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Dietary supplementation of silica nanoparticles effects in growth performance, muscle protein and trace elements concentration in *Anabas tesudineus* fingerlings

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Abstract
The world's fastest-growing food-producing industry is Aquaculture. *Anabas testudineus* is indigenous to Asia. This species grows to maximum length of 25 cm. Silica is important in providing structural support in filtration and feeding mechanisms and helping biomineralization processes in animals. In lower concentrations silica interacts with biological systems beneficially without causing any harm. It is a study of supplementation of dietary silica nanoparticles to *Anabas testudineus* fingerlings at different concentrations to evaluate the growth performance (Length, weight and survival rate), FCR, SGR, changes in concentration of protein level and trace elements (Zinc and iron) for 60 days. In this study the optimum concentration of dietary silica nanoparticles for maximum growth and SGR value was found to be 0.04 g/100 g. It indicates that SiNP has a significant role in the increase in growth performance of Anabas *testudineus* fingerlings. There is a non-significant large positive correlation between the length gain and weight gain of *Anabas testudineus* at particular concentrations of SiNPs (r=0.788, p=0.063). FCR value obtained maximum at 0.06, 0.1 g/100 g SiNPs. Significance of SiNP on FCR value is not clear in this case. SiNP has no significant role on the changes of protein content in the muscle tissues of the *Anabas testudineus* fingerlings. Concentration of essential trace elements such as iron and zinc are increased by the use of silica nanoparticles as dietary supplement.

Keywords: *Anabas testudineus,* silica nano particles, aquaculture, food conversion ratio, specific growth rates

Introduction
The world's fastest-growing food-producing industry is aquaculture. In practically every part of the planet, it is growing, spreading, and becoming more intense. As the world's population rises, the need for aquatic foods also increases. In most parts of the world, aquaculture is seen as a potential solution to close the gap between the supply and demand of aquatic food (Subasinghe, *et al*., 2009) [10]. In this study *Anabas testudineus* is used for experimental study. It is also known as climbing perch, and has the capacity to be grown in any regulated aquatic environment, including ponds (Ahmadi, *et al*., 2018) [1]. *Anabas testudineus* is indigenous to Asia. Its unique traits include a quicker rate of growth, accessory respiratory organs, a shorter culture period (three to four months for marketable size), and a greater survival rate (Kohinoor and Zaher, 2006) [7]. In a few countries, it has also been imported outside of its native region. The maximum length to which this species grows.
Because fish protein contains all of the essential amino acids in the right proportions, it has a high biological value in addition to being highly nutritious (Finegold, 2009) [5]. Fish is one of the most protein-dense foods that people eat (Bostock *et al*., 2010) [3]. Because fish is more readily available, has a stronger flavor, and is digestible, a higher proportion of customers eat it than others because of its value for health. Additionally, they offer vital fatty acids like omega-3 and -6, which people require for their optimal health, boosted immunity, and the avoidance of heart diseases and cancer. Freshwater fish is thought to contribute more than 6% of the yearly animal protein sources used by humans (FAO, 2017) [4]. Aquatic foods constitute a vital part of the global food basket to improve the nutrition, health, and well-being of all people in the world where over 70% of the planet is covered with water and about 30% of

humanity suffers from malnutrition (Srinivasan *et al*., 2017) [9]. Silica is important in providing structural support, filtration and feeding mechanisms support and helping biomineralization processes in animals. Silica nanoparticles are used in medical applications such as drug delivery. In lower concentrations, they interact with biological systems beneficially without causing any harm. They have the ability to enter cells and affect several biological functions, including gene expression, signaling pathways, and cell viability. The present study is to evaluate the growth, survival rate, SGR, FCR and trace elements concentration of *Anabas testudineus* finger lings enriched by different concentration of silica nano particles.

Materials and Methods

Test animal- *Anabas testudineus* fingerling

Anabas testudineus (Climbing Perch) fingerlings of average weight approximately 5.00 g and average length approximately 6 cm purchased from a local agency for development of aquaculture at Ullanam, Kerala. The fingerlings were acclimated for 15 days in a plastic container. Water in the tub changed regularly. The fish *Anabas testudineus* is widely used for its muscle mass which is rich in protein, amino acids, and other nutrients. Nutritional value of the fish is high. Aquaculture system is constructed by using plastic containers of 15L capacity. Six plastic containers were taken for the experiment to accommodate the fishes. Five containers for the series concentrations of SiNP fed fishes and the one for the control. Each container filled with the same measurement of water 10L and provided an equal number of fish (10) fingerlings. Each of the container consists of almost similar size fingerlings. A net was placed over the containers to avoid jumping out of the fish. Total experimental days is 60.

Length-weight measurement and survival rate

After acclimatization the Initial length and weight of anabas measured before starting the experiment. Each weekend the weight of the fish is measured by using a weighing machine. After 60 days of the experiment the final length and weight was also detected. Length of each fish is measured by using a graph paper, transparent glass and scale. The length of the fish was measured by placing it over a glass plate which in turn was placed over a glass plate which in turn was placed over a graph paper and counting the squares in a linear line with the aid of a scale placed on the graph paper. Survival rate is the measure of live fishes in the population in each of the six sets of experimental groups after the experimental period of 60 days. It is calculated by subtracting the number of deaths from the initial population number. Then it is expressed in percentage. Survival rate is calculated by,

Survival=100× (Final fish number/initial fish number)

Analysis of feed conversion ratio (FCR)

It is the traditional indicator of livestock production efficiency. The feed conversion ratio (FCR) is the quantity of feed required to produce one kilogram of fish. Calculated by the weight of feed consumed divided by the animal's weight gain. A lower FCR score denotes greater efficiency.

FCR = Feed given / Animal weight gain.

Analysis of specific growth rate (SGR)

Evaluation of the percentage increase in size and weight necessary for the amount of feed at a certain growth stage about time (SGR) is crucial. It is the coefficient that expresses the daily percentage growth in fish weight.
Calculated by the formula,

SGR = 100 x (ln final weight- ln initial weight)/days.

Estimation of protein

At the end of the experimental period, the protein content of the fish tissue fed with various concentrations of SiNP were calculated by Lowry's method and compared with the control group. Estimation of protein done by Lowry's method (Lowry).

Trace element analysis

Trace element analysis done by using ICP-OES (Inductively coupled plasma-optical emission spectroscopy) to measure the concentrations of trace elements Zinc and Iron.

Statistical analysis

Pearson's correlation coefficient employed to explore linear relationship between length and weight of *Anabas testudineus* at particular concentrations of silica nanoparticles (0.02 to 0.1g/100 g) supplementation and also in control.

Results and Discussion

Length- weight measurement

After 60 days of experiment the changes in length and weight of *Anabas testudineus* (Climbing perch) fingerlings in the presence of different doses of SiNP (0.02 g, 0.04 g, 0.06 g, 0.08 g, 0.1 g) and in control were measured. The results are shown in Table 1.

Table 1: Initial and final length and weight gain of *Anabas testudineus* from 19th Dec 2023 to 16th Feb 2024 (60 days).

Sl. No	SiNP (g/100 g)	Length(cm)		Weight (g)	
		Initial	Final	Initial	Final
1	0.02	6.48±0.108	8.5±0.286	5.264±0.089	12.53±0.0252
2	0.04	6.63±0.09	10±0.382	5.51±0.057	16.45±0.871
3	0.06	6.6±0.152	7.8±0.431	6.103±0.101	9.89±0.401
4	0.08	7.57±0.149	8.4±0.346	7.66±0.236	12.55±0.39
5	0.1	5.11±0.266	7.5±0.286	3.57±0.081	7.36±0.577
6	control	5.01±0.176	8±0.566	3.23±0.073	12.58±0.348

The highest change in length and weight were observed in 0.04g/100g SiNP fed experimental set. It is greater than the change of length and weight in the control. All other concentrations of SiNP show small changes in the length and weight.
Control<0.04 g/100 g SiNP
Control > all other concentration

Feed Conversion Ratio (FCR)

Feed conversion ratio of *Anabas testudineus* during the experimental period from 19th Dec 2023 to 16th Feb 2024 (60 days) were analyzed. The results show that 0.04 g/100g of SiNP is beneficial for the improvement of length and weight of the *Anabas testudineus* fingerlings. All other concentrations such as 0.02, 0.06, 0.08 and 0.1 g/100 g have a role in the reduction of normal length and weight gain when compared to control. So, the most favored concentration of the nanoparticle is 0.04 g/100 g to obtain maximum length and weight. 0.04 g/100 g is the optimum concentration. The gain of length - weight after the supplementation of the series of concentrations (0.02 to 0.1 g/100 g) and the control is represented. The results show that the survival rate of *Anabas testudineus* is high in the 0.1 g/100 g SiNP fed experimental group and in the control group. The lowest FCR is comparable to the findings of Bashar *et al.*, (2021) [2]; Munilkumar and Nandeesha (2007) [8] studied that the lowest FCR in *Oreochromis niloticus*, fed with 2 mg silica nanoparticles.

Survival rate is lowest in 0.02 g/100 g SiNP fed experimental set. Death of fish may be due to the dissolved oxygen depletion, intraspecific competition or any other unfavorable factors.

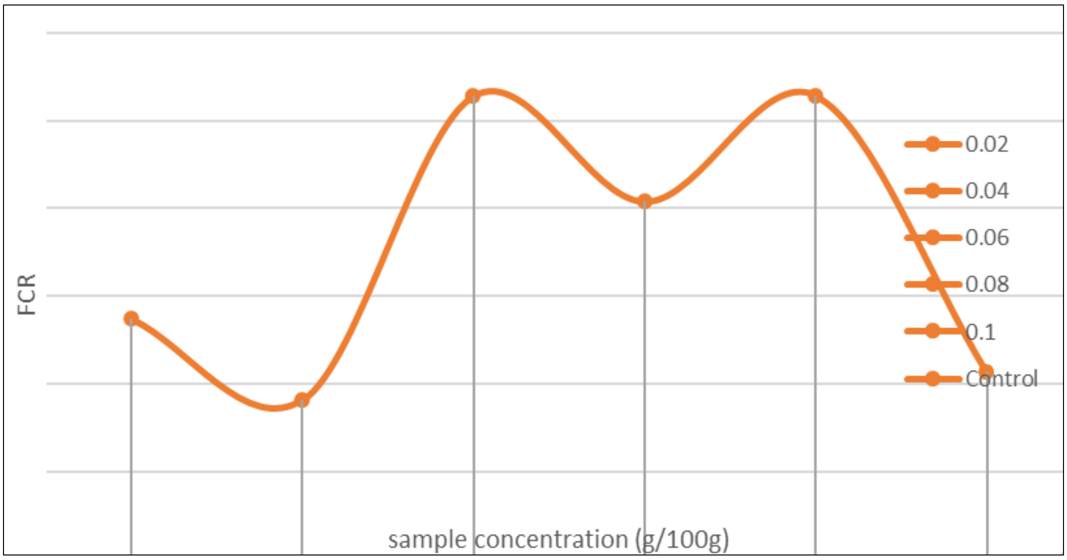


Fig 1: Line diagram showing feed conversion ratio of *Anabas testudines* fed with series of concentrations of SiNP and control from 19th Dec 2023 to 16th Feb 2024

Fig. 1 shows all concentrations except 0.04g/100g with increased feed conversion ratio than control. So, it can be concluded that SiNP has a role in the improvement of feed conversion ratio of *Anabas testudineus* in general.

Analysis of specific growth rate (SGR)

Calculated the specific growth rate of *Anabas testudineus* fed with a series of concentrations of SiNP (0.02g/100g to 0.1g/100g) and control from 19th Dec 2023 to 16th Feb 2024 (60 days). Specific growth rate is maximum at 0.04 g/100g of Silica nanoparticles supplemented fish. High SGR value indicating the rapid growth of the fish. This may be due to the availability of plenty of nutrients genetic factors and may be the water temperature. Lowest value of SGR was observed at 0.06 g/100g SiNP. There is no relationship between FCR at the corresponding concentrations. Having significant increase or decrease with respect to change in body weight of *Anabas testudineus.*

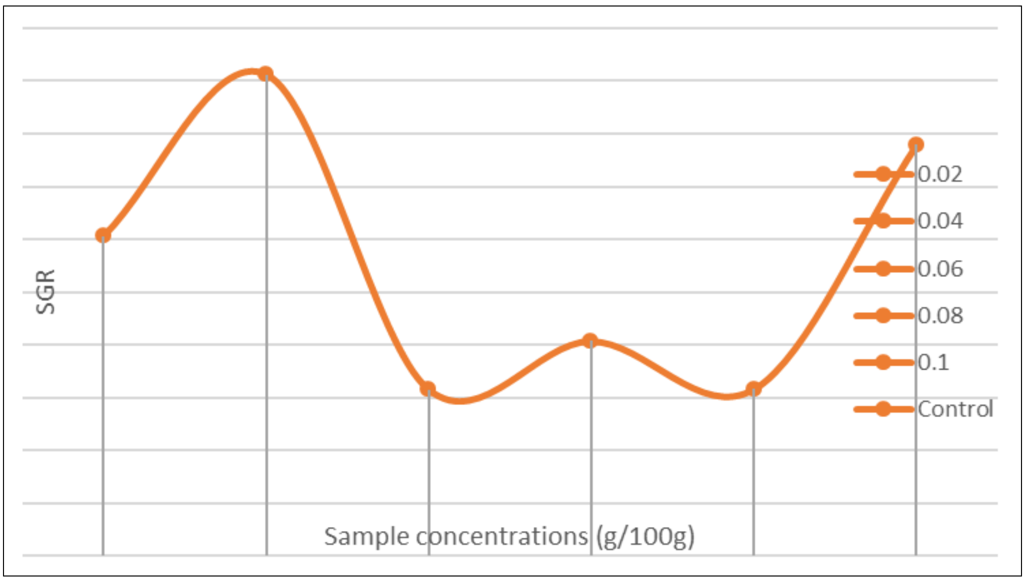


Fig 2: Line diagram showing the SGR values of *Anabas testudineus* corresponding to the concentrations of SiNP

Fig.2 shows that 0.04g/100g SiNP fed experimental sets have the highest SGR value. It is greater when compared with the control group. All other concentrations have a lower SGR value than control. Based on this observation is concluded that the optimum concentration for high SGR value is 0.04 g/100g SiNP as in the case of growth performance. Therefore, SiNP has a role in the improvement of SGR value of *Anabas testudineus.* Jahanbakhshi *et al.*, (2021) [6] were experimenting a study on the effect of feed additives of selenium nanoparticles and selenomethionine in goldfish (*Carassius auratus*). In their study they observed similar results of significant changes in the FCR, SGR and growth performance. Similar result observed when *Labeo rohita* fingerlings were fed a meal with 20 mg ZnO nanoparticles per kg revealed a greater specific growth rate Mohanty, *et al.*, (2019) [11].

Estimation of protein: The protein present in the muscle tissues of *Anabas testudineus* fed with SiNP (0.02g/100g to 0.1g/100g) and control sample were calculated. The experimental period is 60 days from 19th Dec 2023 to 16th Feb 2024. It is represented in Fig.3. Concentrations of protein vary with concentrations of nanoparticles. But these differences have no significance in this study. Because protein content in the fish muscle decreased by the use of dietary silica nanoparticles.

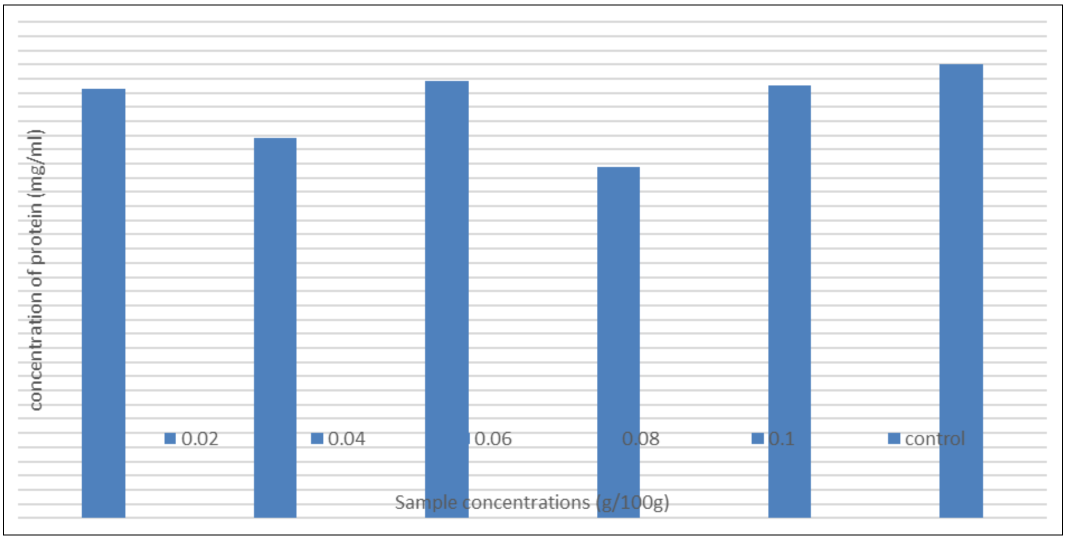
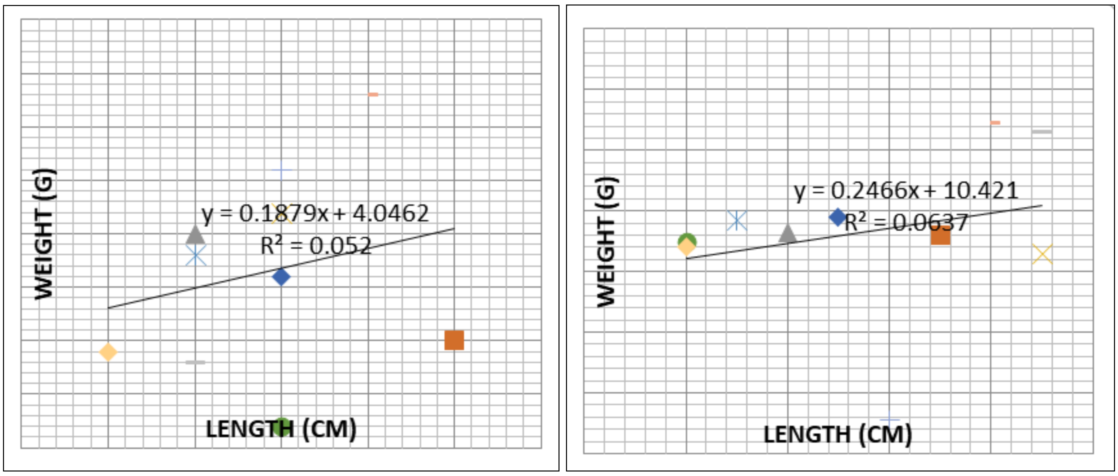


Fig 3: Concentration of muscle protein of *Anabas testudineus* after experimental period

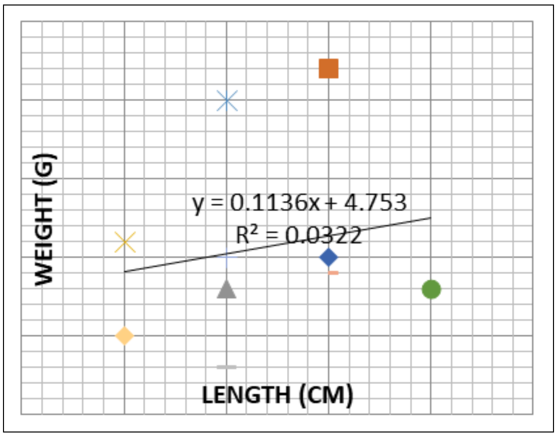
Table 2: Trace elements concentration of *Anabas testudineus* at different concentrations of SiNP and control after 60 days

Sl. No	Parameters	0.02 g/100g SiNP	0.04 g/100g SiNP	0.06 g/100g SiNP	0.08 g/100g SiNP	0.10 g/100g SiNP	Control
1	Iron (Fe), (mg/ml)	8.70	4.63	15.67	4.33	1.42	1.78
2	Zinc (Zn), (mg/ml)	1.23	0.54	1.99	0.51	0.42	0.38

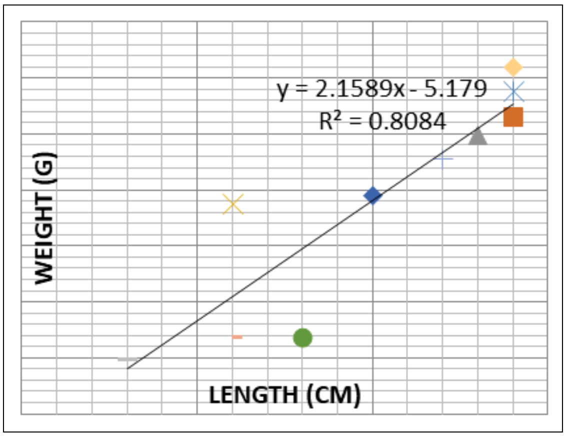
Maximum concentration of iron found at 0.06g/100g of SiNP. But in 0.1g/100g of SiNP concentrations of iron present in the sample is lower than the concentration of the control sample. Zinc concentration is also high in the 0.06g/100g of SiNP. 0.06g/100g is the optimum concentration for trace elements. The changes are in a non-uniform manner across the concentrations of SiNP. Scatter plots resulting in positive correlations between length weight relationship of *Anabas testudineus* (Fig. 4).



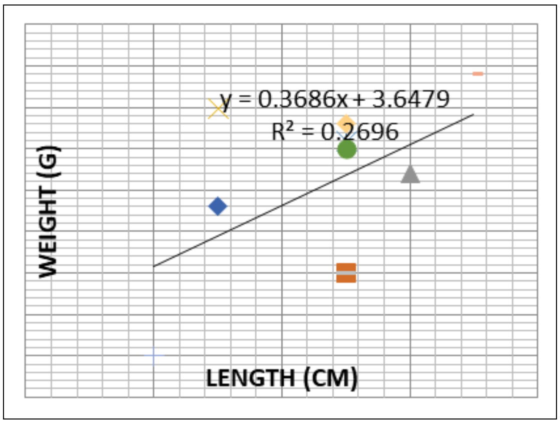
a b


y = 0.1136x + 4.753
R² = 0.0322
WEIGHT (G)
LENGTH (CM)

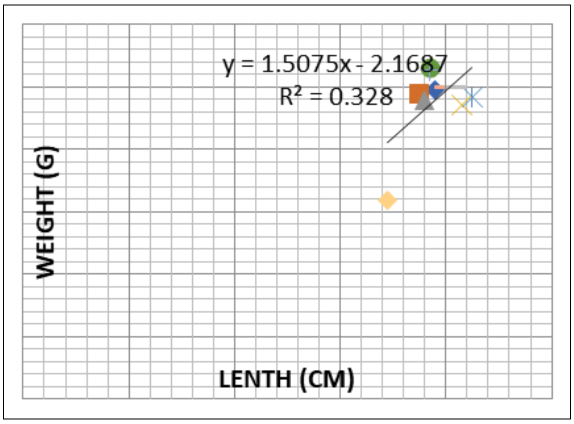
c


y = 2.1589x - 5.179
R² = 0.8084
WEIGHT (G)
LENGTH (CM)

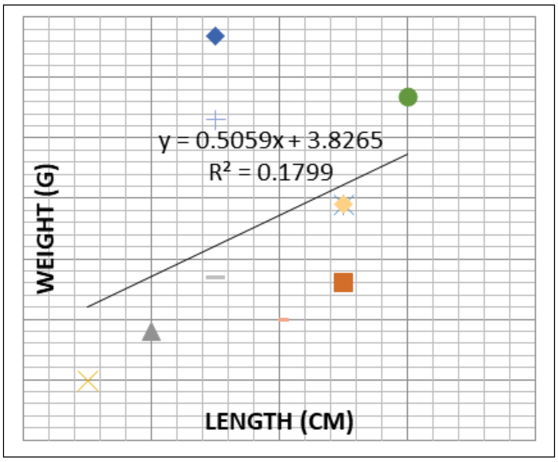
d


y = 0.3686x + 3.6479
R² = 0.2696
WEIGHT (G)
LENGTH (CM)

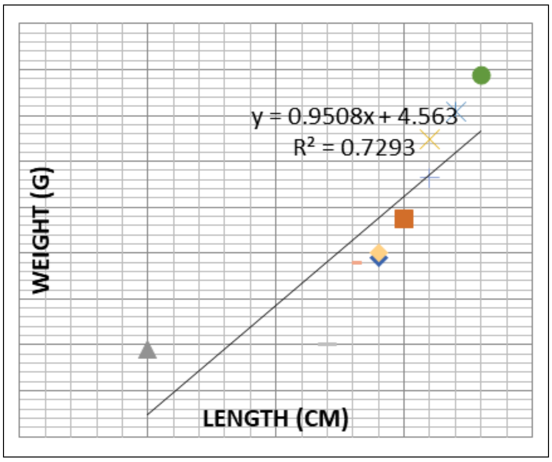
e


y = 1.5075x - 2.1687
R² = 0.328
WEIGHT (G)
LENTH (CM)

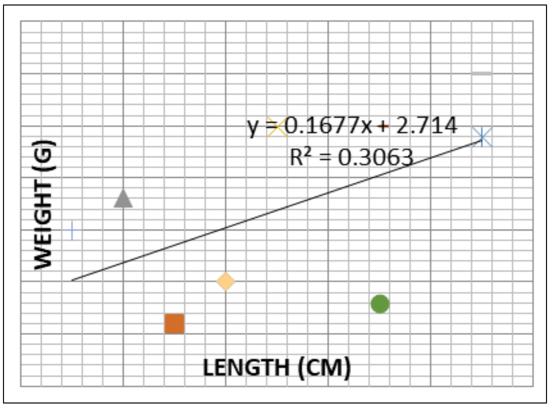
f


y = 0.5059x + 3.8265
R² = 0.1799
WEIGHT (G)
LENGTH (CM)

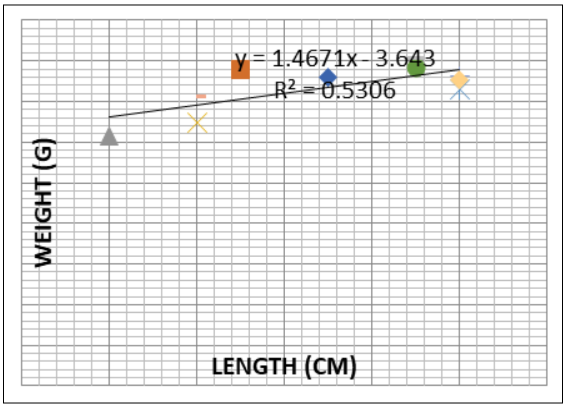
g


y = 0.9508x + 4.563
R² = 0.7293
WEIGHT (G)
LENGTH (CM)

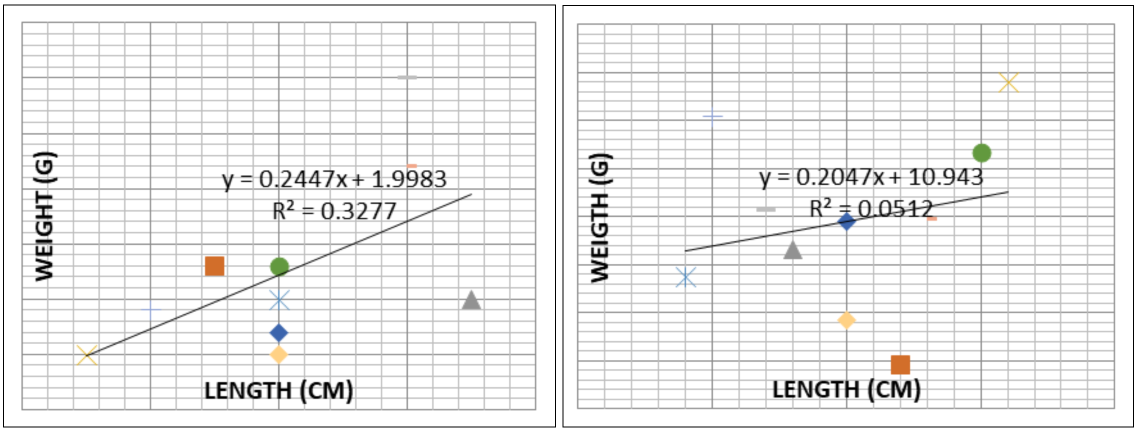
h


y = 0.1677x + 2.714
R² = 0.3063
WEIGHT (G)
LENGTH (CM)

i


y = 1.4671x - 3.643
R² = 0.5306
WEIGHT (G)
LENGTH (CM)

j



k l

Fig 4: Scatter plots resulting in positive correlations between length weight relationship of *Anabas testudineus* fingerlings are shown in figures a to L.

Conclusion

This is a study of supplementation of dietary silica nanoparticles to *Anabas testudineus* fingerlings at different concentrations to evaluate the growth performance (length, weight), FCR, SGR, changes in protein concentration level and trace elements (zinc and iron) concentration. In this study the optimum concentration of dietary silica nanoparticles for maximum growth and SGR value was found to be 0.04 g/100g. It indicates SiNP has a significant role in the increase in growth performance of Anabas *testudineus* fingerlings. There is a non-significant large positive correlation between the length gain and weight gain of *Anabas testudineus* at particular concentrations of SiNPs(r=0.788, p=0.063). FCR value obtained maximum at 0.06, 0.1 g/100g SiNPs. Significance of SiNP on FCR value is not clear in this case. SiNP has no significant role on the changes of protein content in the muscle tissues of the *Anabas testudineus* fingerlings. The changes in the protein concentration are lower than the normal concentration of protein in the control sample. Trace elements such as iron and zinc are essential elements, the concentrations of these elements increased by the use of silica nanoparticles as dietary supplement. Iron and zinc are essential nutrients for human consumption.

Through further research, scientists can clarify the possible advantages and disadvantages of supplementing *Anabas testudineus* with silica nanoparticles, advancing sustainable aquaculture methods and enhancing fish nutrition. Researchers can test whether adding supplements of silica nanoparticles to commercial aquafeed formulations is acceptable. Analyze the scalability, economic feasibility, and practical issues of large-scale aquaculture operations. To assess the relative efficacy and safety of different kinds of nanoparticles or dietary supplements, researchers might conduct comparative studies. This would offer a more comprehensive viewpoint on the application of nanotechnology in aquaculture. Researchers can investigate in more detail how silica nanoparticles interact with trace metals like iron and zinc. Find out how fish absorb, distribute, and use these vital nutrients in response to nanoparticle supplementation. Scholars can establish the ideal silica nanoparticle supplementation dosage and time to maximize growth and nutritional advantages without causing negative side effects. This would require nutritional balance evaluations and dose-response studies. Scholars can Examine the underlying processes that silica nanoparticles use to have an impact on fish development and protein metabolism. This can entail using molecular biology methods to understand dynamics of protein production, metabolic pathways, and variations in gene expression. Students can Research on the potential antimicrobial or antioxidant qualities of nanoparticles, which may help avoid illnesses or reduce their effects on fish health. Scholars can evaluate blood parameters changes and their effects in growth by using silica nanoparticles.

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