and least in T6. The Lipase of Asian Seabass ranged between 196.8433 ±6.72811 mg/dl and 127.0767 ±3.52889 mg/dl with a maximum of T1 and a minimum of T18. The Uric Acid of Asian Seabass ranged between 53.9747± 2.61754 mg/dl and 81.1343± 4.00351 mg/dl with maximum of T1 and minimum in T15. The Albumin of Asian Seabass ranged between 8.2033± 1.0232 mg/dl and 13.3467± 1.06359 mg/dl with higher T15 and lower in T1. The HDL of Asian Seabass ranged between 45.5333±1.5879 mg/dl and 81.2533±2.35502 mg/dl with a maximum of T15 and a minimum of T1. The ASO of Asian Seabass ranged between 22.2467±1.8876 mg/dl and 26.031±3.52772 mg/dl with the greatest of T15 and the finest in T7. The ALP of Asian Seabass ranged between 334.6333±1.65814 mg/dl and 380.4643±14.096 mg/dl with the topmost of T18 and bottom least in T7. The microalbumin of Asian Seabass ranged between 16.4753± 1.05489 mg/dl and 25.5153± 3.9950 mg/dl with better T16 and least in T2. The total protein, mg/dl of Asian Seabass ranged between 7.232±0.96582 mg/dl and 15.0153± 1.02957 mg/dl with greatest of T15 and finest inT1. The C-Reactive protein of Asian Seabass ranged between 11.8577±1.56158 mg/dl and 15.8643±3.00374 mg/dl with extreme T15 and moderate in T1.

The White blood cells of Asian Seabass ranged between 27.351±1.00018 10^9/l and 21.4687±1.02564 10^9/l with better T15 and least in T6. The Lymphocytes of Asian Seabass ranged between 36.46±2.02672 10^9/l and 23.9387±1.48236 10^9/l with the greatest of T15 and finest

Table 6: Protein efficiency ratio of Asian Seabass.

	Ca 0%	Ca 0.5%	Ca 1.0%
P0	0.8497^{ef}±0.10058	0.9643^{ef}±0.02026	1.322^{abcd}±0.2
P0.5	0.92^{ef}±0.04	1.0497^{cdef}±0.30453	1.3673^{abc}±0.18
P01.0	0.9367^{def}±0.07422	1.0561^{cdef}±0.09636	1.651^{a}±0.25958
P01.5	0.999^{cdef}±0.001	0.9407^{cdef}±0.19979	1.6393^{a}±0.2213
P02.0	1.0942^{ef}±0.01684	1.112^{cdef}±.018	1.556^{ab}±0.22
P02.5	0.9337^{ef}±0.3	1.2588^{bcde}±0.18012	1.6221^{a}±0.22

Table 7: Mean growth rate (g) of Asian Seabass.

	Ca 0%(g)	Ca 0.5%(g)	Ca1.0%(g)
P0	31.74^{a}±1	31.6853^{a}±0.97635	31.5133^{a}±3.19569
P0.5	31.5177^{a}±1.72281	31.5041^{a}±3.04547	31.6007^{a}±2
P01.0	31.7807^{a}±1.11058	31.6667^{a}±3.99007	31.9932^{a}±3.9995
P01.5	31.655^{a}±1.00015	31.53^{a}±4.0	31.7214^{a}±1.17903
P02.0	31.6641^{a}±2.02109	31.61330^{a}.95574±	31.7557^{a}±1.60636
P02.5	31.628^{a}±3.04005	31.58^{a}±3	31.8026^{a}±0.74825

Table 8: Thermal unit growth coefficient of Asian Seabass.

	Ca 0%	Ca0.5%	Ca 1.0%
P0	0.705^{a}±0.10065	0.7371 ^{a}±0.03885	0.7832 ^{a}±0.02106
P0.5	0.726 ^{a}±0.10153	0.7455 ^{a}±0.02651	0.8857 ^{a}±0.05064
P01.0	0.745 ^{a}±0.1005	0.7599 ^{a}±0.01956	0.8795 ^{a}±0.04
P01.5	0.7526 ^{a}±0.20051	0.7549 ^{a}±0.02008	0.8608 ^{a}±0.0217
P02.0	0.747 ^{a}±0.20512	0.7539 ^{a}±0.01891	0.8759 ^{a}±0.04097
P02.5	0.705 ^{a}±0.00911	0.774 ^{a}±0.02009	0.8695 ^{a}±0.05412

Table 9: Condition factor of Asian Seabass.

	Ca 0%	Ca 0.5%	Ca 1.0%
P0	1053.947^{bc}±5.00	1053.947^{e}±2.00	1029.412^{h}±2.00
P0.5	1051^{cd}±.1.60	1045.49^{de}±1.00	1014.943^{i}±2.00
P01.0	1061.7^{a} ±2.00	1046.656^{de}±3.00	1091.644^{l}±4.00
P01.5	1057.214^{b}±2.00	1046.939^{g}±1.00	1089.1231^{j}±3.00
P02.0	1050.363^{cd}±1.00	1024.303^{f}±3.00	1087.931^{m}±2.52
P02.5	1053.947^{bc}±2.00	1033.225^{f}±2.00	088.8327^{k}±2.00

in T1. The Midrange absolute count of Asian Seabass ranged between 1.201±0.80023 10^9/l and 4.902±0.55057 10^9/l with the highest of T15 and lowest in T1. The Granulocytes of Asian Seabass ranged between 1.7143±0.39642 10^9/l and 4.5653±0.9518710^9/l maximum in T15 and minimum in T1. The Haemoglobin of Asian Seabass ranged between 0.39±0.35412 g/dl and 0.6487b±2.2 g/dl with the top most of T15 and bottom least in T9. The haematocrit of Asian Seabass ranged between 0.39±0.61335% and 1.7287± 3.91957% with the top of T15 and the bottom in T4. The mean corpuscular volume of Asian Seabass ranged between 72.3±1.12882 fl and 80.4333±1.31449 fl with a maximum of T15 and a minimum of T8. The mean corpuscular haemoglobin concentration of Asian Seabass ranged between 72.3±1.12882 g/dl and 82.4333 ±1.3144949 g/dl with extreme T18 and moderate in T8. The red cells distribution width of Asian Seabass ranged between 71.6233±0.23965% and 82.4033 ±0.24705% with higher T15 and lower in T11. The platelet count of Asian Seabass ranged between 72.3±1.1288210^9/l and 82.4067±2.14326 10^9/l with the top most of T15 and bottom least in T3. The mean platelet volume of Asian Seabass ranged between 0.108±0.3662 fl and 0.2099±0.05499 fl with better of T15 and least in T7. The PDWC of Asian Seabass ranged between 0.188±0.3662% and 0.269± 0.06134% with a maximum of T15 and a minimum of T8. The SOD of Asian Seabass ranged between 29.2291± 7.81508% and 39.764 ±1.26389% with better of T15 and least in T1. The NBT Assay of Asian Seabass ranged between 0.4365±0.11057 mg/dl and 0.6783 ±0.21681 mg/dl with the highest of T15 and the lowest in T2. Similar research work on hematological parameters was also performed in RAS culture under different stocking densities by Ezhilmathi *et al.* (2022).

The serum protein, g/dl of Asian Seabass ranged between 1.5357 ±0.6052 g/dl and1.9297 ±0.73004 g/dl with top most of T15 and bottom least in T3. The Glutathione of Asian Seabass ranged between 0.0615±0.05214 µg/ml and 0.1935±0.04045 µg/ml with a maximum of T15 and a minimum of T6. The peroxidase of Asian Seabass ranged between 0.212±0.16935 µg/ml and 0.428±0.14574 µg/ml with better of T15 and least in T1. The Protease (Liver) of Asian Seabass ranged between 0.2381±0.01963 U mg^{-1} $protein^{-1}$ min and 0.6551±0.05083 U mg^{-1} $protein^{-1}$ min with the highest of T18 and lowest in T2. The Protease (Intestine) of Asian Seabass ranged between 0.1959 ±0.0108 U mg^{-1} $protein^{-1}$ min and 0.5984±0.00994 Umg^{-1} $protein^{-1}$ min with greatest of T18 and finest in T1.

The Protease (Kidney) of Asian Seabass ranged between 0.2268±0.01951 U mg^{-1} $protein^{-1}$ min and 0.5562±0.01007 U mg^{-1} $protein^{-1}$ min with a maximum of T15 and minimum in T1. The Protease (Stomach) of Asian Seabass ranged between 0.2377±0.00932 Umg^{-1} $protein^{-1}$ min and 0.5879±0.00977 U mg^{-1} $protein^{-1}$ min with topmost of T15 and bottom least in T1. The Lipase (Liver) of Asian Seabass ranged between 199.9865±1.0015 Umg^{-1} $protein^{-1}$ min and 241.6831±1.98582 U mg^{-1} $protein^{-1}$ min with extreme T15 and moderate T8. The Lipase (Kidney) of Asian Seabass ranged between 199.5687±1.04359 U mg^{-1} $protein^{-1}$ min and 233.5629±2.0368 U mg^{-1} $protein^{-1}$ min with better of T15 and least in T14. The Lipase (Intestine) of Asian Seabass ranged between 198.826±1.01199 U mg^{-1} $protein^{-1}$ min and 238.4724±2.15626 U mg^{-1} $protein^{-1}$ min with the highest of T15 and lowest in T14. The Lipase (Stomach) of Asian Seabass ranged between 198.7062 ±0.99895 U mg^{-1} $protein^{-1}$ min and 223.4529±2.11973 U mg^{-1} $protein^{-1}$ min with greatest of T15 and finest in T14 (Table 5). The Amylase (liver) of Asian Seabass ranged between 0.0961±0.0083 U mg^{-1} $protein^{-1}$ min and 0.1923±0.01155 U mg^{-1} $protein^{-1}$ min with a maximum of T15 and minimum in T1. The Amylase (Intestine) of Asian Seabass ranged between 0.0961±0.0083 U mg^{-1} $protein^{-1}$ min and 0.1953±0.01155 U mg^{-1} $protein^{-1}$ min with the topmost of T15 and bottom least in T1. The Amylase (Kidney) of Asian Seabass ranged between 0.2048±0.02187 U mg^{-1} $protein^{-1}$ min and 0.4482±0.01887 U mg^{-1} $protein^{-1}$ min with higher T15 and lower in T1. The Amylase (Stomach) of Asian Seabass ranged between 0.065 ±0.01824 U mg^{-1} $protein^{-1}$ min and 0.2173±0.03109 U mg^{-1} $protein^{-1}$ min with extreme T15 and moderate in T2.

The research work on biochemical and hematological changes due to the supplementation of Calcium and phosphorus on the biochemical parameters such as glucose, cholesterol, triglycerides, lipase, uric acid, albumin, HDL, ASO, ALP, micro albumin, total protein and CRP where also found to be in optimum range in the treatment 15 containing 1% level of calcium and phosphorus in the diet. The other hematological parameter such as WBC, LYM, MID, GRA, HGB, HCT, MCV, MCHC, RDWC, PLT, MPV and PDWC. also proves the results. In the present trials, it was evident that the supplementation of calcium and phosphorus each with 1% in the diet of Asian Seabass in freshwater RAS system is proven to be the best among all other treatments. An increase in minerals supplementation beyond 1% level of calcium and phosphorous did not increase the weight gain this may be due to the dietary requirement of Calcium and Phosphorous in the diet of Asian Seabass is only up to 1% in freshwater-based RAS System is at the optimum levels.

CONCLUSION

The inclusion of calcium and phosphorus at the rate of 1% each in the diet of Asian seabass for culture in a freshwater-based RAS system significantly increased the growth rateand survival rate in comparison with other percentages of inclusions. The biochemical and hematological parameter also proves the results and hence, it is concluded that mineral nutrition is one of the vital components of fish nutrition, especially in the Recirculating Aquaculture System and supplementation of Calcium and Phosphorus is vital.

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Conflict of interest

We declare that there is no conflict of interest.

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