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EFFECT OF CREATINE SUPPLEMENTATION ON THE BEHAVIOURAL PATTERN AND EXTERNAL MORPHOLOGY OF KOI CARP (*Cyprinus rubrofuscus*)

Harsha Padwal[1], Barkha Mishra[2], Pooja Mishra[3], Azim Mollah[4]

[1]Assistant. Professor, B. K. Birla Night College, Kalyan

[2,3,4]Student, B. K. Birla Night College, Kalyan

ABSTRACT:

The present study investigates the impact of creatine supplementation on the behavioural patterns and external morphology of Koi *(Cyprinus rubrofuscus*), a widely cultured ornamental fish species. Creatine, a naturally occurring nitrogenous organic acid, is known in for its role in cellular energy metabolism and has been shown to influence various physiological processes in vertebrates. Despite its popularity as a dietary supplement in human and livestock nutrition, limited research exists of creatine on the aquatic organisms, particularly ornamental fish. In this experimental study, Koi specimens were subjected to a controlled environment and divided into three groups: a control group receiving a standard diet and two experimental groups supplemented with creatine. Behavioural observations were analysed by noticing the changes in swimming patterns, feeding behaviour, and overall activity levels. Additionally, external morphological parameters such as body shape, fin development, and coloration were assessed to detect any noticeable variations between the control and experimental groups. Preliminary findings suggest that creatine supplementation may influence the behavioural repertoire of Koi, including alterations in swimming dynamics and feeding habits. Moreover, external morphological analyses reveal potential changes in fin morphology and coloration patterns in response to creatine supplementation. This research contributes to our understanding of the physiological effects of creatine in aquatic organisms, shedding light on its potential applications in aquaculture and the ornamental fish industry. Further investigations are warranted to elucidate the underlying mechanisms and long-term consequences of creatine supplementation on Koi and other ornamental fish species.

: Creatine supplementation, Koi Carp (*Cyprinus rubrofuscus*), Behavioural pattern, External morphology

INTRODUCTION:

Vertebrate muscle tissue contains the amino acid derivative creatine (H_2N) (HN)CN(CH_3) (CH_2CO_2H). The substance helps the body recycle adenosine triphosphate (ATP), which supplies energy to the brain and muscles.

A scientific review that combines data on creatine's many uses, physiological effects on fish, and possible advantages as an aquaculture supplement was released by researchers from the Leibniz-Institute of Freshwater Ecology and Inland Fisheries and the Thünen Institute of Fisheries Ecology in Germany.

The application of creatine as a supplement in fish nutrition is still mostly unknown. However, because creatine has so many physiological uses, it can be a useful addition to aquaculture species' feed.

Fish have up to five times the amount of creatine per body weight than terrestrial animals, which is also explained by the fact that fish have a higher proportion of skeletal muscle to body weight. The study's authors claim that since creatine has been shown to have benefits in humans and other terrestrial species, supplementing fish with it could be a fruitful area of investigation.

As an aquafeed, creatine is essential for fish muscle and can enhance the health and performance of fish bred in aquaculture systems. By boosting body mass, creatine supplementation of fish feeds encourages the formation of muscle and may enhance feed utilization, especially in plant-based diets. Additional function of creatine is in osmoregulation during salinity adaptation in fish.

We especially use Koi carp (*Cyprinus rubrofuscus*) for the physiological observation of the effects of creatine, and our supply of creatine is supplemental powder, or creatine monohydrate. because to its wide availability, affordable price, abundance of colour variations, and extended life (up to 30 years or more). More than 100 decorative species of koi (*Cyprinus rubrofuscus*), which are housed as pets in both indoor and outdoor freshwater ponds worldwide, are most recognized for their vibrant body patterns. In the beginning, koi were grown in China and Japan as food fish.

Koi are strong fish with large bodies. When fully grown, most koi measure about 90 cm (36 inches) in length and weigh about 16 kg (35 pounds). These are versatile, omnivorous cold-water fish that eat algae, plants, and other invertebrates in addition to insects and crustaceans. Male koi reach sexual maturity somewhat before female koi, which happens between the ages of three and six. During a single spring or summer spawning session, females can produce hundreds of thousands of eggs. These eggs are externally fertilized and hatch between four and seven days later. Koi typically live 30 to 40 years in the wild, but some have been kept in captivity for almost 70 years.

By adding and comparing various quantities of creatine monohydrate and observing the effects on the physiology of koi from normal settings, when no additional creatine is supplied, our main focus will be on the physiological changes that the fish undergo over the course of 20 days. This study aims to provide useful insights for the ornamental fish business in addition to adding to the corpus of knowledge already available on the physiological effects of creatine in aquatic creatures. Through the clarification of possible changes in the external morphology and behavioural patterns of Koi upon creatine supplementation, this study hopes to

facilitate the development of optimal and well-informed Koi husbandry techniques. A thorough study of creatine's effects on these interesting species becomes increasingly important as the demand for aesthetically beautiful and healthy Koi continues to climb.

MATERIALS AND METHODS:

Sr.No.	Material	Chemicals
1.	Source of creatine	Creatine monohydrate **(**$C_4H_9N_3O_2$) power supplement
2.	Species of fish	Koi Carp (*Cyprinus rubrofuscus)*
3.	Components used in fish tank	Aquarium products: Anti-white spot formula, Dichlorination
4.	Added minerals	Rock salt
5.	Aquafeed	**Tropical fish food:** Ingredients: Wheat flour, rice, corn, fish meal, gluten meal, dried yeast, fish oil, spirulina, vitamins and minerals.

Table no. 1

PHYSIO CHEMICAL TESTS:

Sr.No.	Tests	Methods
1.	pH	The pH was Measured using pH meter
2.	Hardness	The hardness of water sample was measured by EDTA Titrimetric Method (Ambast, 1990)
3.	Ammonia N_2	Nessler's Ammoniacal test method (Julius Neßler,1827)
4.	Biological oxygen demand (BOD)	Winkler Titration Method (Lajos Winkler ,1863)
5.	Salinity	The salinity was measured using Refractometer
6.	Odour	Samples were compared on the basis of their ammoniacal smell
7.	Transparency	The water transparency was checked to determine the turbidity of water sample before and after the addition of fish (with creatine supplementation)

Table no. 2

OBSERVATIONS:

A. Analysis of water

- The following tests were carried out and the observations were done before and after addition of Koi (*Cyprinus rubrofuscus*) in fishtank after every 5th day (1. In control tank- without supplementation of creatine. 2. In Tank A and Tank B with the supplementation of Creatine in different quantities).
- It was concluded that creatine supplementation had a positive effect on the muscle and scale growth of Koi.

1. Control tank (No Creatine supplementation)

Tests	Before addition of fish	After addition of fish (intervals of five days)
Colour	Colourless	Slightly yellow
Odour	Odourless	Ammoniacal
Transparency	Transparent	Yellowish in colour, less transparent
pH	7.2	6.9
Hardness	150mg/L	155mg/L
BOD	3.75mg/L	14.38mg/L
Ammonia test	Light orange	Dark orange

Table no.3

2. Tank A (Creatine supplementation- 5 gm)

Tests	Before addition of fish	After addition of fish (intervals of five days)
Colour	Colourless	Slightly yellow
Odour	Odourless	Ammoniacal
Transparency	Transparent	Yellowish in colour, less transparent
pH	7.1	6.78
Hardness	150mg/L	162mg/L
BOD	3.75mg/L	13.68mg/L
Ammonia test	Light orange	Dark orange

Table no.4

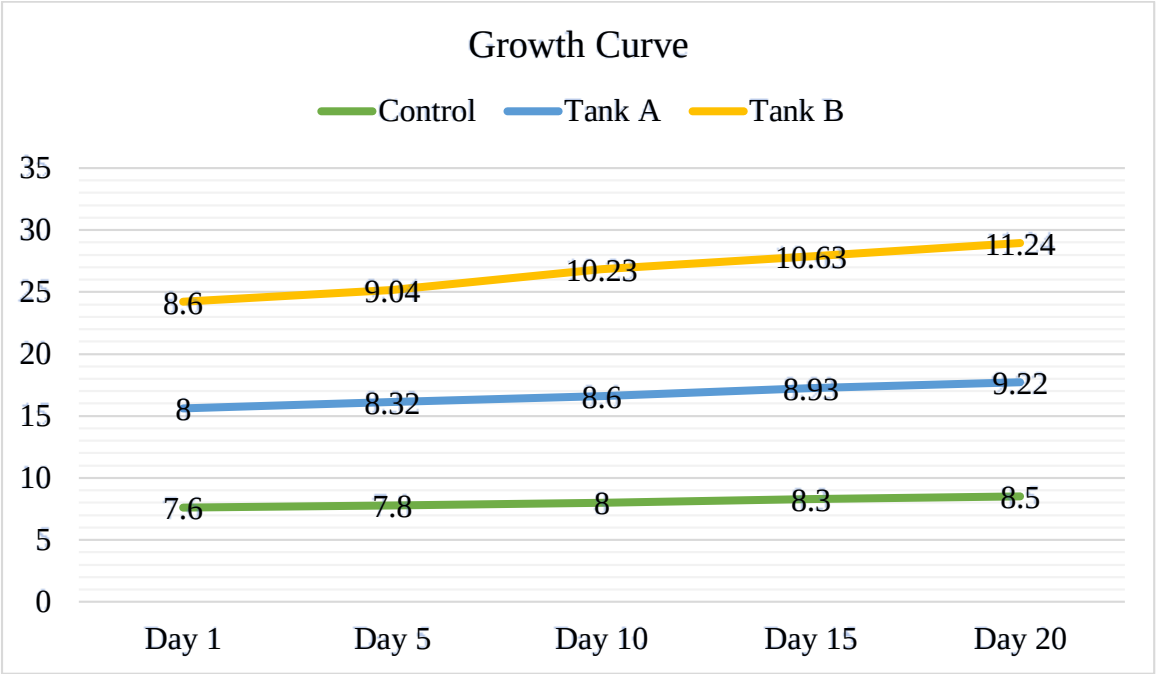
3. Tank B (Creatine supplementation- 10gm)

Tests	Before addition of fish	After addition of fish (intervals of five days)
Colour	Colourless	Slightly yellow
Odour	Odourless	Ammoniacal
Transparency	Transparent	Yellowish in colour, less transparent
pH	7.1	6.73
Hardness	150mg/L	166mg/L
BOD	3.75mg/L	13.4mg/L
Ammonia test	Light orange	Dark orange

Table no. 5

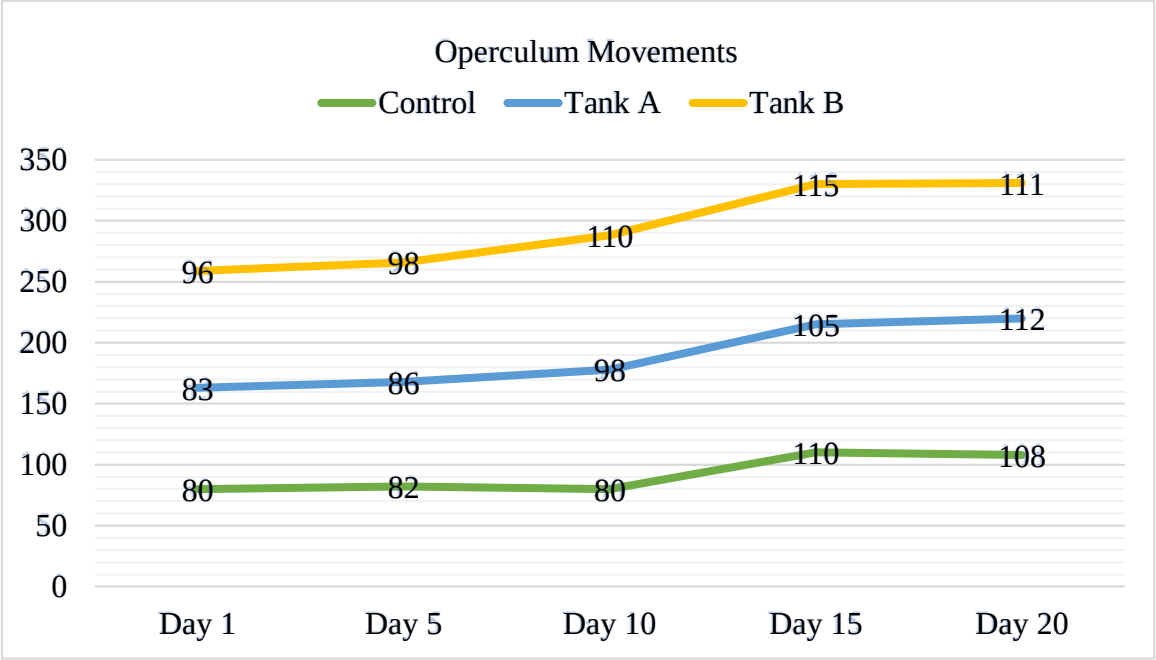
B. External morphology and Behavioural pattern of Koi

❖ Morphological growth (average increase in the size)



Since the tail and fins span one-third of the body, their length and width have slightly expanded.

❖ Operculum Movements (average)



❖ Behavioural Observation and Feeding Pattern

a. Despite receiving two feedings a day, they seem a little more agitated and hungrier during feedings because of the creatine.

b. The smaller fish is more energetic during feedings but occasionally appears a little sleepy (perhaps because its high creatine content).

c. After ten days, they have mostly adjusted to creatine and have stopped being as aggressive.

d. Constantly moving and looking for food at the bottom.

- **Osmoregulation Adaptation:** Since creatine aids in osmoregulation, which helps the fish adjust to brackish water, the salinity is gradually raised in intervals of five days for the tanks supplemented with the creatine. These fishes appear to be acclimated to the salinity.
- **Observation in Colour pattern:** The dark patches have grown larger and have begun to spread. Also, orange and yellow accentuate colours, especially in the dark, the colour has also become more vibrant.

GENERAL CONCLUSION:

The provided research emphasizes the potential benefits of creatine supplementation in Koi carp (*Cyprinus rubrofuscus*), focusing on its physiological effects, growth performance, and potential applications in aquaculture.

1. Physiological Role of Creatine: The study highlights the role of creatine in vertebrate muscle tissue, particularly in recycling adenosine triphosphate (ATP), the energy currency of cells. This underscores the importance of creatine in providing energy to the brain and muscles, crucial for various physiological functions.

2. Potential Advantages in Aquaculture: The research suggests that creatine supplementation could be beneficial for aquaculture species, citing its numerous physiological uses and potential advantages. Fish, in particular, have a higher proportion of skeletal muscle to body weight compared to terrestrial animals, indicating the potential significance of creatine supplementation in fish nutrition.

3. Focus on Koi Carp: The study specifically targets Koi carp (*Cyprinus rubrofuscus*) for physiological observation, emphasizing their suitability as a model organism for studying the effects of creatine supplementation. Koi carp, known for their vibrant body patterns and popularity in ornamental fish business, serve as an ideal species for this investigation.

4. Methodology and Duration of Study: The research involves the addition and comparison of various quantities of creatine monohydrate over a 20-day period to observe its effects on the physiology of Koi carp. This longitudinal approach allows for the thorough examination of physiological changes in response to creatine supplementation.

5. Implications for Aquaculture Industry: The study aims to provide useful insights for the ornamental fish business, suggesting that understanding the effects of creatine supplementation on Koi carp could facilitate the development of optimal husbandry techniques. As the demand for aesthetically pleasing and healthy Koi continues to rise, such research becomes increasingly important for the aquaculture industry.

Overall, this research underscores the potential significance of creatine supplementation in enhancing the health and performance of Koi carp in aquaculture settings, offering valuable insights for both scientific understanding and practical applications in the ornamental fish industry.

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These references provide a comprehensive overview of the research on creatine supplementation in Koi carp, including its physiological effects, growth performance, and potential applications in aquaculture.