Dept.of Animal & Clinical Nutrition,
Fac. of Vet.Med.,Assiut Univ.

NUTRITIONAL CONSIDERATIONS CONCERNING THE REQUIREMENTS OF NILE TILAPIA FOR RIBOFLAVIN AND PANTOTHENIC ACID

(With 7 Tables and 2 Figures)

By
ABDEL-BASET N. SAYED

(Received at 13/2/2002)

الاعتبارات الغذائية لاحتياجات أسماك البلطي النيلي من الريبوفلافين وحمض البانتوثينك

عبدالباسط نصر سيد

في هذه الدراسة تم إجراء تجربتين متتاليتين مدة كل منها ١٠ أسابيع علي أسماك البلطي النيلي وذلك لتقدير المستوي المطلوب من كل من الريبوفلافين وحمض البانتوثينك لاسماك البلطي النيلي للحصول علي أحسن نمو وتحويل غذائي ولتفادي ظهور أي أعراض نقص مرضية علي هذه الأسماك. لذلك تم تغذية خمس مجموعات من الاسماك علي عدد خمس علائق في التجربة الأولى محتوية مستويات صفر, ٥, ١٠, ١٥, ٢٠ مجم ريبوفلافين/كجم عليقة وكذلك تغذية عدد ستة مجموعات من الاسماك علي عدد ستة علائق أخرى في التجربة الثانية محتوية مستويات صفر, ٥, ١٠, ١٥, ٢٠, ٤٠ مجم بانتوثينات الكالسيوم/كجم عليقة. وقد أوضحت النتائج : عدم وجود أي فروق معنوية في الزيادة في وزن الجسم ومعدل استهلاك العليقة بين مجموعات الأسماك التي تمت تغذيتها علي علائق محتوية ١٠ مجم ريبوفلافين/كجم عليقة أوأكثر في حين أن المجموعة التي تمت تغذيتها علي عليقة خالية من الريبوفلافين كان وزنها أقل وظهرت عليها بعض الأعراض المرضية مثل فقدان الشهية وبعض التقرحات علي الزعانف والذيل وتقزم في الجسم مع ظهور عتامة علي العين في بعض الأسماك مقارنة بالمجموعات الأخرى التي غذيت علي العلائق التي تحتوي علي نسب مختلفة من الريبوفلافين والتي لم تظهر عليها أية أعراض مرضية. هـذا وقد حظيت المجموعات *التي* غذيت علي العلائق التي تحتوي علي ١٠ مجم ريبوفلافين/كجم عليقة أو أكثر بأعلى قيم بينما سجلت المجموعة التي تمت تغذيتها علي عليقة خالية من الريبوفلافين أقل قيم في كل من البروتين المخزون ومعدل كفاءة تحويل البروتين مقارنة بالمجموعات الأخرى. أما بالنسبة لحمض البانتوثينك فقد أوضحت النتائج عدم وجود أي فروق معنوية في كل من معدلات النمو واستهلاك العليقة بين مجموعات الأسماك التي غذيت علي العلائق المحتوية علي ١٥ مجم بانتوثينات الكالسيوم أوأكثر من هذا المستوي , بينما سجلت مجموعة الأسماك التي غذيت علي عليقة خالية من بانتوثينات الكالسيوم ضعف النمو والتحويل الغذائي مع ظهور فقدان الشهية ووجود بعض الإفرازات المرضية علي الخياشيم مع ظهور بعض البقع النزفية علي الزعانف والذيل وزيادة نسبة النفوق, كذلك وجدت بعض هذه

الأعراض المرضية بصورة مخففة علي بعض الأسماك التي غذيت علي العليقة التي تحتوي علي ٥ مجم بانتوثينات الكالسيوم بينما لم تسجل أي من هذه الأعراض علي المجموعات التي غذيت علي مستويات أعلي من بانتوثينات الكالسيوم. أما بالنسبة للبروتين المخزون في الجسم وكفاءة تحويله فلا توجد أي فروق معنوية بين مجموعات الاسماك التي تمت تغذيتها علي ١٠ مجم بانتوثينات الكالسيوم أوأكثر بينما سجلت المجموعة التي غذيت علي عليقة خالية من بانتوثينات الكالسيوم أقل قيم في هذه المعدلات.، نستخلص من هذه النتائج أن الريبوفلافين وحمض البانتوثينيك ضروريان للنمو وكفاءة التحويل الغذائي وحيوية الأسماك بالإضافة *إلى* زيادة كفاءة ومعدل تخزين البروتين في جسم الأسماك. لذلك فان اسماك البلطي تحتاج *إلى* حوالي ١٠ مجم ريبوفلافين و ١٣,٨ مجم حمض البانتوثينيك/كجم عليقة (١٥مجم بانتوثينات الكالسيوم) للحصول علي اعلي أداء ونمو وان أي زيادة عن هذه الكميات ليس لها أي تأثير اوفائدة بينما تحتاج *إلى* ٥ مجم ريبوفلافين و ٩,٢ مجم حمض البانتوثينيك /كجم عليقة لمنع ظهور أي علامات نقص مرضية علي الأسماك.

SUMMARY

In this study a series of two feeding experiments were conducted with Nile tilapia (*O.niloticus*) fingerlings to determine the required level of either riboflavin or pantothenic acid for maximum growth and efficiency of feed utilization. In two experiments durated 10 weeks each, five purified diets were formulated in experiment I to contain 0, 5, 10, 15 and 20 mg riboflavin/kg diet. In experiment II, six experimental diets having 0, 5, 10, 15, 20 and 40 mg Ca *d*-pantothenate/kg diet were formulated. There were no significant ($P< 0.05$) differences in weight gain and feed intake were observed between fish groups fed diets containing either 10 mg riboflavin/kg or more. The weight gain was nearly doubled in unsupplemented group, it was nearly seven times the initial in the most optimal level (10 mg riboflavin) of supplementation. Fish fed the riboflavin unsupplemented diet performed poorly in terms of growth parameters and exhibited typical signs of deficiency as anorexia, lethargy, fin erosion, short body dwarfism and high incidence of cataracts. No deficiency signs were observed in fish fed the riboflavin-supplemented diets. Mortality was about 17% in the unsupplemented group and a survival of 100% due to riboflavin supplementation. The highest values for protein retention and protein efficiency ratio were recorded with group fed on diet containing 10-mg riboflavin/kg diet, while the lowest values with unsupplemented group. Fish fed the pantothenic acid free-diet demonstrated poor growth and feed utilization, anorexia, gill exudate, sluggishness, hemorrhage in the fins and tail and increased mortality. Mild signs were observed in fish fed on diet containing 5 mg Ca *d*-pantothenate, whereas no deficiency signs were observed on fish fed diets supplemented with 10 mg Ca *d*-

pantothenate/kg diet or more. The survival was found to be 83.15% in group II (5 mg/kg) and 100% in the other supplemented groups, it decreased to 63.15% when the basal diet was not supplemented with pantothenic acid. No significant ($P<0.05$) differences was observed in the terms of growth parameters and feed intake between fish groups fed on diets containing 15 mg Ca *d*-pantothenate and those fed diets with higher levels. Based on these data, riboflavin and pantothenic acid are essential for growth, efficiency of feed utilization and survival of fish, in addition, it increases protein retention and efficiency ratio. Tilapia fish need as far as it is concluded, about 10 mg riboflavin/kg diet and 13.8 mg pantothenic acid/kg diet for optimum performance and increasing the level of supplementation does not have any significant benefit, however a level 5 mg riboflavin/kg diet and 9.2 mg pantothenic acid/kg diet are sufficient to prevent the appearance of gross deficiency signs.

Key Words:- *Riboflavin, Pantothenic acid, Requirements, Tilapia*

INTRODUCTION

Nutritional research on tilapia is very slow because tilapia production has traditionally relied on natural pond food organisms for much of their nutritional needs. Under this type of culture system, nutritionally complete diets were not necessary, thus basic dietary requirements for tilapia has not been priority area of research. However, the increasing importance of tilapia in commercial aquaculture and the recent emergence of its intensive culture have created a need for research on its nutritional requirements.

Riboflavin functions as a component of two coenzymes (FMN & FAD) serving as prosthetic groups of oxidation-reduction enzymes involved in the metabolism of keto-acids, fatty acids and amino acids in the mitochondrial electron transport system (NRC, 1993). It plays an important role in the respiration of poorly vascularized tissues and in the retinal pigment during light adaptation (Pike and Brown, 1984). A number of riboflavin deficiency signs has been reported in various species of fish, but the only clinical symptoms that are common among species are anemia, poor growth in addition high mortality (Arai *et al.*,1972; Murai & Andrews, 1978; Takeuchi *et al.*, 1980; Hughes *et al.*, 1981 and NRC, 1993). Cataracts have been routinely observed in riboflavin-deficient salmonids (Takeuchi *et al.*, 1980; Hughes *et al.*,1981 and Amezaga & Knox, 1990), channel catfish (Dupree, 1966) and blue tilapia (Soliman and Wilson, 1992b). Short body dwarfism associated with riboflavin deficiency has been described in channel catfish (Murai and Andrews, 1978) and blue tilapia (Soliman and Wilson, 1992b).

Dietary riboflavin requirement values expressed as mg/kg diet are 3-12 for rainbow trout (Takeuchi *et al.*, 1980; Woodward, 1982,1985; Hughes *et al.*,1981; Amezaga & Knox, 1990; NRC, 1993); 3-9 mg for channel catfish (Murai & Andrews, 1978; NRC, 1993; Serrini *et al.*,1996); 4-7 mg for common carp (Takeuchi *et al.*, 1980; NRC, 1993); 6 mg for blue tilapia (Soliman & Wilson, 1992b); 5 mg for Red hybrid tilapia (Lim *et al.*,1993) and 2.5-3.5 mg for broiler chicks (NRC, 1994).

Pantothenic acid is a component of coenzyme A which, especially as acetyl-Co-A, is of considerable importance in the metabolism of carbohydrate, protein and fat. A deficiency of pantothenic acid impairs the metabolism of mitochondria rich cells that undergo rapid mitosis and high energy expenditure. Thus, deficiency signs have been found to appear within 10-14 days in rapidly growing fish (Hosokawa, 1989). Clubbed gills, anemia and high mortality have been observed in salmonids (Poston & Page, 1982 and Karges & Woodward, 1984), channel catfish (Murai & Andrews, 1979; Brunson *et al.*, 1983 and Wilson *et al.*, 1983). Slow growth, anorexia, lethargy, hemorrhage, skin lesion and anemia were observed in common carp and Japanese eel (Ogino, 1967 and Arai *et al.*, 1972).

Dietary calcium d-pantothenate levels required for maximum growth and prevention of deficiency signs have been reported to be 10-20 mg/kg for trout (Poston & Page, 1982 and NRC, 1993), salmon (Halver, 1972), channel catfish (Murai & Andrews, 1979; Wilson *et al.*, 1983 and NRC, 1993), red sea bream (Yano *et al.*, 1988), blue tilapia (Soliman & Wilson, 1992a); 30 mg/kg for common carp (NRC, 1993) and 5 mg/kg for broiler chicks (NRC, 1994). Environmental factors such as water temperature, oxygen availability and fish density may affect the pantothenic acid requirement of fish; therefore, the recommended levels of this vitamin in commercial feeds for trout and salmon have been increased to 40-50 mg/kg (Halver, 1980 and NRC, 1981) and to 30 mg/kg for channel catfish (Wilson *et al.*, 1983).

To compensate the incomplete information about the needs of tilapia for the aforementioned vitamins (riboflavin and pantothenic acid), the present study was conducted to determine the levels of dietary riboflavin and pantothenic acid required to promote optimum growth, feed conversion and to prevent gross deficiency signs.

MATERIALS and METHODS

In this study, two experiments, 10 weeks each were conducted to determine the minimum requirements of tilapia fish for riboflavin (Exp.I) and pantothenic acid (Exp.II) needed for optimum growth and efficient feed conversion.

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 5 ±0.01g.

Five hundred and thirty five fish were used in this study of which 20 fish used for analysis at the start of each experiment, while the rest was divided into 33 groups each of 15 fish, 15 groups for experiment I and 18 for experiment II. The groups were distributed into separate aquaria, each was aerated and in which dechlorinated tap water at about 26 ± 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.

To meet the 20 fish composition samples at the start of the experiments, 5 fish were randomly removed from each aquarium at the end of each experiment for body analysis comparison (AOAC, 1984).

Five experimental diets were formulated in experiment I to contain 0, 5, 10, 15 and 20 mg riboflavin/kg diet. For experiment II, six diets were formulated to contain 0, 5, 10, 15, 20 and 40 mg Ca *d*-pantothenate (92% pantothenic acid)/kg diet. The quantities of the vitamins were added on the expense of corn starch in the basal diet. Each of the dietary treatments in each experiment was applied to randomly assigned three groups of fish.

2-Experimental diets:

A basal diet was formulated according to *NRC (1993)* from purified ingredients to contain 35.69% crude protein and 3448 Kcal digestible energy /kg diet. Three specialized vitamin mixtures were manufactured by Hoffman La Roach Co., Germany on request; the first is free of riboflavin for exp. I and the second free of pantothenic acid for exp. 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 on the basal diet to which a complete premix was added for 2 weeks as a conditioning period.

Each of the experimental diets was fed at satiation all over the period which continued for 10 weeks. The dry ingredients of each diet were thoroughly mixed, pelleted and dried at room temperature. All diets were stored at -20°C until feeding.

3-Parameters evaluated:

Body composition and body length were measured, while weight gain, feed conversion, 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 need to start with fish of comparable condition. The results of experiment I are displayed in Tables 2,3,4 and Fig.1, while that of experiment II in Tables 5, 6, 7 and Fig.2.

Experiment I:

1-Fish weight:

The five groups started the experiment by a weight ranging from 5.00 to 5.30g and attained an end weight in the supplemented groups of 31.13g in group II to 40.50g in group III, and with a difference of 9.37g and an average of 36.79g. In the unsupplemented group (I), the final weight reached only 14.32g with only 38.92% of the other group's average. So, the weight gain was nearly doubled in group I (9.22g), it was nearly seven times (35.35g) the initial in the most optimal level of supplementation in group III. The fish increased by 180.8% in the unsupplemented group and increased to 522.6% in group II and to about 641.6% on average for the last three supplemented groups.

The relation of body weight with length was expressed as "condition factor" and found to be 3.15 in group I, while it ranged from 1.85 to 2.10 in the supplemented groups. The fish fed the unsupplemented diet had a body dwarfism due to shortening of the individual vertebrae as been also reported in channel catfish (Murai & Andrews, 1978), blue tilapia (Soliman & Wilson, 1992b) and red hybrid tilapia (Lim *et al.*, 1993) which fed on free riboflavin diets. They indicated that this abnormal growth of spinal vertebrae might be related to hypothyroidism. However, they added that, there might be differences among species in riboflavin deficiency and hypothyroidism interactions.

The retarded growth in group I was due to the decreased feed intake which was recorded to be 26.28g compared with about 56.07g of the supplemented group's average. There was no significant differences (P<0.05) between the fish group fed on 5 mg riboflavin/kg diet or more in feed intake, while the group fed on unsupplemented diet was consumed an amount less than half that consumed by the supplemented groups. The riboflavin free group consumed 49.6% of that consumed by the 10 mg-group which was reflected in attaining the lowest final body weight which reached only 35.36% of that attained by the best 10 mg-riboflavin group. The food consumed was utilized with different efficiencies reaching an index of 2.85 in group I and improved to 2.15, 1.50, 1.74 and 1.80 in the supplemented four 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 17% in the first group and a survival of 100% due to riboflavin supplementations. High mortality rates have been reported for salmonids fed riboflavin deficient diet (Takeuchi *et al.*, 1980; Hughes *et al.*, 1981 and Amezaga & Knox, 1990) and common carp (Aoe *et al.*, 1967), whereas lower rates were observed in channel catfish (Murai and Andrews, 1978) and blue tilapia (Soliman and Wilson, 1992b).

In the present experiment (Table 2), lens cataracts (74.95%) were detected only in the fish fed unsupplemented riboflavin diet. These agreed with that reported in salmonids (Takeuchi *et al.*, 1980; Hughes *et al.*, 1981 and Amezaga & Knox, 1990), channel catfish (Dupree, 1966), blue tilapia (Soliman and Wilson, 1992b) and red hybrid tilapia (Lim *et al.*, 1993) fed on the riboflavin free diets. However, Murai and Andrews (1978) did not observe cataract in channel catfish and rainbow trout fed riboflavin deficient diet.

2-Protein parameters and body composition:

The fish at the start of the experiment had 23.50% DM and decreased to 21.50% in group I, while it increased to 24.30% in group II and about 25.73% on average in the last three groups with the lowest where riboflavin is lacking and the highest at the level of 10 mg riboflavin /kg diet used. As to the other body components, protein was decreased from 72.20% to 53.17%, 59.80%, 69.10%, 66.20% &

64.23% in groups I, II, III, IV & V respectively. The fat increased from 12.10% to 13.10% in group I, about 15.18% on average in groups II & III and 16.52% in the last two groups, while the ash increased from 14.13 to about 16.37% on average in groups I & II, and decreased to be about 13.65% on average in last three groups with the significantly ($P<0.05$) differences between the experimental groups.

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 group III got significantly ($P<0.05$) the highest values. The protein intake followed the level of feed intake and reached 9.38g in group I, and an average of 20.01g for the supplemented groups with no statistically significant ($P<0.05$) differences among the four intakes. Using the data of body composition, the protein retention was calculated and found to be 0.79g in group I and nearly five times (3.67g) in group II, while it reached an average of about 5.78g (nearly seven times) in the last three 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 0.98, 1.30 and 1.87 in groups I, II & III respectively compared with an average of about 1.59 in the last two groups. Besides, as a ratio for the protein retained and the protein fed expressed in percentage, the value were 8.42, 18.30 & 34.13% for the groups I, II & III respectively compared with an average of about 26.50% in the last two groups.

Riboflavin 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 10 mg riboflavin/kg diet for optimum performance and increasing the level of supplementation does not have any significant benefit. This value is in the range of 3-12 mg riboflavin/kg diet that reported for rainbow trout (Takeuchi *et al.*, 1980; Hughes *et al.*, 1981; Woodward, 1982 & 1985 and Amezaga & Knox, 1990), channel catfish (Murai & Andrews, 1978) and higher than 4-7 mg/kg in which reported for common carp (Takeuchi *et al.*, 1980), blue tilapia (Soliman and Wilson, 1992b) and red hybrid tilapia (Lim *et al.*, 1993).

Experiment II:

1-Fish weight:

In this experiment, six levels of calcium *d*-pantothenate (92% pantothenic acid) supplementation were tested. Statistically the 15, 20

& 40 mg/kg diet supplementation did not differ significantly (P<0.05) in weight or feed intake effect. The fish increased by 149% in the unsupplemented first group and increased to 353.8% & 485.4% in groups II & III respectively and found to be about 556.4% as an average for the last three groups.

Feed intake was 15.64, 36.88 and 40.07g and used with a conversion indices of 2.05, 1.74 and 1.37 for the group I, average of groups II & III and average of the last three groups respectively. The unsupplemented group consumed 38.76% of that consumed by the 15 mg - supplemented group which was reflected on its attaining the lowest final body weight reaching only 35.21% of that attained by the best 15 mg Ca *d*-pantothenate. The condition factor found to be 2.98 in-group I, while it ranged from 2.54 to 2.90 in the supplemented groups.

While the survival was 83.15% in group II and 100% in the other supplemented groups, it decreased to 63.15% when the basal diet was not supplemented. Mortalities began to observe in the unsupplemented group during the 5th week. The high mortality associated with pantothenic acid deficiency is probably due to reduced oxygen uptake by the fish as a result of severe hyperplasia of the gill lamellae as reported by Soliman & Wilson (1992a). In addition, Fish fed the pantothenic acid unsupplemented diet began to exhibit deficiency signs include anorexia, poor growth, anaemia, gill exudate, sluggishness, lethargy, hemorrhages in the fins and tail and increased mortality. The same observations due to pantothenic acid deficiency have been reported for other species of fish as rainbow trout, carp, eel, blue tilapia and channel catfish as reported by authors (Ogino, 1967; Arai *et al.*, 1972; Murai & Andrews, 1979; Poston & Page, 1982; Brunsen *et al.*, 1983; Wilson *et al.*, 1983; Karges & Woodward, 1984; Hosokawa, 1989 and Soliman & Wilson, 1992a). Murai and Andrews (1979) suggested that the anaemia associated with pantothenic acid deficiency might be related to anorexia. Fish fed the 5 mg Ca d-pantothenate/ kg diet level started to exhibit these deficiency signs by the end of the 8th week but the signs were less severe. The results of the study would support this suggestion, in that reduced food consumption was evidenced by the low feed efficiency value for fish fed the pantothenic acid free diet. Similar observations have been reported for the parrot fish fed a pantothenic acid free diet (Ikeda *et al.*, 1988).

2-Protein parameters and body composition:

The fish at the start of the experiment had 23.50% DM and increased to about 24.11% on average in groups I & II and 25.26% in the last four groups with the lowest when pantothenic acid is lacking and the highest at the level of 15 mg/kg diet. As to the other body components, protein decreased from 72.20 to about 56.66% on the average in groups I & II and 64.11% in the last four groups, while fat increased from 12.10 to 14.31% in group I and to be about 15.68% on average in the supplemented groups. The ash increased from 14.13 to about 15.38% on average in the first three groups, while decreased to about 13.78% in the last three groups

In protein parameters, group I was the worst followed by group II, while the last four groups did not differ significantly ($P<0.05$) and got the highest values. The protein intake followed the level of feed intake and reached 5.85g in group I, 12.94g in group II and an average of about 14.07g for the last four groups with no statistically significant differences ($P<0.05$) among the four intakes. Using the data of body composition, the protein retention found to be 0.83g in group I and nearly three times (2.49g) in group II, while it reached an average of about 4.57g in the last four groups (nearly six times). The efficiency of protein utilization reached only 1.37 and 1.42 in groups I & II respectively compared with an average of about 2.00 in the last four groups. And as a ratio for the protein retained and the protein fed expressed in percentage, the values were 14.87%, 19.24% in groups I & II respectively and to be about 32.41% on average for the last four groups. Pantothenic acid serves as a precursor for Co-enzyme A which is essential for the metabolism of the major dietary nutrients such as carbohydrate, lipid and protein. This metabolic role may explain the reduced growth and protein utilization data observed with the fish fed the pantothenic acid free diet. Addition of dietary pantothenic acid to the basal diet improved these parameters. Similar observations have been reported for other species of fish (Murai & Andrews, 1979; Poston & Page, 1982; Wilson *et al.*, 1983 and Soliman & Wilson, 1992a).

Pantothenic acid 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 13.8 mg pantothenic acid/kg diet (15 mg Ca d-pantothenate) for optimum performance and increasing the level of supplementation does not have any significant benefit. This value is in the range of 10-20 mg/kg diet that reported for trout (Poston & Page, 1982), salmon (Halver, 1972), channel catfish (Murai & Andrews, 1979 and Wilson *et al.*, 1983), Red Sea bream (Yano *et al.*, 1988) and blue tilapia (Soliman and Wilson, 1992a) and lower than 30 mg/kg diet that required for common carp (NRC, 1993).

The requirement of fish for riboflavin and pantothenic acid may vary depending on the balance of other dietary ingredients, caloric density and environmental conditions under which the fish are raised.

It could be concluded that, riboflavin and pantothenic acid are essential for Nile tilapia and the requirement for prevention of gross deficiency signs are 5 mg riboflavin/kg diet and 9.2 pantothenic acid/kg diet, however, maximun growth performance could be attained by the levels of 10&13.8 mg/kg diet for riboflavin and pantothenic acid respectively.

REFERENCES

Amezaga, M.R. and Knox, D. (1990): Riboflavin requirements in on growing rainbow trout. Aquaculture, 88:87-98.

Aoe, H.; Masuda, I.; Saito, I. and Knon, A. (1967): Water soluble vitamin requirements of carp.I. Requirement for vitamin B2. Bull. Jpn. Soc. Sci. Fish., 33:355-360.

Arai, S.; Nose, T. and Hashimoto, Y. (1972): Qualitative requirements of young eels for water soluble vitamins and their deficiency symptoms. Bull.freshwater Fish. Lab.,22:69-83.

Association of Official Analytical Chemists (1984): Official Methods of Analysis.14th ed., AOAC, Washington, DC.

Brunson, M.W.; Robinette, H.R.; Bowser, P.R. and Wellborn, T.L. (1983): Nutritional gill disease associated with starter feeds for channel catfish fry. Prog.Fish.Cult., 45:119-120.

Duncan, D.B. (1955): Multiple range and multiple F test. Biometrics, 11:42

Dupree, H.K. (1966): Vitamins essential for growth of channel catfish. Bureau of Sport Fish. and Wildl.Tech.Paper No 7:1-12.

Fulton, T. (1902): Rate of growth of sea fishes. Sci. Invest. Fish Div.Scot.Rept., 20.

Halver, J.E. (1972): The Vitamins. In: J.E.Halver (Editor), Fish Nutrition. Academic Press, New York, NY, pp.29-103.

Halver, J.E. (1980): The Vitamins. In: Fish Feed Technology. ADCP/REP/80/11, Food and Agriculture Organization (FAO), Rome, pp.65-103

Hosokawa, H. (1989): The vitamin requirement of fingerling yellowtail. Ph.D. Disseratation.Kochi Univ., Japan.

Hughes, S.G.; Rumsey, G.L. and Nickum, J.G. (1981): Riboflavin requirement of fingerling rainbow trout.Prog.Fish.Cult., 43(4):167-172.

Ikeda, S.; Ishibashi, Y.; Murata, O.; Nasu, T. and Harada, T. (1988): Qualitative requirements of Japanese parrot fish for a water-soluble vitamins. Nippon Suisan Gakkaishi, 54:2029-2035.

Karges, R.G. and Woodward, B. (1984): Development of lamellar epithelial hyperplasia in gills of pantothenic acid deficient rainbow trout. J.Fish Biol., 25:57-62.

Lim, C.; Leamaster, B. and Brock, J.A. (1993): Riboflavin requirement of fingerling red hybrid tilapia growth in seawater. Aquaculture, 100-107.

Murai, T. and Andrews, J.W. (1978): Riboflavin requirement of channel catfish fingerlings.J. Nutr., 108:1512-1517.

Murai, T. and Andrews, J.W. (1979): Pantothenic acid requirements of channel catfish fingerlings. J.Nutr., 109:1140-1142.

National Research Council (1981): Nutrient requirements of coldwater fishes. National Academy Press, Washington, DC.

National Research Council (1993): Nutrient requirements of Warmwater fishes. National Academy Press, Washington, DC.

National Research Council (1994): Nutrient requirements of poultry.9th ed. National Academy Press, Washington, DC.

Ogino, C. (1967):-B-vitamins requirements of carp.2. Requirements for riboflavin and pantothenic acid. Bull.Jap.Soc.Fish., 33(4):351-354.

Pike, R.H. and Brown, M.L. (1984): Nutrition: An integrated Approach, 3rd ed., pp., 84-136. John Wiley and Sons, New York, NY.

Poston, H.A. and Page, J.W. (1982): Gross and histological signs of dietary deficiencies of biotin and pantothenic acid in lake trout. Cornell Vet., 72:242-261.

Serrini, G.; Zhang-Zhan, Wilson, R.P. and Zhang, Z. (1996): Dietary riboflavin requirement of fingerling channel catfish. Aquaculture, 139 (3-4): 285-290.

Soliman, A.K. and Wilson, R.P. (1992a): Water soluble vitamin requirements of tilapia.1.Pantothenic acid requirement of blue tilapia. Aquaculture, 104:121-126.

Soliman, A.K. and Wilson, R.P.(1992b): Water-soluble vitamin requirements of tilapia.2.Riboflavin requirement of blue tilapia. Aquaculture, 104:309-314.

Takeuchi, L.; Takeuchi, T. and Ogino, C. (1980): Riboflavin requirements in carp and rainbow trout. Bull. Jpn. Soc. Sci. Fish,46:733-737.

Wilson, R.P.; Bowser, P.R. and Poe, W.E. (1983): Dietary pantothenic acid requirement of fingerling channel catfish. J.Nutr.,113:2224-2228.

Woodward, B. (1982): Riboflavin supplementation of diets for rainbow trout. J.Nutr., 112:908-913.

Woodward, B. (1985): Riboflavin requirement for growth, tissue saturation and maximal flavin-dependent enzyme activity in young rainbow trout at two temperatures. Aquaculture, 115: 79-84.

Yano, T.; Nakao, M.; Furuichi, M. and Yone, Y. (1988): Effect of dietary choline, pantothenic acid and vitamin C on the serum complement activity of red sea bream. Nip.S.Gak.,54:141-144.

Table 1: Composition of the basal diet

Ingredients	%
Physical composition	
Casein, (vitamin free)[1]	30
Gelatin	12
Corn starch	25
Dextrin	21
Corn oil	5
Mineral mixture*	4
Vitamin mixture**	3
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., Hoffmann 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; biotin, 0.02; folic acid, 0.09; B_{12}, 0.0013; starch (85.552 in exp.I & 85.662 in exp.II).

Table (2):- Growth performance and feed utilization efficiency of tilapia fish fed on experiment diets with different levels of riboflavin (Exp.I)

Parameters	Group / Riboflavin (mg/kg diet) I 0	II 5	III 10	IV 15	V 20
Initial weight (g)	5.10±1.01	5.00±1.00	5.15±1.05	5.30±1.07	5.20±1.05
Final weight (g)	14.32±1.10	31.13±1.17	40.50±1.35	38.32±1.30	37.20±1.25
Weight gain (g)	9.22±1.15^{c*}	26.13±1.25^{b}	35.35±1.25^{a}	33.02±1.18^{a}	32.0±1.20^{a}
Weight gain (%)	180.8	522.6	686.4	623.0	615.4
Feed intake (g)	26.28±1.10^{b}	56.18±1.50^{a}	53.03±1.45^{a}	57.45±1.70^{a}	57.60±1.55^{a}
Feed conversion index	2.85	2.15	1.50	1.74	1.80
Body length (cm)	7.69	11.40	12.98	12.59	12.40
Condition factor[1]	3.15	2.10	1.85	1.92	1.95
Survival (%)	83.15	100	100	100	100
Cataract (%)	74.95	0.00	0.00	0.00	0.00

[1]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

Parameters	Group / Riboflavin (mg/kg diet) I 0	II 5	III 10	IV 15	V 20	Blank group
Dry matter	21.50±1.05^{c*}	24.30±1.05^{b}	26.14±1.20^{a}	25.85±1.15^{a}	25.20±1.20^{a}	23.50±1.10
Crude protein	53.17±1.20^{d}	59.80±1.15^{c}	69.10±1.50^{a}	66.20±1.48^{ab}	64.23±1.40^{b}	72.20±1.45
Crude fat	13.10±1.02 ^{c}	15.10±1.05 ^{b}	15.25±1.05 ^{b}	16.83±1.10 ^{a}	16.20±1.05 ^{a}	12.10±1.05
Ash	16.72±1.03 ^{a}	16.01±1.05 ^{a}	13.05±1.02 ^{b}	13.70±1.05 ^{b}	14.20±1.02 ^{b}	14.13±1.05

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

Table (4): Protein parameters of tilapia fish fed on the experimental diets with different levels of riboflavin (Exp.I)

Parameters	Group / Riboflavin (mg/kg diet)				
	I 0	II 5	III 10	IV 15	V 20
Protein intake (g)	9.38±1.01^{b*}	20.05±1.10^{a}	18.93±1.05^{a}	20.50±1.10^{a}	20.56±1.15^{a}
Protein retention (g)	0.79±0.01^{c}	3.67±0.05^{b}	6.46±0.08^{a}	5.71±0.03^{a}	5.17±0.02^{a}
Protein efficiency ratio	0.98±0.02^{d}	1.30±0.01^{c}	1.87±0.01^{a}	1.61±0.02^{b}	1.56±0.01^{b}
App.net prot.utilization	8.42±1.00^{d}	18.30±1.10^{c}	34.13±1.25^{a}	27.85±1.20^{b}	25.15±1.15^{b}

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

Table (5): Growth performance and feed utilization efficiency of tilapia fish fed on experimental diets with different levels of Ca *d*-pantothenate (Exp.II)

Parameters	Group / Ca *d*-pantothenate (mg/Kg diet)					
	I 0	II 5	III 10	IV 15	V 20	VI 40
Initial weight (g)	5.12±1.02	5.20±1.05	5.15±1.02	5.17±1.05	5.29±1.05	5.32±1.07
Final weight (g)	12.75±1.08	23.60±1.10	30.15±1.15	36.21±1.30	34.81±1.18	32.50±1.20
Weight gain (g)	7.63±1.10^{d*}	18.4±1.15^{c}	25.0±1.20^{b}	31.04±1.30^{a}	29.52±1.20^{a}	27.18±1.25^{a}
Weight gain (%)	149.0	353.8	485.4	600.4	558.0	510.9
Feed intake (g)	15.64±1.05^{c}	36.25±1.40^{b}	37.50±1.30^{b}	40.35±1.35^{a}	40.44±1.50^{a}	39.41±1.25^{a}
Feed conversion index	2.05	1.97	1.50	1.30	1.37	1.45
Body length (cm)	7.54	9.34	10.31	11.25	10.82	10.80
Condition factor	2.98	2.90	2.75	2.54	2.57	2.58
Survival (%)	63.15	83.15	100.0	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 / Ca *d*-pantothenate (mg/Kg diet)						Blank group
	I 0	II 5	III 10	IV 15	V 20	VI 40	
Dry matter	23.92±1.05^{b*}	24.30±1.05^{b}	25.03±1.01^{a}	25.50±1.15^{a}	25.37±1.10^{a}	25.12±1.10^{a}	23.50±1.10
Crude protein	55.12±1.15^{b}	58.20±1.10^{b}	62.50±1.30^{a}	65.98±1.30^{a}	64.10±1.30^{a}	63.85±1.35^{a}	72.20±1.45
Crude fat	14.31±1.08 ^{b}	14.72±1.05 ^{a}	15.65±1.05 ^{a}	16.23±1.07 ^{a}	16.01±1.05 ^{a}	15.81±1.03 ^{a}	12.10±1.05
Ash	15.97±1.05 ^{a}	15.60±1.04 ^{a}	14.57±1.02 ^{a}	13.54±1.07 ^{b}	13.78±1.05 ^{b}	14.02±1.02 ^{b}	14.13±1.05

*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 with different levels of Ca *d*-pantothenate (Exp.II)

Parameter	Group / Ca *d*-pantothenate (mg/Kg diet)					
	I 0	II 5	III 10	IV 15	V 20	VI 40
Protein intake (g)	5.58±1.03^{c*}	12.94±1.10^{b}	13.38±1.10^{a}	14.40±1.15^{a}	14.43±1.20^{a}	14.07±1.10^{a}
Protein retention (g)	0.83±0.01^{c}	2.49±0.01^{b}	3.87±0.01^{a}	5.24±0.04^{a}	4.81±0.02^{a}	4.36±0.03^{a}
Protein efficiency ratio	1.37±0.02^{c}	1.42±0.01^{b}	1.87±0.01^{a}	2.16±0.02^{a}	2.05±0.01^{a}	1.93±0.02^{a}
App.net prot.utilization	14.87±1.10^{c}	19.24±1.15^{b}	28.92±1.20^{a}	36.39±1.25^{a}	33.33±1.20^{a}	30.99±1.15^{a}

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

Fig .1 .Weight gain and feed intake of fish fed on different levels of riboflavin

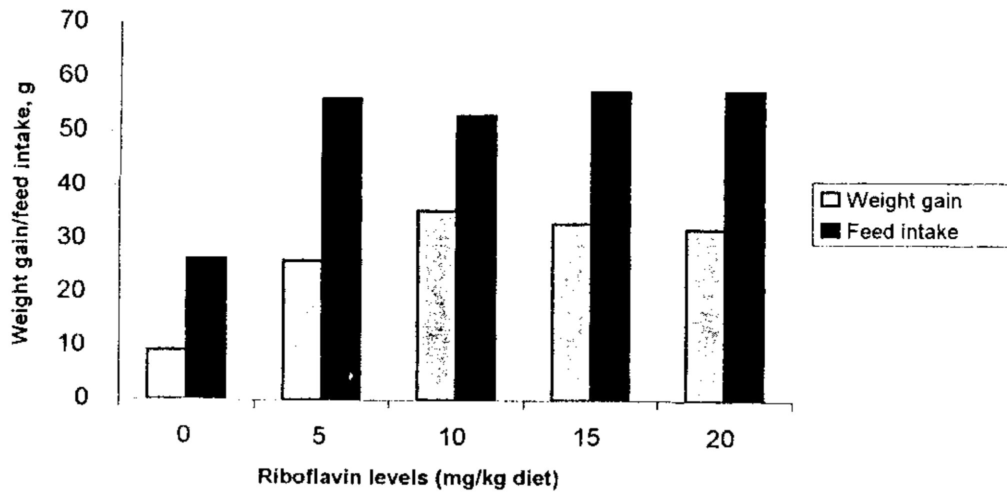

Fig .2 .Weight gain & feed intake of fish fed on different levels of Ca d- pantothenate