International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 5 (2013) pp. 8-17**
http://www.ijcmas.com

Original Research Article

Dietary magnesium requirement of *Mystus vittatus*

C. Kandeepan*

Fish Nutrition Laboratory, PG and Research Department of Zoology, A.P.A College of Arts and Culture, Palani - 624 602, Tamil Nadu. India

**Corresponding author e-mail:* kansbiotech@gmail.com

Keywords

Mystus vittatus; Magnesium; Enzymes; Dietary requirement

A B S T R A C T

The requirement of *Mystus vittatus* for dietary magnesium was investigated by feeding them with a basal diet containing ($MgCo_3$: 0.1 to 1.0 /100g) different levels of magnesium, 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 magnesium. The basal diet of 50% contained waste silkworm pupae used as the main source of protein with fishmeal and oilcake. The highest growth rate (14.101 mg/g live fish /day) occurred in the fish fed on a diet supplemented with 0.079% magnesium. 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 magnesium levels. Judging from the growth rate of the fish, adequate magnesium content of the diet of *Mystus vittatus* was estimated to be 0.079%.

Introduction

In most of the countries where hatchery and culture practices are better-established supplementary feeding with the feedstuffs formulated into pellets form the backbone of the intensive fish culture. 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 (Jeyachandran and Paul Raj, 1976; Nandeesha *et al.*,1990; Kandeepan and Arunachalam, 2003). Hence, it is felt that the silkworm pupae may be utilized to replace the fishmeal in formulating fish diet. Jeyachandran and Paul Raj (1977) have stated that the silkworm pupae as raw materials induced the growth of carps when used instead of fishmeal in the 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. Magnesium is essential for the activation of a great number of enzymes involved in protein and carbohydrate metabolism (Lall, 1979) and for the maintenance of intra and extra cellular homeostasis in fish (Houston, 1985).

Feeding efficiency ratio and dietary protein utilization were significantly influenced by dietary requirements of magnesium for *Cyprinus carpio*, *Salmo gairdneri* and *Ictalurus punctatus* range from 0.04 to 0.08 of dry weight (Dabrowska *et al.,* 1989a; Ogino and Chiou 1976; Ogino *et al.,*1978; Gatlin *et al.,* 1982). Fishmeal, the major source of protein in formulated diet contains 0.04 to 0.07% magnesium. This satisfies the level of the suggested magnesium requirement for fish. But Satoh *et al.,* (1983a, b) have found poor growth and feed efficiency together with deficiency symptoms in fish, which was fed on diet without magnesium supplementation. Lall (1979) has stated that the uptake of magnesium from the environment is insufficient to satisfy the metabolic demand of freshwater fishes. The survey of literature on the requirements of magnesium for fishes indicates that the supplementation of magnesium is necessary. Hence, the present study was conducted to determine the dietary magnesium level and their effects on the growth, proximate composition, enzymes and whole body mineral content of *Mystus vittatus*.

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 magnesium 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 *Mystus vittatus* collected from local ponds near Palani, TamilNadu, 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 ($28 \pm 1^{\circ}C$). The acclimation period lasted for 15 days. Individuals showing signs of disease or injury were removed during the acclimation period. Stockfishes 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 & 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 *M.vittatus* range of 1.17 to 2.15 g were selected from the stock. Six different groups (Table 2) (M-M1, M-M2, M-M3, M-M4, M-M5, M-M6 and M-M7), 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 magnesium supplementation such as M1, M2, M3, M4, M5, M6 and M7 (Table II). 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 magnesium, 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 and 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 magnesium 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

Mystus vittatus grows well on the diet of M2.The results revealed that the fish exhibited maximum growth i.e., 14.101 mg live weight/ g live fish/ day when fed on diet M2. Then there was a gradual decrease in the growth rate of fish as a function of increase in the magnesium content of diet and reached the level of 10.461 mg live weight/ g live fish/ day when the fish was fed on diet with 0.337% magnesium. (Table. 3). To be more appropriate, the growth rate of *M. vittatus* when reared on different diets supplemented with different levels of magnesium is expressed in terms of energy value in Table 3. In terms of energy also *M. vittatus* exhibited the same trend of growth as observed in terms of live weight. The fish group, which exhibited maximum growth in terms of energy value, found as M-M2 i.e., the fish group which received 0.079% magnesium when compared to all other fish groups, which received diets with higher levels of magnesium supplementation (Table.3).

The growth rate of this group was 72.408 Joules/ g live fish/day. This level was gradually decreased to 52.038 J/ g live fish/day in the fish group (M-M7), which received the diet with 0.0.337% magnesium. 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 M-M2, which gave maximum growth, might have contained the sufficient amount of magnesium.

Hence, it is concluded that the diets of that groups with 0.079 mg were considered as the optimum requirement for *M. vittatus*. Hence, this diet was considered as optimum one. The optimum requirement of these fishes is compared to that of other fishes studied. The optimum magnesium requirement for *O.niloticus* ranges from 0.059 to 0.077% for better growth and feed conversion (Dabrowiske *et al.*, 1989b).

Further, they have stated that growth increased with increasing magnesium concentration up to 0.1% in the diet. For *O.aureus*, the magnesium requirement ranges from 0.05 to 0.065% for better growth rate (Reigh *et al.*, 1991). When compared to these values, the optimum dietary requirement of magnesium for *M. vittatus* is greater. This may be due to the fact as suggested by Dabrowska *et al.* (1989b). In our present investigation, the protein content of diet is around 40%. Hence, the magnesium requirement of *M. vittatus* through diet is greater. The growth of *M. vittatus* at optimum level of magnesium has been verified by means of field trials. The growth rate of *M. vittatus* is less (8.826 mg/g live fish/day) in the field when compared to the results obtained (14.101 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 *M. vittatus*, which were reared on diets supplemented with different levels of magnesium, was presented in Table 4 and Fig.1.The fish group M-M2, which were

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

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

Diets with Magnesium supplementation	Feed mixture (g)	Magnesium source supplemented ($MgCo_3$) (g)	Total Magnesium Content (%)
M1 Control	100	0.0	0.051
M2	100	0.1	0.079
M3	100	0.2	0.109
M4	100	0.4	0.165
M5	100	0.6	0.223
M6	100	0.8	0.279
M7	100	1.0	0.337

Figure.1 Effect of magnesium supplementation to diet on body composition of *M. vittatus.*

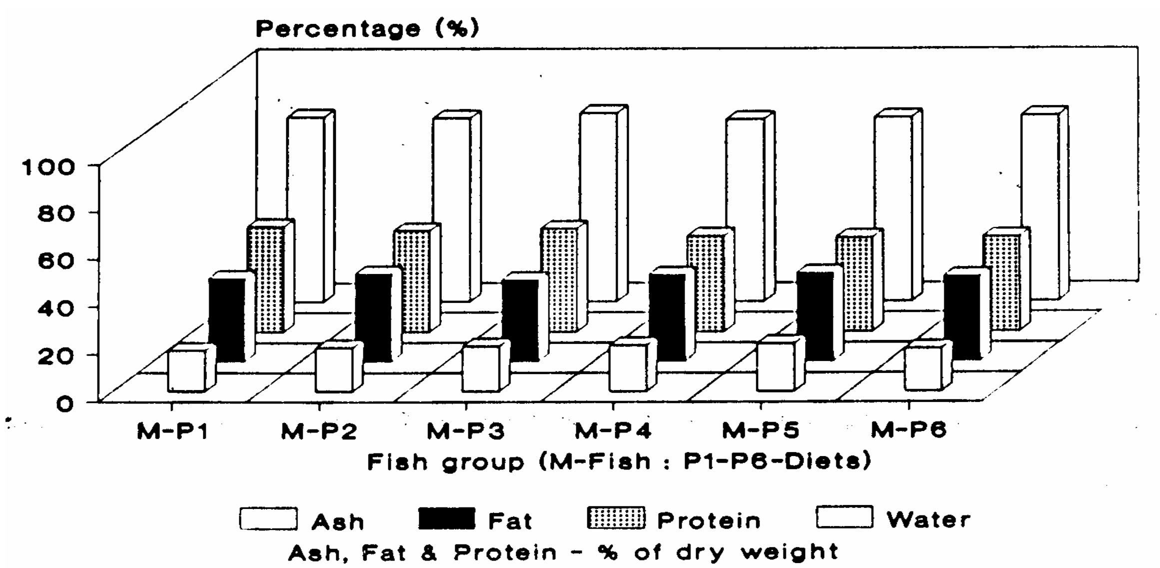

Table.3 Effects of different levels of magnesium supplementation in diets on growth (live weight gained and in terms of energy gained) of *M. vittatus* (1.180 ± 0.150 g). Each value is the average (± SD) performance of 3 groups, consist of 10 individuals.

Diets	Magnesium (%)	Total growth (g)	**Growth rate** (mg/ g live fish /day)	Total energy gained (KJ)	Rate of energy gained (J/G live fish/day)
M-M1 ©	0.051	7.36 ± 0.25	10.226 ±1.14	36.246 ± 0.66	51.744 ± 3.44[a]
M-M2	0.079	10.13±0.85	14.101 ± 1.75	50.113 ± 0.82	72.408 ± 1.85[b]
M-M3	0.109	8.16 ± 0.16	11.768 ±1.37	40.248± 0.75	61.032 ± 4.52[a]
M-M4	0.165	7.82 ± 0.17	10.492 ± 0.29	46.545 ± 1.13	60.283 ± 5.06[a]
M-M5	0.223	7.33± 1.17	10.979 ± 2.39	38.364± 0.71	59.022 ± 2.99[a]
M-M6	0.279	8.09 ± 0.50	10.884± 0.36	40.012 ± 0.99	56.028 ± 2.91[a]
M-M7	0.337	7.28± 0.39	10.461 ± 1.41	36.011± 0.83	52.038 ± 2.56[a]

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

Table.4 Effects of dietary magnesium on body composition, enzymes and whole body mineral content of *M.vittatus*

Fish group	DM (%)	Protein (%)	Transaminases (μ moles Pyruvate/ mg protein/hr)		Fat (%)	Lipase (units/ g live tissue)	Whole body mineral content (%)		
			GOT	GPT			P	Ca	Mg
M-M1 ©	0.051	55.60±1.25	1.52±0.12	1.88±0.09	24.6	1.08±0.84	1.985[ab]	3.142[bc]	0.119[a]
M-M2	0.079	58.25±1.52	1.32±0.08	1.65±0.07	20.6	1.22±0.75	2.079[b]	3.119[bc]	0.126[b]
M-M3	0.109	56.95±1.75	1.48±0.05	1.69±0.06	21.5	1.13±0.02	1.988[ab]	3.260[c]	0.131[bc]
M-M4	0.165	54.40±1.05	1.54±0.06	1.82±0.05	23.2	0.97±0.01	2.015[ab]	3.014[ab]	0.138[cd]
M-M5	0.223	54.75±1.50	1.54±0.07	1.82±0.04	21.4	1.13±0.02	1.913[a]	2.962[a]	0.140[d]
M-M6	0.279	53.60±1.68	1.62±0.02	1.85±0.05	22.8	0.98±0.02	2.105[ab]	3.053[ab]	0.143[d]
M-M7	0.337	52.85±1.15	1.69±0.03	1.89±0.02	23.6	0.92±0.01	2.017[b]	3.251[d]	0.145[d]

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

fed on diet with 0.079% magnesium, exhibited protein content 58.25%. This level was more when compared to other fish groups, which received diets with higher levels of magnesium 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 = 111.626 – 1.127X.

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 magnesium supplementation, contained more protein. Viola *et al.*, (1986a) have reported that the protein 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 & 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 conformed by the present observation on the water content and fat content of fishes, which are reared on diets containing different levels of magnesium. The ash content did not vary significantly (Figure.1). The ash content does not show any significant variation as observed by Arunachalam (1986) in *M.vittatus*, which were reared at different environmental conditions.

The activity of transaminases (GOT and GPT) and lipase in the muscle of *M. vittatus* when reared on diets supplemented with different levels of magnesium were presented in Table 4. Generally, the activity of GOT is less when compared to the activity of GPT in all fish groups received different diets. In *M. vittatus* which was fed on control diet (0.051% magnesium) the activity of GOT was 1.52μ moles pyruvate/ mg protein /hr and of GPT was 1.88 μ moles pyruvate/mg protein/hr. This enzyme level fluctuated without showing any regular trend according to the level of magnesium 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 24.6% lipid receiving control diet was 1.08-units/ g live tissue. This level decreased to 0.92 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 *M. vittatus* which was 0.119% in the fish receiving control diet with 0.051% magnesium increased up to 0.145% when the dietary magnesium level increased to 0.337% (Table.4). As per the statistical analysis (One-way Anova and Student Newman – Keul's test), the dietary magnesium levels have the significant effects on magnesium retention in *M. vittatus*. But the variation in whole body phosphorus and calcium was not consistent with the level of magnesium (Table.4). Similar observations have been made on whole body magnesium content of fishes, fed on graded levels of magnesium. Ogino and Chiou (1976), who have studied the effect of dietary magnesium level on whole body content of magnesium content increased with dietary magnesium levels and that the other minerals like calcium and phosphorus did not show any consistent changes in relation to dietary magnesium levels. Ogino *et al.,* (1978) have also reported that the whole body magnesium content of rainbow trout increased from 0.084 to 0.146%, when it was fed on diets supplemented with increasing levels of magnesium from 0.046% to 0.779%, while other minerals did not show drastic changes. Dabrowska *et al.,* (1989b) have found that the dietary magnesium levels did not affect calcium 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).

References

Arunachalam, S., 1986. Physiological studies on Freshwater catfish. Ph.D.Thesis. Madurai Kamaraj University, Madurai, India.pp. 148.

Association of Official Analytical Chemists (AOAC)., 1975. Official Methods of Analysis Washington. DC.USA. pp.1094.

Dabrowska, H., K.D. Gunther and Meyer – Burgdorff, K. 1989a. Availability of various magnesium compounds to tilapia (*Oreochromis niloticus*). Aquacul. 76: 269 – 276.

Dabrowska, H., K. Meyer – Burgdorff and Gunther, K. D. 1989b. Interaction between dietary protein and magnesium level in tilapia (*Oreochromis mossambicus*). Aquacul. 76: 269 – 276.

Gatlin, D. M., E.H. Robinson, W.E. Poe, and Wilson, R. P.1982. Magnesium requirement of fingerling channel catfish and signs of magnesium deficiency. J. Nutri. 112:1182 – 1187.

Goel, K. A., S.K. Tyagi, and Awasti, A. K. 1985. Enzymological probes in liver, kidney and muscles of *Heteropnestes fossilis* under phenylene Brown Chemicoazo stress. Indian. J. Fish. 32 (4): 488 – 491.

Jana, B., 1980.Calorific values as functions of mainbody constituents in some freshwater teleosts. Indian. J. Fish. 27: 269 – 272.

Jeyachandran, P., and Paul Raj, S. 1976. Experiments with artificial feeds on *Cyprinus carpio* fingerlings J. Inland. fish. Society. India. 8:33 – 37.

Jeyachandran, P., and Paul Raj, S. 1977. Formulation of pelleted feeds and feeding trials with common carp. J. Inland. fish. Society. India. 9:46-52.

Jeyaraman, J. 1981. Laboratory manual in biochemistry. Wiley Eastern Ltd. p.77-78.

Fisher, Z. 1979.Selected problems of fish bioenergetics. PP.17 – 44. In: Proceedings of World Symposium on Finfish Nutrition and Fishfeed Technology, Vol 1 Eds: J.E.Halver and K.Tiews H.Heenemann GMBH and Co, Berlin, Germany.

Fiske,C.H. and Subbarow,Y. 1925. The colorimetric determination of calcium.Journal of Biological Chemistry, **66**: 375 – 400.

Houston, A. H., 1985.Erthrocyte magnesium in freshwater fishes. Magnesium. 4: 106 - 128.

Kandeepan, C., and Arunachalam, S. 2003. Phosphorus supplementation to fish diet containing silk worm pupae as a protein source. Proceedings of International Seminar on Recent Advances In Biotechnology, 24th and 25th Feb.2003.P.R.College, Tanjore, India pp.48 – 55.

Kandeepan, C., and Arunachalam, S. 2005. Dietary phosphorus requirement of *Oreochromis mossambicus*. J. Ecobiol. 17(6):571 – 579.

Kandeepan, C., and Poongulali, C. 2009. Dietary magnesium requirement of the *Oreochromis mossambicus*. J. Natural. Sci.Technol.Life Sci. Bioinfor. 1: 67-72.

Lall, S. P., 1979. Minerals in finfish nutrition. p.85-97. In Proceedings of World Symposium on Finfish Nutrition and Fish feed Technology, Eds:J.E.Halver and K. Tiews, Vol I, H. Heenemann GMbH and Co, Berlin, Germany.

Muthukumar, P., and Kandeepan, C. 2009. Effects of diets containing different proportions of chicken intestine waste on growth of *Mystus cavicius*. J. Natural. Sci.Technol.Life Sci. Bioinfor. 1:78–80.

Nandeesha, M. C., G.K. Srikanth, P. Kesavanath, J. Vargesh and Shetty, H. P. C. 1990. Growth performance of *Cyprinus carpio* var *communis* fed on diets containing different levels of deoiled silkworm pupae. In: Proc. Second Asian Fiseries Forum, Eds.R.Hirano and I.Hanyu. Asian Fish Soc.Manila, and the Philippines. pp 271-274.

Ogino, C., and Chiou, J. Y. 1976. Mineral requirements in fish II. Magnesium requirement of carp. Bull. Japanese. Soci. Sci. Fish. 42: 71-75.

Ogino, C., F. Takashima and Chiou, J. Y. 1978. Requirement of Rainbow trout for dietary magnesium. Bull. Japanese. Soci. Sci. Fish. 42: 793 – 799.

Ogino,C., and Takeda, 1976. Mineral requirements in fish III.Calcium and Calcium requirements in carp. Bull. Japanese. Soci. Sci. Fish. 42: 793- 799.

Oser, B. L., 1965 Hawk's Physiological Chemistry 14th ed. Tata McGraw Hill Publishing Co., Ltd.Bombay.NewDelhi. pp.1-1472.

Poongulali, C., and Kandeepan, C.2008. Dietary calcium requirement of the catfish *Mystus vittatus*. J. Ecobiol. 23(2):187 - 195.

Reigh, R. C., E.H. Robinson and Brown, P. B. 1991. Effects of dietary magnesium on growth and tissue content of blue tilapia *Oreochromis aureus.* J. World. Aquacul.Society. 2(3):192-199.

Satoh, S., H. Yamamoto, T. Takeuchi and Watanabe, T.1983a. Effects on growth and mineral composition of rainbow trout of deletion of trace elements or magnesium from fish meal diet. Bull. Japanese. Soci. Sci. Fish. 49:425 – 429.

Satoh, S., H. Yamamoto, T. Takeuchi and Watanabe, T. 1983b. Effects on growth and mineral composition of carp of deletion of trace elements or magnesium from fish meal diet. Bull. Japanese. Soci. Sci. Fish. 49:431 – 435.

Sree Ramulu Chetty, C., R. Chandramohan Naidu, Y. Srinivasa Reddy, P. Aruna and Swami, K.S. 1980. Tolerance limits and detoxification mechanisms in the fish Tilapia mossambica subjected to ammonia toxicity. Indian. J. Fish. 27 (1-2): 177-182.

Tacon, A.G. J., and De Silva, S.S. 1983. Mineral composition Yn of some commercial fish feeds available in Europe. Aquacul. 31: 11-20.

Tacon, A.G. J., D. Knox, and Cowey, C. B. 1984. Effects of different dietary levels of salt mixtures on growth and body composition in carp. . Bull. Japanese. Soci. Sci. Fish. 50: 1217-1222.

Takeuchi, M., and Nakazoe, J. 1981. Effects of dietary calcium on lipid content and its composition in carp. . Bull. Japanese. Soci. Sci. Fish. 47:347-652.

Venkatesh, B., A.P. Mukerji, P.K. Mukhopadhyay and Dehadraai, P.V. 1986. Growth and metabolism of the catfish Clarias batrachus (Linn.) fed with different experimental diets. Proc.Indian Aca. Sci.95 (4): 457 – 462.

Viola, S., G. Zohar and Arieli, Y. 1986a. Phosphorus requirement and its availability from different sources for intensive pond culture in Israel part 1. Tilapia. Bamidgeh 38 (1): 3 – 12.

Wooten, I. D. P., 1964. In: Microanalysis in medical biochemistry. J and A Churchill Ltd., London W.1.