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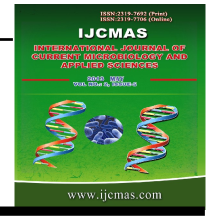

Original Research Article

Dietary calcium requirement of *Oreochromis mossambicus*

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A B S T R A C T

The requirement of *Oreochromis mossambicus* for dietary calcium was investigated by feeding them with a basal diet containing ($CaCo_3$:1 to 8.0 /100g) different levels of calcium, in the laboratory condition for the period of 60 days. To investigate the growth of the fish, field trial was also conducted in the fish diet supplemented with optimum level of calcium. The basal diet of 50% contained waste silkworm pupae used as the main source of protein with fishmeal and oilcake. The highest growth rate (30.164 mg/g live fish /day) occurred in the fish fed on a diet supplemented with 1.097% calcium. The proximate composition (protein, fat, ash and water), enzymes transaminases (GOT and GPT), lipase and whole body mineral (Phosphorus, Magnesium and Calcium) content of the tested fishes were varied according to the dietary calcium levels. Judging from the growth rate of the fish, adequate calcium content of the diet of Oreochromis mossambicus was estimated to be 1.097%.

Keywords

Oreochromis mossambicus; Calcium; Enzymes; Dietary requirement.

Introduction

Tilapia is one of the most widely cultured fish in the world. Currently, farmed tilapia represents more than 75% of world tilapia production (FAO, 2009), and this contribution has been exponentially growing in recent years. Several factors have contributed to the rapid global growth of tilapia. Tilapia is easily cultured and highly adaptable to a wide range of environmental conditions. Considerable work has been done in India on supplementary feeding as a fish culture practice. In India attention is being given for silk production. In silk reeling centres, the silkworm pupae are discarded. This silkworm pupae is rich in protein as well as fat (Kandeepan and Arunachalam, 2003). Hence, it is felt that the silkworm pupae may be utilized to replace the fishmeal in formulating fish diet. According to Venkatesh *et al.,* (1986) the diet containing silkworm pupae enhances the growth of catfish when compared to meat meal and groundnut oilcake. Quality of food is the nutritional level of food. The fish food should contain all the essential nutrients as components. The fishes, which are reared on such food, will grow normally without any deficiency diseases. Hence it is stated that the efficient and economically effective fish husbandry depends on fish nutrition and diet development. Research and development organizations focus their attention on the basic nutritional requirements of cultivated species and the development of least count formulations based on commonly available ingredients. So much attention is being given to basic and applied fish nutrition to furnish useful data for formulating artificial fish diets. (Muthukumar and Kandeepan, 2009). Minerals required by fish are calcium, magnesium, phosphorus (Kandeepan and Arunachalam, 2003, 2005, Poongulali and Kandeepan, 2008, Kandeepan and Poongulali 2009) and a number of trace elements such as copper, iodine, iron, manganese, selenium and zinc. Calcium as the function primarily structural components of hard tissue (e.g bone, exoskeleton, scales and teeth). In addition to its structural function, calcium is essential for blood clotting, proper nerve impulse transmission, osmoregulation and as a cofactor for enzymatic processes. (NRC. 1993). This satisfies the level of the suggested calcium requirement for fish. But the low levels of calcium have found, poor growth and feed efficiency together with deficiency symptoms in fish.

The survey of literature on the requirements of calcium for fishes indicates that the supplementation of calcium is necessary. Hence, the present study was conducted to determine the dietary calcium level and their effects on the growth, proximate composition, enzymes and whole body mineral content of *Oreochromis mossambicus*.

Materials and Methods

Feeds

The percentage composition and biochemical composition of basal diet are presented in Table 1. The basal diet was formulated from 50% silkworm pupae, 20% fishmeal, 19% oil cake, 10% mydha and 1% vitamin premix.

These four components are dried in sunlight and subsequently dried at 60^{o}C for 8 hours in hot air oven. Then they were powdered and sieved. The basal feed mixture was added to source for calcium to prepare different diets as given in Table 2. These components were mixed and pelleted in a pelletizer, without steam and stored in bins.

Experimental Design

Juveniles of *Oreochromis mossambicus* collected from local ponds near Palani, TamilNadu, India were brought to the laboratory and acclimated to the laboratory condition and feeding schedule, keeping them in fibre glass (acrylic) aquaria (60X30X30cm) at room temperature (28 ±1°C). The acclimation period lasted for 15 days. Individuals showing signs of disease or injury were removed during the acclimation period. Stock fishes were fed *ad-libitum* with chopped pieces of fresh goat liver twice a day for a period of two hours at each time (8 –10 AM and 6-8 PM) and gradually switched over to formulated diet. Un-eaten food was removed from the tank after the feeding schedule. Aquarium water was changed daily to keep it clean. Well-acclimated juveniles of *Oreochromis mossambicus* range of 1.22 to 2.21 g were selected from the stock. Six different groups (Table 2) (O-C1, O-C2, O-C3, O-C4, O-C5, O-C6 and O-C7), each with 10 individuals were introduced into rectangular acrylic tanks (60X30X30cm) containing 20 litres of tap water. The six different groups were fed on an *ad-libitum* diet of formulated diets (approximately 5% of its live body weight and the food given was adjusted according to the increment in the weight of fish) with different levels of calcium supplementation such as C1, C2, C3, C4, C5, C6 and C7 (Table 2). Triplicate was maintained for each diet. The test fish were fed twice a day at 8 AM and 6 PM for two hours at each time. The aquarium water was renewed daily to keep it clean. The rearing experiment was carried out for 60 days. Each group was weighed accurately on the first day of experimentation for their live weight. To find out the initial dry weight of test individuals, five sample individuals of similar body weight and experimental state were sacrificed and dried to weight constant in hot air oven. The test individuals were weighed periodically on 15 days interval period. On the day of termination of experiment, the test individuals were weighed in live condition and dried to weight constant to estimate the final dry weight of test individuals. The dried samples of test fish were stored in a desicator for subsequent biochemical analysis. To investigate the growth of fish when fed on artificial diet supplemented with optimum level of calcium, field trials were conducted in outdoor cement tanks at ground level with the dimension of 230X160X88cm. According to their size, the density of fingerlings reared was adjusted.

Data processing and evaluation of growth

Growth = Final live weight – Initial live weight.

Growth rate = Wt. gained by the fish / Initial Wt. of fish X No. of expt. days

Gain in energy content (J) = Final energy content – Initial energy content

Conversion rate = Gain in energy content (J) / Initial wt. of fish X No. of expt. Days.

Analyses

The feeds and tested fishes were assayed for moisture (AOAC 1975), Protein (Jayaraman, 1981), Fat (Soxhlet with Chloroform and Methanol), Ash (560^{O}C), Energy (Fisher 1979; Jana 1980), Phosphorus (Fiske & Subbarow, 1925), Calcium and Magnesium (Tacon and De Silva, 1983). Transaminases (GOT and GPT) and lipase were estimated in tested fishes by Wooten (1964) and Oser (1965) method, respectively.

Statistical analysis

Data from the calcium requirement studies were subjected to analysis of variance and Student Newman - Keul's Multiple Range Test (SPSS/PC) to determine differences in means (P< 0.05

Results and Discussion

Oreochromis mossambicus grows well on the diet of C1.The results revealed that the fish exhibited maximum growth i.e., 30.164 mg live weight/ g live fish/ day when fed on diet C1. Then there was a gradual decrease in the growth rate of fish as a function of increase in the calcium content of diet and reached the level of 6.039 mg live weight/ g live fish/ day when the fish was fed on diet with 4.297% calcium(Table 3).

To be more appropriate, the growth rate of *Oreochromis mossambicus* when reared on different diets supplemented with different levels of calcium is expressed in terms of energy value in Table 3. In terms of energy also *Oreochromis mossambicus* exhibited the same trend of growth as observed in terms of live weight. The fish group, which exhibited maximum growth

Table.1 Composition and biochemical composition of basal diet

Ingredient	Percent
Silkworm pupae	50
Fish meal	20
Oil cake	19
Mydha	10
Vitamin Premix	01

Biochemical composition	Percent
Moisture	08.3 ± 0.21
Protein	45.3 ± 0.32
Lipid	23.7 ± 0.20
Ash	21.6 ± 0.39
Minerals	
Calcium	01.097
Magnesium	00.051
Phosphorus	00.714
Energy value (KJ)	22.363

Figure.1 Effect of calcium supplementation to diet on body composition of *O.mossambicus.*

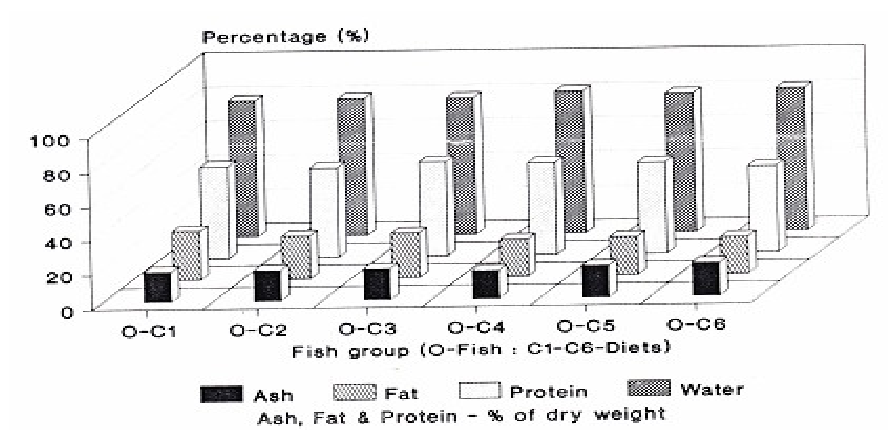

Table. 2 Diets with basal feed mixture and calcium supplementation and their total calcium content

Diets with Calcium supplementation	Feed mixture (g)	Calcium source supplemented (Ca Co_3) (g)	Total Calcium Content (%)
C1 Control	100	0	1.097
C2	100	1	1.497
C3	100	2	1.897
C4	100	4	2.697
C5	100	6	3.497
C6	100	8	4.297

in terms of energy value, found as O-C1 i.e., the fish group which received 1.097% calcium when compared to all other fish groups, which received diets with higher levels of calcium supplementation (Table 3). The growth rate of this group was 77.744 Joules/ g live fish/day. This level was gradually decreased to 56.028 J/ g live fish/day in the fish group (C1-C6), which received the diet with 4.297% calcium. One- way analysis of variance of growth rate of different groups of individuals revealed that the different diets have significant effects on growth and hence, the groups of fish were heterogenous.

Further, the growth rate data were subjected to Student Newman - Keul's test and classified in to two homogenous subsets at 0.05 level of significance. From this, it is clear that the diet O-C1, which gave maximum growth, might have contained the sufficient amount of calcium. Hence, it is concluded that the diets of that groups with 1.097 mg were considered as the optimum requirement for *Oreochromis mossambicus*. Hence, this diet was considered as optimum one. The optimum requirement of these fishes is compared to that of other fishes studied. The optimum calcium requirement for *Oreochromis aureus* ranges from 0.24 to 1.5 g kg-1 for better growth and feed conversion (Robinson *et al.*, 1987). Further, they have stated that growth increased with increasing calcium concentration up to 0.1% in the diet. Rodgers (1984) showed that ambient calcium concentration had a pronounced effect on calcium dynamics. Schwarz (1995) considered that calcium requirements of fish are usually adequately supplied from the water and the calcium content in the food can thus be minimized. When compared to these values, the optimum dietary requirement of magnesium for *Oreochromis mossambicus* is lesser amount. In our present investigation, the protein content of diet is around 40%. Hence, the calcium requirement of *Oreochromis mossambicus* through diet is lesser. The growth of *Oreochromis mossambicus* at optimum level of calcium has been verified by means of field trials. The growth rate of *Oreochromis mossambicus* is less in the field when compared to the results obtained (30.164 mg/g live fish/day) in the laboratory (Table 3). In the field the fish encounter changes in physical, chemical and biological parameters such as the changes in temperature, dissolved O_2 and CO_2 and pH of water. Because of all, the growth is less when compared to the growth obtained in the laboratory.

The proximate chemical composition of *Oreochromis mossambicus*, which were reared on diets supplemented with different levels of calcium, was presented in Table 4 and Fig.1.The fish group O-C1, which was fed on diet with 1.097% calcium, exhibited protein content 58.25%. This level was more when compared to other fish groups, which received diets with low levels of calcium supplementation. The fat content of different fish groups did not have any definite relationship with protein content; it had inverse relationship with water. The correlation coefficient (r) calculated for these two parameters were -0.809 and the regression equation developed was $Y = 171.66 - 1.1750X$.Generally, the fast growing fishes contain more amount of protein. In the present observation also the fishes, which grow fast, contain more amount of protein. It appeared that the fish groups, which received optimum level of dietary calcium supplementation, contained more protein. Viola *et al.* (1986a) have reported that the protein

Table.3 Effects of different levels of calcium supplementation in diets on growth (live weight gained and in terms of energy gained) of *O. mossambicus* (1.470 ± 0.150 g). Each value is the average (± SD) performance of 3 groups, consist of 10 individuals.

Diets	Calcium (%)	Total growth (g)	**Growth rate** (mg/ g live fish /day)	Total energy gained (KJ)	Rate of energy gained (J/G live fish/day)
O-C1 ©	1.097	31.6 ± 1.00	30.164 ±0.86	36.246 ± 0.66	77.744 ± 3.44[a]
O-C2	1.497	26.40±2.55	26.331 ±1.47	50.113 ± 0.82	72.408 ± 1.85[b]
O-C3	1.897	15.26 ±2.60	18.693 ±0.87	40.248± 0.75	61.032 ± 4.52[c]
O-C4	2.697	15.10 ± 0.18	17.480 ± 1.69	46.545 ± 1.13	60.283 ± 5.06[c]
O-C5	3.497	12.90± 0.19	13.672 ±1.39	38.364± 0.71	59.022 ± 2.99[d]
O-C6	4.297	05.82 ±2.49	06.039±0.17	40.012 ± 0.99	56.028 ± 2.91[a]

©=Control Diet; Means in columns that have different superscripts are significantly different ($P<0.05$). Field trial = Optimum Calcium diet (0.165%), Total growth (12.122 g), Growth rate (8.826 mg/ g live fish / day)

Table.4 Effects of dietary Calcium on body composition, enzymes and whole body mineral content of *Oreochromis mossambicus*.

Fish group	DC (%)	Protein (%)	Transaminases (μ moles Pyruvate/ mg protein/hr)		Fat (%)	Lipase (units/ g live tissue)	Whole body mineral content (%)		
			GOT	GPT			P	Ca	Mg
O-C1 ©	1.097	53.70±1.55	1.21±0.06	1.52±0.03	28.6	1.34±0.03	1.696[ab]	3.002[a]	0.110[a]
O-C2	1.497	52.40±1.87	1.32±0.03	1.53±0.04	25.1	1.62±0.05	1.598[b]	3.152[b]	0.122[b]
O-C3	1.897	55.20±1.63	1.09±0.04	1.30±0.06	26.2	1.55±0.04	1.756[ab]	3.419[c]	0.115[bc]
O-C4	2.697	54.10±1.58	1.15±0.03	1.38±0.04	21.6	1.86±0.05	1.999[ab]	3.324[c]	0.127[cd]
O-C5	3.497	53.60±1.45	1.23±0.05	1.54±0.03	22.5	1.78±0.04	1.954[a]	3.659[d]	0.132[d]
O-C6	4.297	51.00±1.52	1.59±0.03	1.59±0.02	21.6	1.86±0.02	1.978[ab]	3.781[d]	0.129[d]

© = Control Diet, DC (%) = Dietary calcium
Means in columns that have different superscripts are significantly different ($P<0.05$).

content of tilapia was high when it was reared on optimum level of phosphorus. According to them, this may be due to activation of protein synthesizing enzymes. Further they have stated that the fat content was correspondingly decreased and this may be due to the inhibition of breakdown of fat by β oxidation. Similar observation has been made by a number of authors in different species of fishes (Tacon *et al.*, 1984). Takeuchi and Nakazoe (1981) have also observed that the fat content decreases as the phosphorus level increases in the diet (See Tacon *et al*., 1984; Arunachalam, 1986). This fact also confirmed by the present observation on the water content and fat content of fishes, which are reared on diets containing different levels of calcium. The ash content did not vary significantly (Figure.1). The ash content does not show any significant variation as observed by Arunachalam (1986) in *Oreochromis mossambicus*, which were reared at different environmental conditions.

The activity of transaminases (GOT and GPT) and lipase in the muscle of *Oreochromis mossambicus* when reared on diets supplemented with different levels of calcium were presented in Table IV. Generally, the activity of GOT is less when compared to the activity of GPT in all fish groups received different diets. In *Oreochromis mossambicus* which was fed on control diet (1.097% calcium) the activity of GOT was 1.21μ moles pyruvate/ mg protein /hr and of GPT was 1.52 μ moles pyruvate/mg protein/hr. This enzyme level fluctuated without showing any regular trend according to the level of calcium present in the diets. But the fluctuation was according to the level of protein content of fish. Similar observation has been made in different species (Sree Ramulu Chetty *et al*.,1980; Goel *et al.*, 1985). This appears that the transaminases show only normal activity in well growing fishes. The activity of transaminases increases, when the protein content decreases. This statement is also confirmed in this present study. The same trend was observed in the level of fat and the activity of lipase. The lipase activity in the fish with 28.6% lipid receiving control diet was 1.34-units/ g live tissue. This level decreased to 0.28 units/ g live tissue as the fat content increased to 23.6% (Table 4). Only due to increase in the activity of lipase, the fat content decreases. From the whole body mineral retention studies in *Oreochromis mossambicus* which was 0% in the fish receiving control diet with 1.097% calcium increased up to 4.297% when the dietary calcium level increased to 4.30% (Table 4).

As per the statistical analysis (One-way Anova and Student Newman – Keul's test), the dietary calcium levels have the significant effects on calcium retention in *Oreochromis mossambicus*. But the variation in whole body phosphorus and magnisium was not consistent with the level of calcium (Table 4). Similar observations have been made on whole body calcium content of fishes, fed on graded levels of calcium. Ogino *et al.*, (1976) have also reported that the whole body calcium content of rainbow trout increased from 0.084 to 0.146%, when it was fed on diets supplemented with increasing levels of calcium from 0.046% to 0.779%, while other minerals did not show drastic changes. Robinson *et al.*, (1984, 1986) have found that the dietary calcium levels of *Tilapia aurea* and *Ictalurus punctatus* did not affect magnesium and phosphorus concentration. In the present observation, it is derived that the mineral, which was given as supplementation with diet, increased in the body according to the levels of supplementation, while other minerals did not show any consistent changes. The body mineral content is more than the dietary level of the same, and this may be for normal health and mineralisation (Ogino and Chiou, 1976; Robinson *et al.* 1986).

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