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STUDY ON NIACIN AND PYRIDOXINE REQUIREMENTS OF NILE TILAPIA FISH

(With 7 Tables and 2 Figures)

By
H. A. ABDEL-RAHEEM

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دراسة علي احتياجات اسماك البلطي النيلي من النياسين والبيرودوكسين

حسن عباس عبدالرحيم

في هذه الدراسة تم إجراء تجربتين لتقدير مستويات كل من فيتامين النياسين والبيرودوكسين
التي تحتاجها اسماك البلطي النيلي للحصول علي اعلي معدلات نمو ولتفادي ظهور أي
أعراض مرضية علي هذه الأسماك. أستغرقت كل تجربة ١٠ أسابيع وفيها تم تغذية خمس
مجموعات من أسماك البلطي علي خمس علائق تحتوي علي صفر, ٦, ١٢, ٢٤, ٤٨ مجم
نياسين/كجم عليقة في التجربة الأولى و غذيت أربعة مجموعات أخرى من الأسماك علي
علائق تحتوي علي صفر, ٣, ٦, ١٢ بيرودوكسين هيدروكلوريد/كجم عليقة في التجربة
الثانية و كان متوسط أوزان الأسماك ٥ جم . وقد وجد أن : مجموعة الأسماك التي غذيت
علي العليقة الخالية من النياسين ظهرت عليها أعراض النقص المرضية مثل فقدان الشهية
وفقد في وزن الجسم وعدم الاتزان مع ظهور تقرحات علي الجلد والزعانف مع نسبة نفوق
عالية.، عدم وجود أي فروق معنوية في الزيادة في وزن الجسم ومعدل التحويل الغذائي
وكفاءة استخدام البروتين بين مجموعات الأسماك التي غذيت علي علائق تحتوي علي ١٢
مجم نياسين/كجم عليقة اواكثر وحظيت المجموعة التي غذيت علي ١٢ مجم نياسين/كجم
عليقة علي اعلي معدلات نمو وتحويل غذائي.، عدم وجود أي فروق معنوية في معدلات
النمو والتحويل الغذائي في مجموعات الأسماك التي غذيت علي علائق تحتوي علي ٦ مجم
بيرودوكسين هيدروكلوريد/كجم عليقة اواكثر مع ظهور أعراض النقص الغذائي علي
مجموعة الأسماك التي غذيت علي العليقة الخالية من البيرودوكسين هيدروكلوريد مثل فقدان
الشهية وبعض الأعراض العصبية بالإضافة إلى ضعف النمو.، نستخلص من هذه الدراسة
إلى أن النياسين والبيرودوكسين ضروريان للنمو وكفاءة التحويل الغذائي وحيوية الاسماك
بالاضافة الي زيادة كفائة ومعدل تخزين البروتين في جسم الاسماك. لذلك فان اسماك البلطي
تحتاج الي حوالي ١٢ مجم نياسين و ٦ مجم بيرودوكسين هيدروكلوريد للحصول علي
اعلي اداء ونمو.

SUMMARY

In this study two experiments were conducted to determine the levels of niacin and pyridoxine which required for tilapia fish for maximum growth and prevention of deficiency signs. Fingerlings tilapia (average weight 5 gm) were fed diets supplemented with 0, 6, 12, 24 & 48 mg niacin/kg diet (Exp. I) and 0, 3, 6 & 12 mg pyridoxine HCl /kg diet (Exp.II) for 10 weeks each. Fish fed the niacin unsupplemented diet exhibited anorexia, loss of gain, incoordination, skin and fin lesions and high mortality rate. No significant differences (P>0.05) in weight gains, feed efficiency and protein utilization were observed in the fish groups fed on the diets containing 12 mg niacin/ kg diet or more; however, growth rate and feed efficiency recorded maximum values at the level of 12 mg niacin/kg diet. There were no significant differences (P>0.05) in the growth parameters, survival and feed utilization in the fish groups fed on the diets containing 6 mg pyridoxine HCl or more. Fish fed on the diets devoid of pyridoxine HCl had anorexia, nervous manifestations, poor growth, ataxia and rapid breathing. It could be concluded that, niacin and pyridoxine are essential for growth, efficiency of feed utilization and survival of fish and increase protein retention and efficiency ratio. Tilapia fish need about 12 mg niacin/kg diet and 6 mg pyridoxine HCl/kg diet for optimum performance and increasing the level of supplementation does not have any significant benefit.

Key Words:- *Niacin, Pyridoxine. Requirements, Tilapia*

INTRODUCTION

Natural feed is usually rich in vitamins, while this may not be the case with the formulated one. Vitamin deficiency appears, therefore mainly in intensive culture systems, stocked at high density of fish, where supplementary feed is the major, if not the only, source of feed. Vitamin requirements of tilapia have not been widely studied. The only available information on vitamin requirements, allowances established for other warm-water fish are used. Niacin functions as a component of the two coenzymes (NAD, NADP) which are essential for several oxidation-reductions processes in metabolism. They are also involved in various energy yielding and biosynthetic pathways. The conversion of tryptophan to niacin is limited in fish and niacin must be fed to prevent deficiency and improve growth. Channel catfish and common carp showed skin and fin lesions, skin hemorrhages, anemia and high mortality when fed niacin deficient diets and poor growth rates were found in tilapia fed a niacin-deficient diet (Keshamaru, 1981). In short, common symptoms of niacin deficiency in most fishes studied are muscular weakness and spasms coupled with poor growth and poor feed conversion. All deficiency symptoms could be prevented and maximum rates of growth obtained, if niacin was supplemented at the rate of 28mg/kg diet for carp (Aoe *et al.*, 1967), 14 & 9 mg/Kg diet for channel catfish (Andrews & Murai, 1978 and Wingkeong *et al.*, 1997) and 10 mg for rainbow trout (Poston & Wolfe, 1985). Mortality and gross deficiency symptoms were prevented by 11.6 mg niacin/kg diet. They also added that 6.6 mg/kg diet was sufficient to prevent anemia in channel catfish (Andrews & Murai, 1979).

Pyridoxal phosphate is the coenzyme of many aminotransferases involving amino acids such as transamination, decarboxylation and dehydration. Also, it has a function in the biosynthesis of porphyrins and in the catabolism of glycogen. Since pyridoxine requirement is related to protein intake and metabolism, adequate pyridoxine in the diets is essential for good fish health and rapid growth (Halver, 1985). Carp fed diet with an inadequate supply of pyridoxine exhibited nervous disorders, ataxia, loss of appetite, fluid accumulation in the abdominal cavity, exophthalmia, and retarded growth in youngs and weight loss in adults as found by Ogino (1965). He said that a minimum daily requirement of 5.4 mg pyridoxine HCl/kg diet is needed for carp. Channel catfish which received a diet deficient in pyridoxine exhibited anorexia, nervous disorders and ultimately 100% mortality. At 2.2mg/kg diet the deficiency symptoms were prevented but the maximum rate of growth was only attained in response to 3mg pyridoxine/kg diet (Andrews & Murai, 1979).

As the informations about the needed amounts of niacin and pyridoxine required for tilapia are scarce, the present study was undertaken to estimate the niacin and pyridoxine requirements of Nile tilapia.

MATERIALS and METHODS

Two experiments, 10 weeks each were conducted to determine the sufficient amount of niacin (Exp.I) and pyridoxine (Exp.II) for optimum growth and efficient feed conversion in tilapia fish.

1-Fish and experimental design :

The tilapia fish fingerlings (*O.niloticus*) were obtained from the Aquatic Animal Research Unit, Fac.of Vet.Medicine, Assiut Univ., with an average initial weight of about 5g.

Two hundred and forty five fish were used in Exp.I, while two hundred fish in Exp.II. 20 fish were used for the initial body analysis at the start of each experiment and the rest was divided into 15 equal groups in Exp.I and 12 equal groups in Exp.II, each of 15 fish. Each aquarium was aerated and dechlorinated tap water at about 26°C±1°C was used. The dissolved oxygen and pH of water were measured and found to be about 3.8 mg/L and 7.2 respectively.

In addition to the 20 fish composition sample at the start of the experiments, 5 fish were randomly removed from each aquarium at the end for the end body analysis (AOAC, 1984).

Five experimental diets were formulated in exp. I to contain 0, 6, 12, 24 and 48 mg niacin/kg diet and four diets in exp.II to contain 0, 3, 6 & 12 mg pyridoxine HCl/kg diet. The quantity of the vitamin used was substituted for corn starch in the basal diet.

2-Experimental diets :

A basal diet was formulated from purified ingredients to contain 35.69% crude protein and 3448 Kcal digestible energy /kg diet (NRC, 1993). Three specialized vitamin mixtures were manufactured by Hoffman La Roche Co., Germany on request, the first is free of niacin for experiment I and the second free of pyridoxine for experiment II, while the third is an all-vitamin premix containing all the vitamins including the two tested ones (Table, 1).

Prior to the initiation of the experiment, the fish were fed the basal diet to which a complete premix was added for 2 weeks as a conditioning period.

Each of the dietary treatments in each experiment was applied to randomly assigned three groups of fish. Each of the experimental diets were fed at satiation allover the period which continued for 10 weeks in each experiment.

In preparing the diets, ingredients were thoroughly mixed, pelleted and dried at room temperature. All diets were stored at -20°C until immediately prior to feeding.

3-Parameters evaluated:

Weight gain, feed conversion, specific growth rate, condition factor, protein efficiency ratio and net protein utilization were calculated.

4-Statistical analysis:

Statistical analysis of the data was performed using analysis of variance and Duncan's multiple range test (Duncan, 1955).

RESULTS and DISCUSSION

The fish of all the groups were firstly fed for two weeks on vitamin-supplemented basal diet in order to replete any depleted vitamin if any and to start with fish of comparable condition. The results of experiment I are illustrated in Tables 2,3, 4 & Fig.1, while that of experiment II in Tables 5, 6, 7 & Fig.2.

Experiment I:

1-Fish weight:

The five groups started the experiment by a weight ranging from 5.20 to 5.33g and attained an end weight in the supplemented groups of 24.85g in group II to 30.59g in group III, and with a difference of 5.74g and an average of 28.56g. In the unsupplemented group, the final weight reached only 15.20g with only 53.22% of the other group's average. So while the weight gain was nearly doubled in group I, it was nearly five times the initial in the most optimal level of supplementation in group III (488.3%).

The relation of body weight with length was expressed as "condition factor" and found to be 2.91 in group I while it ranged from 2.73 to 2.80 in the other supplemented groups.

The retarded growth in group I was due to the decreased feed intake which was recorded to be 20.94g compared with 36.04g in group II to the highest intake of 43.16g in group III. The food consumed was utilized with different efficiencies reaching an index of 2.10 in group I and improved to be 1.85, 1.70, 1.73 and 1.72 in the other 4 groups.

It is not only the body weight gain and efficiency of food utilization to be considered but also the survival of the fish which showed a mortality of about 37% in the first group and a survival of 100% due to niacin supplementations. In addition, the fish fed the unsupplemented diet had anorexia, loss of body weight, skin and fin lesion, lethargy, reduced coordination, exophthalmia and high mortality rate. The same observations have been reported for rainbow trout (Halver, 1957), common carp (Aoe *et al.*, 1967) and channel catfish (Andrews & Murai, 1978; Wingkeong *et al.*, 1997). Data from this experiment indicated that 6 mg niacin/kg diet was sufficient for prevention of gross deficiency symptoms and mortality in tilapia fish, but not for optimal performance.

2-Protein parameters and body composition:

The fish at the start of the experiment had 24.10% DM and increased from 25.45 to 26.54% in the different five groups with the lowest where niacin is lacking and the highest at maximum level used (48mg/kg diet). As to the other body components protein decreased from 71.25 to about 65.64% on the average while fat and ash increased from 11.31 to an average of 15.3% and from 15.63 to an average of 17.34% respectively and with no significant differences between groups. On contrary, Poston (1969) reported that high dietary intake of niacin increased liver fat, decreased body fat and tended to reduce growth rate in brook trout.

From the body composition and feed intake data protein intake and efficiency of utilization could be calculated. In protein parameters group I was the worst followed by group II while the last 3 groups did not differ significantly and got the highest values. The protein intake followed the level of food intake and reached 7.47g in group I, 12.86g in group II and an average of 15.02g for the last 3 groups with statistically significant differences among the three intakes.

Using the data of body composition, the protein retention was calculated and found to be 1.71g in group I and nearly doubled 3.36g in group II, while it reached an average of 4.28g in the last 3 groups. The efficiency of protein utilization was measured by the number of grams of body weight gained for every gram of protein fed which reached only 1.33 & 1.51 in groups I & II respectively compared with an average of 1.63 in the last 3 groups. And as a ratio for the protein retained and the protein fed expressed in percentage the values were 22.89, 26.13 and 28.44% for the groups I, II & last 3 groups respectively.

Niacin supplementation is essential for growth, efficiency of feed utilization and survival of fish. It increases protein retention and efficiency ratio. Tilapia fish need, as far as it is concluded, about 12 mg niacin/kg diet for optimum performance and increasing the level of supplementation does not have any significant benefit. This value is higher than 10 mg/kg diet for rainbow trout (Poston & Wolfe, 1985) and 9 mg/kg diet for channel catfish (Wingkeong *et al.*, 1997) and lower than 28 mg/kg for carp (Aoe et al, 1967) and 14 mg /kg for channel catfish (Andrews & Murai, 1978).

While tryptophan is an in vivo precursor of niacin, brook trout and channel catfish cannot efficiently convert tryptophan to niacin. Results from this experiment suggests that the same conversion inefficiency may exist in tilapia.

Experiment II:

1-Fish weight:

In this experiment 3 levels of pyridoxine supplementation were tested. Statistically the 6 & 12 mg/kg diet supplementation did not differ in weight or feed intake effect. The fish increased by 278.6% in the unsupplemented first group and increased to 447.5% in group II and 497.4% as an average for the last two groups. Feed intake was 28.01, 39.17 and 43.41g for group I, II and an average of last two groups respectively and used with a conversion indices of 1.89, 1.69 & 1.64. The condition factors were 2.97, 2.79 & an average of 2.73 for the groups I, II and last two respectively.

While the survival in the supplemented groups was 100% it decreased to 75.32% when the basal diet was not supplemented. Fish fed the unsupplemented pyridoxine HCl had anorexia, ataxia, hyperirritability, edema of the peritoneal cavity and rapid breathing. The same signs have been reported for other species of fish (Ogino, 1965; Halver, 1972; Smith *et al.*, 1974; Andrews & Murai, 1979; Herman, 1985). Fish fed the diet containing 3 mg pyridoxine or more showed no deficiency signs and mortality rate was nill.

2-Protein parameters and body composition:

The fish at the start of experiment had 24.10% DM and increased from an average of 25.12% in groups I & II to an average of 26.80% in the last two groups with the lowest when pyridoxine is lacking and the highest at 6mg pyridoxine HCl/kg diet. As to the other body components protein decreased from 71.25% to about an average of 61.71% in groups I & II and an average of 67.03% in the last two groups, while fat increased from 11.31 to 13.40% in group I and 14.7% on the average in the last three groups. In addition, ash was decreased from 15.63 to 11.88% in group I and increased to 15.79% in group II and 18.10% on the average in the last 2 groups with statistically significant differences between groups.

In protein parameters, group I was the worst followed by group II, while the last two groups did not differ and got the highest values. The protein intake followed the level of feed intake and reached 10g in group I, 13.98g in group II and an average of 15.53g for the last two groups with statistically significant differences among groups. Using the data of body composition, the protein retention was found to be 2.18g in group I and nearly doubled 3.64g in group II, while it reached an average of 4.86g in the last two groups. The efficiency of protein utilization reached only 1.48 & 1.72 in groups I & III respectively compared with an average of 1.68 in groups II & IV. The ratio for the protein retained and the protein fed expressed in percentage the values were 21.80, 26.04, 32.47 & 30.09% for the groups I, II, III & IV respectively.

Pyridoxine supplementation is essential for growth, efficiency of feed utilization and survival of fish. It increases protein retention and efficiency ratio. Tilapia fish need, as far as it is concluded, about 6 mg pyridoxine HCl/kg diet for optimum performance and increasing level of supplementation does not have any significant benefit. This level in the range (5-6 mg) with that reported for carp (Ogino, 1965), higher than 3 mg for channel catfish (Andrew & Murai, 1979) and much lower than that required by several species of fish (Halver, 1972; Jurss *et al.*, 1988).

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Table 1: Composition of the basal diet.

Ingredients	%
a-**Physical composition**:-	
Casein (vitamin free)[1]	30
Gelatin	12
Corn starch	25
Dextrin	21
Corn oil	5
Mineral mixture*	4
Vitamin mixture**	3
b-**Chemical composition**:-	
Protein (%)	35.69
Digestible energy (Kcal/kg diet)	3448
Calcium (%)	0.62
Phosphorus (%)	0.55

[1] Sigma Chemical Co.

*Min.mix. (Merck Co.) supplies the following minerals in g/100g :-$MgSO_4$, 12.75; $CaHPO_4$, 72.79; $ZnSO_4$, 0.55; $MnSO_4$, 0.25; Ca I_2O_6, 0.02; KCl, 5.0; $FeSO_4$, 2.5; $CuSO_4$, 0.08; $CoSO_4$, 0.05; NaCL, 6.0; $CrCl_3.6H_2O$, 0.01.

**Vit.mix., Hoffman La Roche Co. (as g/100g premix):- ascorbic acid, 4.167; inositol, 0.5; choline chloride, 7.5; niacin, 0.45; Ca *d*-pantothenate, 0.41 ; riboflavin, 0.3 ; pyridoxine HCl, 0.10 ; thiamine mononitrate, 0.10; retinyl acetate, 0.06; cholecalciferol, 0.083; menadione, 0.167; DL-α tocopheryl acetate, 0.80; biotine, 0.02; folic acid, 0.09; B_{12}, 0.0013; starch (85.702 in Exp.I & 85.352 in Exp.II).

Table 2: Growth performance and feed utilization efficiency in tilapia fish fed on experimental diets with different levels of niacin (Exp.I)

Parameter	Group/Niacin (mg/Kg diet)				
	I 0	II 6	III 12	IV 24	V 48
Initial weight (g)	5.23±1.02	5.37±1.05	5.20±1.03	5.25±1.05	5.33±1.08
Final weight (g)	15.20±1.10	24.85±1.10	30.59±1.15	29.63±1.30	29.15±1.25
Weight gain (g)	9.79±1.05[c*]	19.48±1.10[b]	25.39±1.20[a]	24.38±1.15[a]	23.82±1.10[a]
Weight gain (%)	190.6	362.8	488.3	464.4	446.9
Feed intake (g)	20.94±1.20[c]	36.04±1.20[b]	43.16±1.30[a]	42.18±1.25[a]	40.97±1.25[a]
Feed conversion index	2.10	1.85	1.70	1.73	1.72
Body length (cm)	8.05	9.61	10.36	10.28	10.16
Condition factor[1]	2.91	2.80	2.75	2.73	2.78
Survival (%)	63.12	100	100	100	100

[2] Condition factor = body weight × 100 / length^3 (Fulton, 1902).

*Figures in the same row having the same superscripts are not significantly different ($P < 0.05$).

Table 3: Whole body composition (% on DMB) of tilapia fish at the start and end of experiment I

Parameter	Group/Niacin (mg/Kg diet)					Blank group
	I 0	II 6	III 12	IV 24	V 48	
Dry matter	25.45±1.10*	25.61±1.15	26.34±1.10	26.50±1.10	26.54±1.20	24.10±1.10
Crude protein	66.31±1.30	66.28±1.35	67.25±1.45	64.27±1.20	64.10±1.35	71.25±1.30
Crude fat	15.21±1.00	15.58±1.05	15.20±1.03	15.35±1.05	15.15±1.05	11.31±1.02
Ash	17.10±1.02	16.45±1.05	16.35±1.02	17.98±1.04	18.82±1.05	15.63±1.03

*N.B. There was a no significant difference ($P>0.05$) between the different treatments in this table.

Table 4: Protein parameters of tilapia fish fed on the experimental diets in Exp.I.

Parameter	Group/Niacin (mg/Kg diet)				
	I 0	II 6	III 12	IV 24	V 48
Protein intake (g)	7.47±1.01[c]	12.86±1.10[b]	15.40±1.15[a]	15.05±1.13[a]	14.62±1.10[a]
Protein retention (g)	1.71±0.02[c]	3.36±0.01[b]	4.56±0.03[a]	4.19±0.01[a]	4.10±0.02[a]
Protein efficiency ratio[1]	1.33±0.01[c]	1.51±0.03[b]	1.65±0.02[a]	1.62±0.01[a]	1.63±0.02[a]
App.net prot.utilization[2]	22.89±1.20[c]	26.13±1.25[b]	29.61±1.30[a]	27.66±1.50[a]	28.04±1.45[a]

[1]Protein efficiency ratio = g gain / g protein fed.

[2]App. net protein utilization = (final total body protein – initial total body protein / total protein fed) × 100

*Figures in the same row having the same superscripts are not significantly different ($P>0.05$).

Table 5: Growth performance and feed utilization efficiency in tilapia fish fed on experimental diets with different levels of pyridoxine HCl (Exp.II)

Parameter	Group/Pyridoxine HCl (mg/Kg diet)			
	I 0	II 3	III 6	IV 12
Initial weight (g)	5.32±1.02	5.18±1.03	5.27±1.05	5.38±1.05
Final weight (g)	20.14±1.10	28.36±1.00	32.54±1.10	31.06±1.20
Weight gain (g)	14.82±1.20c*	23.18±1.15b	27.27±1.10a	25.68±1.10a
Weight gain (%)	278.6	447.5	517.5	477.3
Feed intake (g)	28.01±1.15c	39.17±1.25b	44.45±1.35a	42.37±1.30a
Feed conversion index	1.89	1.69	1.63	1.65
Body length (cm)	8.79	10.05	10.63	10.41
Condition factor	2.97	2.79	2.71	2.75
Survival (%)	75.32	100.0	100.0	100.0

*Figures in the same row having the same superscripts are not significantly different ($P<0.05$).

Table 6: Whole body composition (% on DMB) of tilapia fish at the start and end of experiment II.

Parameters	Group/ Pyridoxine HCL (mg/Kg diet)				Blank group
	I 0	II 3	III 6	IV 12	
Dry matter	25.10±1.05b*	25.13±1.15b	27.10±1.10a	26.50±1.20a	24.10±1.10
Crude protein	60.21±1.10b	63.20±1.15b	68.13±1.25a	65.93±1.35a	71.25±1.30
Crude fat	13.40±1.02 b	14.70±1.00 a	14.30±1.05 a	15.10±1.03 a	11.31±1.02
Ash	11.88±1.03 c	15.79±1.05 b	17.30±1.04 a	18.89±1.05 a	15.63±1.02

*Figures in the same row having the same superscripts are not significantly different ($P<0.05$).

Table 7: Protein parameters of tilapia fish fed on the experimental diets in Exp.II

Parameters	Group/ Pyridoxine HCL (mg/Kg diet)			
	I 0	II 3	III 6	IV 12
Protein intake (g)	10.00±1.05 c	13.98±1.10 b	15.86±1.20 a	15.19±1.10 a
Protein retention (g)	2.18±0.0 c	3.64±0.02 b	5.15±0.07 a	4.57±0.04 a
Protein efficiency ratio	1.48±0.0 c	1.66±0.02 b	1.72±0.03 a	1.69±0.01 b
App.net prot.utilization	21.80±1.15 d	26.04±1.20 c	32.47±1.50 a	30.09±1.30 b

*Figures in the same row having the same superscripts are not significantly different ($P>0.05$).

Fig.1.Weight gain & feed intake of fish groups fe on different levels of niacin

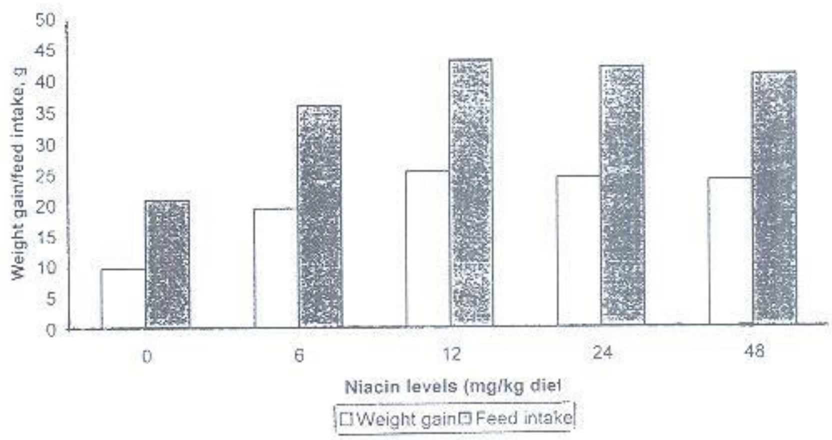

Fig.2.Weight gain & feed intake of fish groups fed on different levels of pyridoxine HCl