Vitamin B₆ requirements of tilapia *Oreochromis niloticus* x *O. aureus* fed two dietary protein concentrations

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A feeding trial was conducted to determine the adequate level of dietary vitamin B₆ (pyridoxine, PN) for juvenile tilapia *Oreochromis niloticus* x *O. aureus* fed diets containing different dietary protein concentrations. Purified diets with eight levels of PN (0, 2, 4, 8, 12, 20, 50, 100 mg/kg diet) at either 28 or 36% protein concentration were each fed to three replicate groups of tilapia (mean weight: 0.73 ± 0.01 g) for 8 weeks. Results indicated that in the 28% protein group, fish fed 4 mg PN/kg diet had a more significant ($p < 0.05$) weight gain than fish fed 0 and 100 mg PN/kg diet; feed efficiency ratio (FER) of fish fed 4 mg PN/kg diet was higher than fish fed 0 and 8–100 mg PN/kg diets. In the 36% protein group, fish fed 12–50 mg PN/kg diets had higher weight gain than fish fed 0, 100 mg PN/kg diets and fish fed 20 mg PN/kg diet had higher FER than fish fed 0–4 mg PN/kg diets. Hepatic alanine aminotransferase (ALT) activity generally increased with increasing dietary vitamin B₆ and leveled off at 12 mg PN/kg and 20 mg PN/kg in the 28% protein and 36% protein groups, respectively. Tilapia fed 100 mg PN/kg diet at 28% protein concentration showed normocytic normochromic anemia symptoms. Weight gain and ALT activity analyzed by broken-line regression indicated that the optimum dietary vitamin B₆ requirement in juvenile tilapia were 1.7–9.5 mg PN/kg and 15.0–16.5 mg PN/kg diet in 28% and 36% protein diets, respectively.

Key words: vitamin B₆, fish, tilapia, *Oreochromis*

Vitamin B₆ in the form of pyridoxal phosphate participates as a prosthetic group of enzymes in a large number of metabolic reactions, particularly those associated with the metabolism of proteins or amino acids. A dietary requirement for pyridoxine has been demonstrated in salmonid fishes,^{1–3} channel catfish,⁴ carp⁵ and yellowtail.⁶ Signs of pyridoxine deficiency include poor growth, anorexia, ataxia, oedema and epileptiform fits and other nervous disorders.

The quantitative requirement of pyridoxine has been studied in a few fish species. For example, 1–2.5 mg/kg, 1.97 mg/kg, 3 mg/kg, 5–6 mg/kg, 5.4 mg/kg, 2 mg/kg and 10–15 mg/kg have been reported as the requirement for turbot,⁷ gilthead sea bream,⁸ channel catfish,⁹ red sea bream,¹⁰ carp,⁵ and rainbow trout,¹¹ respectively.

There is little information about the vitamin B₆ requirements within fish species with respect to different protein concentrations. Phillips *et al.*¹² noted that brook trout fed a higher protein diet developed deficiency signs in a much shorter period of time than did groups fed lower levels of protein. This may indicate that fish reared on a high dietary protein diet could have a higher vitamin B₆ requirement. In contrast, Hardy *et al.*¹³ noted no effect of dietary protein level on the vitamin B₆ requirement of chinook salmon.

Tilapia are mainly lacustrine fish which are well adapted to enclosed waters; they produce high yields and thus are an important human protein source in many tropical and subtropical countries. Their vitamin B₆ requirements have

not been established. The purpose of this study was to estimate the dietary vitamin B₆ requirements of juvenile hybrid tilapia *Oreochromis niloticus* x *O. aureus* at two dietary protein concentrations using growth indices supported by measurement of hepatic alanine aminotransferase activity.

Materials and Methods

Diet Preparation

Formulations and proximate analysis¹⁴ of the experimental diets are given in Table 1. These formulations are similar to that of Shiau and Suen,¹⁵ which has been shown to be adequate for tilapia. Vitamin-free casein (Sigma Chemical, St. Louis, MO) was used as the protein source and was added at either 30 or 40% to the diet providing 28 and 36% protein, respectively. The vitamin mixture was similar to that used by Shiau and Hsu,¹⁶ except that it did not contain vitamin B₆. To each diet, pyridoxine hydrochloride (Sigma Chemical) was added at the expense of small amounts of cellulose to provide concentrations of 0, 2, 4, 8, 12, 20, 50, and 100 mg/kg diet. The pyridoxine concentrations of the experimental diets were determined by HPLC¹⁷ to be 0.07, 0.10 (unsupplemented control); 1.3, 1.6 (2 mg/kg); 3.1, 3.3 (4 mg/kg); 6.3, 6.5 (8 mg/kg); 9.7, 9.9 (12 mg/kg); 15.5, 15.7 (20 mg/kg); 40.7, 40.9 (50 mg/kg) and 82.6, 82.8 (100 mg/kg) mg/kg diet in the 28% and 36% protein diets, respectively. The diets were prepared and handled as de-

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Table 1. Formulation and composition of the experimental diets

Ingredient	28% protein	36% protein
g/100 g		
Formulation		
Casein (Vitamin free)	30	40
Corn Starch	44	34
Premix* ^{1,2,3,4}	26	26
Proximate composition		
Moisture	11.5	10.4
Crude protein	28.3	36.8
Ether extract	7.7	7.1
Ash	3.0	3.2
Crude fiber	6.4	7.1
N-free extract* ⁵	43.1	35.4

*¹ Premix contained (g/100 g): corn oil, 7; cod liver oil, 4; vitamin mix, 2; mineral mix, 4; carboxymethyl cellulose, 2; cellulose, 7.

*² Pyridoxine was added to the diets at the expense of cellulose to provide concentrations of 0, 2, 4, 8, 12, 20, 50, 100 mg/kg diet.

*³ Vitamin mix supplied the following (per g mixture): thiamin·HCl, 5 mg; riboflavin, 5 mg; calcium pantothenate, 10 mg; nicotinic acid, 6.05 mg; biotin, 0.6 mg; folic acid, 1.5 mg; cyanocobalamin, 0.01 mg; inositol, 200 mg; L-ascorbic acid, 3.95 mg; choline chloride, 400 mg; menadione, 4 mg; all-*rac*- α -tocopherol acetate, 40 mg; p-aminobenzoic acid, 5 mg; retinyl acetate, 68.8 μ g; cholecalciferol, 0.4685 μ g; and α -cellulose to 1 g.

*⁴ Mineral mix supplied the following (g/100 g mixture): calcium biphosphate 13.58; calcium lactate, 32.7; ferric citrate, 2.97; magnesium sulfate, 13.7; potassium phosphate dibasic, 23.98; sodium biphosphate, 8.72; sodium chloride, 4.35; AlCl₃·6H₂O, 0.015; KI, 0.015; CuCl₂, 0.01; MnSO₄·H₂O, 0.88; CoCl₂·6H₂O, 0.1; ZnSO₄·7H₂O, 0.3.

*⁵ Obtained by subtraction.

scribed by Shiau *et al.*¹⁸⁾

Experimental Procedure

Male tilapia, *Oreochromis niloticus* x *O. aureus*, were supplied from the Far East Hatchery Co. (Cha-Yi, Taiwan). Upon arrival, they were acclimated to laboratory conditions for 4 wk in a plastic tank (74W × 95L × 45H cm, 60 l) and fed a commercial diet (Lucky Star, Hung Kuo Industrial Co., Taipei, Taiwan). At the beginning of the experiment, 10 fish (mean weight: 0.73 ± 0.01 g) were stocked in each aquarium (30.5W × 61.0L × 55.5H cm). There were a total of 16 treatments. Each experimental diet was fed to fish in three aquaria. Each aquarium was part of a closed-recirculated system with a common water reservoir maintained at 26 ± 1°C. The water was circulated at 2 l/min per tank through two separate biofilters to remove impurities and reduce ammonia concentrations. Water quality was checked regularly, and throughout the experiment the fish were maintained under humane conditions.¹⁹⁾

The fish were fed 5% of their body weight per day. This amount was close to the maximum daily ration for tilapia according to the daily rations consumed by the tilapia during the acclimation period. The daily ration was subdivided into two equal feedings and fed at 0900 and 1700 h. Fish were weighed once every 2 wk and the daily ration adjusted accordingly. The light cycle was 12 h (0800–2000 h) light: 12 h dark. The fish were fed the test diets for an 8-wk period.

At the end of the feeding trial, fish were weighed and percent weight gain and feed efficiency ratio were calculated as previously described.¹⁵⁾ Two fish from each aquarium were selected randomly, anesthetized and killed.²⁰⁾ Livers

were removed and pooled for subsequent immediate enzyme assay. Alanine aminotransferase (ALT, EC 2.6.1.2) was determined according to the method described by Casillas *et al.*²¹⁾ Two other fish were then taken randomly from the remaining fish of each aquarium for blood analysis. Blood samples were collected directly into the diluting pipettes or capillary tubes by making an incision through the caudal vein. The red blood cell (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined according to Agrawal and Mahajan.²²⁾

Statistical Analysis

Data were analyzed by two-way ANOVA with two protein concentrations and eight pyridoxine levels as main effects using the SAS/PC statistical software (SAS Institute, Cary, NC) and significance was set at $p < 0.05$. This analysis allowed examination of the main effects of both protein and pyridoxine as well as any interactions occurring between the two factors. When a significant main effect was found without the interaction effect, individual mean differences were determined by Duncan's test. Broken-line analysis²³⁾ was used to estimate the adequate level of pyridoxine for maximal growth and liver ALT activity.

Results

Fish fed the control diet without pyridoxine supplementation exhibited symptoms of anorexia and ataxia after 3 weeks. Mortality increased and oedema was observed in the dead fish of the control group. There were no symptoms of vitamin B₆ deficiency in any of the fish fed diets supplemented with pyridoxine.

Weight gain, feed efficiency ratio (FER) and survival rate are summarized in Table 2. In the 28% protein group, weight gain was highest for fish fed diet supplemented with 4 mg PN/kg diet, followed by fish fed 2, 8–50 mg PN/kg diet and the lowest for fish fed 100 mg PN/kg diet and the unsupplemented control diet. The differences between the highest and the lowest groups were significant ($p < 0.05$); FER was highest for fish fed diet supplemented with 4 mg PN/kg, followed by 2 mg PN/kg diet, then 12 mg PN/kg diet, then 8, 20–100 mg PN/kg diet and the lowest for fish fed the unsupplemented control diet. The differences between 4 mg PN/kg diet and the other diets except 2 mg PN/kg, as well as those fed 2, 12 mg PN/kg diet and the unsupplemented control group were significant. In the 36% protein group, weight gains were highest for fish fed diets supplemented with 12, 20 and 50 mg PN/kg diet, followed by 2, 4 and 8 mg PN/kg diet, then 100 mg PN/kg diet and diet without vitamin B₆ supplementation. The differences between 12, 20, 50 mg PN/kg diet and 100 mg PN/kg diet, the unsupplemented control diet, were significant; FER was highest for fish fed diet with 20 mg PN/kg, followed by 8, 12, 50 and 100 mg PN/kg, then 2 mg PN/kg, then 4 mg PN/kg and finally the unsupplemented control diet. The differences between 20 and 2, 4, the unsupplemented control diet, as well as those between 2, 8, 12, 50, 100 mg PN/kg and the unsupplemented

Table 2. Weight gain, feed efficiency ratio (FER) and survival rate of tilapia fed the 28% or 36% protein diet supplemented with various concentrations of vitamin B₆*¹

	Dietary pyridoxine, mg/kg diet								pooled SEM
	0	2	4	8	12	20	50	100	
Weight gain, g/100 g initial wt									
28% protein	241.6 ^a	287.6 ^{ab}	304.9 ^b	255.1 ^{abx}	278.3 ^{abx}	251.8 ^{abx}	268.6 ^{abx}	227.5 ^{ax}	21.3
36% protein	256.8 ^a	308.3 ^{ab}	300.7 ^{ab}	315.0 ^{aby}	364.8 ^{by}	376.9 ^{by}	375.1 ^{by}	298.1 ^{ay}	21.9
FER, g gain/g feed									
28% protein	0.81 ^a	1.00 ^{bc}	1.08 ^c	0.93 ^{ab}	0.97 ^b	0.94 ^{ab}	0.92 ^{ab}	0.88 ^{ab}	0.07
36% protein	0.67 ^a	0.83 ^b	0.80 ^{ab}	0.89 ^{bc}	0.90 ^{bc}	1.00 ^c	0.92 ^{bc}	0.88 ^{bc}	0.05
Survival rate, %									
28% protein	43.33 ^a	95.00 ^b	100.00 ^b	96.67 ^b	93.33 ^b	96.67 ^b	96.67 ^b	90.00 ^b	6.66
36% protein	46.67 ^a	86.67 ^b	86.67 ^b	100.00 ^b	96.67 ^b	100.00 ^b	83.33 ^b	80.00 ^b	10.33

*¹ Values are means from three groups of fish with 10 fish per group (n=3).^{abc} Significant ($p < 0.05$) differences among vitamin B₆ concentrations within protein level;^{xy} significant ($p < 0.05$) differences between protein level within vitamin B₆ concentration.

Table 3. Maximal activities of alanin aminotransferase (ALT) in liver of tilapia fed 28% or 36% protein diet supplemented with various concentrations of vitamin B₆*¹

	Dietary pyridoxine, mg/kg diet								pooled SEM
	0	2	4	8	12	20	50	100	
ALT, Mol pyruvate × 10 ⁶ /(mg liver · h)									
28% protein	1.46 ^a	2.00 ^{ab}	2.16 ^{ab}	2.97 ^{bc}	3.79 ^c	3.57 ^c	3.81 ^c	3.92 ^c	0.35
36% protein	1.80 ^a	2.40 ^{ab}	2.87 ^{bc}	3.64 ^{cd}	3.87 ^{de}	4.66 ^e	4.69 ^e	4.83 ^e	0.33

*¹ Values are means from three groups of fish with 2 fish per group (n=3).^{abcde} Significant ($p < 0.05$) differences among vitamin B₆ concentrations within protein level;^{xy} significant ($p < 0.05$) differences between protein level within vitamin B₆ concentration.

Table 4. Red blood cell (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in blood of tilapia fed 28% or 36% protein diets supplemented with various concentrations of vitamin B₆*¹

	Dietary pyridoxine, mg/kg diet								pooled SEM
	0	2	4	8	12	20	50	100	
RBC, per L									
28% protein	1.35 ^b	1.53 ^b	1.53 ^b	1.29 ^b	1.16 ^b	1.27 ^b	1.29 ^b	0.40 ^{ax}	0.13
36% protein	1.53	1.10	1.26	1.52	1.14	1.26	1.19	1.25 ^y	0.20
Hb, g/L									
28% protein	43.7 ^b	44.3 ^b	45.5 ^b	37.0 ^b	35.0 ^b	35.7 ^b	48.5 ^b	15.5 ^{ax}	4.1
36% protein	45.0	40.0	41.0	50.0	39.0	42.3	39.3	50.0 ^y	4.9
Ht, Vol fraction									
28% protein	0.18 ^b	0.20 ^b	0.20 ^b	0.19 ^b	0.18 ^b	0.20 ^b	0.20 ^b	0.05 ^{ax}	0.01
36% protein	0.21	0.19	0.20	0.21	0.19	0.20	0.20	0.21 ^y	0.01
MCV, μm ³									
28% protein	133.03	132.97	133.25	130.85	137.75	137.80	138.80	137.55	4.87
36% protein	137.87	137.45	140.40	140.33	139.25	135.45	131.45	133.80	5.12
MCH, pg/RBC									
28% protein	28.85	28.93	29.90	29.05	30.25	28.10	30.80	32.00	2.23
36% protein	29.40	32.10	28.55	32.15	29.97	32.47	28.10	28.28	4.01
MCHC, g/L									
28% protein	215.0	217.7	229.5	222.0	215.0	203.7	220.3	257.0	28.7
36% protein	213.0	234.5	203.0	233.0	230.5	234.5	212.5	222.5	18.0

*¹ Values are means from three groups of fish with 10 fish per group (n=3).^{ab} Significant ($p < 0.05$) differences among vitamin B₆ concentrations within protein level;^{xy} significant ($p < 0.05$) differences between protein level within vitamin B₆ concentration.

control diet were significant. Fish fed 36% protein diet supplemented with 8, 12, 20, 50 and 100 mg PN/kg diet had significantly higher weight gain than those of fish fed 28% protein diet. The survival of fish fed diets supplemented

with vitamin B₆ were significantly higher than fish fed the unsupplemented control diet in both the 28 and 36% protein groups. There was no difference in survival of the fish among the vitamin B₆ supplemented diets.

Fig. 1. Weight gain percent and hepatic alanine aminotransferase (ALT) activity of *Oreochromis niloticus* × *O. aureus* fed diets containing various concentrations of pyridoxine (PN) after 8 weeks feeding.

Each point represents the mean of three groups of fish, with 10 fish per group ($n=3$). Requirements derived with the broken-line analysis for weight gain and ALT activity are 1.7, 9.5 and 16.5, 15.0 mg PN/kg diet in 28% and 36% protein diets, respectively.

Hepatic alanine aminotransferase (ALT) activity in fish (Table 3) generally increased with increasing vitamin B₆ supplementation in the diet. In the 28% protein group, ALT activities were the highest for fish fed diets supplemented with > 12 mg PN/kg, followed by 8 mg PN/kg, then 2 and 4 mg PN/kg, and finally the unsupplemented control group. The differences between > 12 mg PN/kg and < 4 mg PN/kg as well as between 8 mg PN/kg and the unsupplemented control group were significant. In the 36% protein group, the highest ALT activities were in fish fed diets supplemented with > 20 mg PN/kg diet, followed by 12 mg PN/kg, then 8 mg PN/kg, then 4 mg PN/kg, then 2 mg PN/kg, and finally the unsupplemented control group. The differences between > 20 mg PN/kg and < 8 mg PN/kg, 12 mg PN/kg and < 4 mg PN/kg, 8 mg PN/kg and < 2 mg PN/kg, as well as between 4 mg PN/kg and the unsupplemented control diet were significant.

As shown in Table 4 in the 28% protein group, red blood cell (RBC), hemoglobin concentration (Hb) and hematocrit (Ht) of blood were significantly lower in tilapia fed diet supplemented with 100 mg PN/kg diet than fish fed diets supplemented with the other PN levels and the unsupplemented control diet. These three blood parameters were lower in the 28% protein group than those in the 36% protein group at the 100 mg/kg level of PN supplementation. Blood mean corpuscular volume (MVC), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in tilapia were not affected by dietary treatments. These suggest a normocytic normochromic anemia in tilapia fed 28% protein diet supplemented with 100 mg/kg diet of pyridoxine.

When broken-line analysis was used, based on weight gain of the fish and the liver alanine aminotransferase (ALT) activity (Fig. 1), because the breakpoints at 1.7 mg/kg (weight gain), 9.5 mg/kg (ALT activity) of 28% protein group and 16.5 mg/kg (weight gain), 15.0 mg/kg (ALT activity) of 36% protein group gave the least mean square error, the adequate amount of dietary vitamin B₆ for juvenile tilapia is estimated to be 1.7–9.5 mg/kg and 15.0–16.5 mg/kg for 28% and 36% dietary protein levels, respectively.

Discussion

Our result clearly demonstrated that the vitamin B₆ requirement in tilapia appears to be associated with dietary protein concentration for maximum growth of fish. For maximum growth of fish, an optimum protein in feed is necessary. But the optimum level of dietary protein does not necessarily lead to the most economical production of fish due to the high cost of the protein component of the diet. Thus, a suboptimum dietary protein level is commonly used in fish diet and this is considered to result in more cost-effective fish production than the use of diets which contain an optimum protein content. The optimum protein requirements for tilapia have been determined by several investigators and estimates of 30%,²⁴ 30–35%,²⁵ and 40%²⁶ have been reported. In the present study, 36% was selected as the optimum protein level for tilapia, whereas 28% was selected as suboptimum. When the requirement of the growing tilapia for vitamin B₆ is expressed on the base of dietary protein, it is equivalent to 0.61–3.39 mg PN/100 g protein in 28% protein diet and 4.17–4.58 mg PN/100 g protein in 36% protein diet.

Only two studies of the vitamin B₆ requirements in fish in relation to dietary protein concentrations have been reported in the literature. Phillips *et al.*¹² did not exactly determine the requirements but indirectly inferred that the vitamin B₆ requirement in brook trout may reflect the protein levels in the diet. Hardy *et al.*¹³ reported that chinook salmon fed either high or low protein diets did not exhibit a growth response to incremental supplementation of dietary pyridoxine. It should be noted that the protein levels of high and low protein diets in the study of Hardy *et al.*¹³ were 60% and 30%, respectively. These figures were much higher and lower than the NRC²⁷ requirement of this species. This could have confounded a clear interpretation of the results. In addition, the increment vitamin B₆ supplements used in Hardy's study were 5, 10, 20 and 40 mg/kg

feed. The initial of the vitamin B₆ supplements was possibly too high to detect differences in the growth response of the salmon. This may explain the result of no effect of dietary protein level on the vitamin B₆ requirement of chinook salmon.

It has been speculated that salmonids, because of their carnivorous nature, logically will have a high dietary vitamin B₆ requirement in comparison to species with lower protein requirements.²⁸⁾ However, this speculation has been challenged recently as no correlation was apparent as the author states²⁸⁾ when interspecies comparison was performed between dietary protein and vitamin B₆ requirement for maximal weight gain of several fish species.²⁹⁾ It is important to acquire much evidence in the field of fish nutrition. Our results add to a growing body of evidence that the vitamin B₆ requirement is not related to the protein nitrogen requirement of a species.

Cowey³⁰⁾ suggested that the nutrient requirement of cultured fish be established by biochemical measurements, such as a particular enzyme's activity. Many vitamins function, either as such or in a modified form, as coenzymes and measurements of relevant tissue enzyme activities have frequently been used to provide supplementary evidence concerning the requirement of an animal for a given vitamin. However, pyridoxal phosphate serves as a coenzyme for many enzymes and there is at present no means of knowing which of these enzymes is most critically affected by the stress of a restricted vitamin B₆ intake. In the present study, hepatic ALT activity was measured for estimating the vitamin B₆ requirement of tilapia. In the 36% protein groups, ALT activity and weight gain agree very well, while in the 28% protein groups, considerable higher vitamin B₆ requirements were obtained based on ALT activity than that of weight gain. Nevertheless, the lower vitamin B₆ requirement in tilapia fed 28% protein diet than fish fed 36% protein diet was consistent in both parameters.

The occurrence of anemia has been established in chinook salmon *Oncorhynchus tshawytscha*,²⁾ coho salmon *O. kisutch*³¹⁾ and rainbow trout *Salmo gairdneri*³²⁾ The absence of anemia in pyridoxine deficient tilapia found in the present study has also been reported in channel catfish *Ictalurus punctatus*⁹⁾ and turbot *Scophthalmus maximus*⁷⁾ indicating there may be a species difference among fishes as is the case in terrestrial animals.³³⁾ Severe anemia is characteristic of pyridoxine deficiency in ducks, swine, and dogs but not in chicks or turkey poults. As suggested by Andrews and Murai,⁹⁾ the occurrence of anemia in pyridoxine deficiency may be related to the severity of other deficiency symptoms; i.e., in some species the anemia may be precluded by the early cessation of growth and death prior to the onset of anemia.

Anemia occurring in tilapia fed high dietary levels of pyridoxine is a somewhat unusual phenomenon. This phenomenon has also been reported in channel catfish.⁹⁾ It is noted, however, that normocytic, normochromic anemia was observed in the present study with tilapia, whereas microcytic, normochromic anemia was reported in catfish fed high levels of pyridoxine. The reason for the difference is not known. While there have been numerous publications documenting vitamin B₆ toxicity symptoms in dogs, humans, and rats, very few toxicity symptoms have

been reported as the result of high dietary levels of pyridoxine in fish. Future study is needed in this regard. It should also be noted that the anemia in tilapia fed diets containing high levels of pyridoxine only occurred in the 28% protein group. In an earlier study with catfish,⁹⁾ no information was provided as to the protein concentration used in the diet, but it was certain that only one protein concentration was included in that study. The extend to which the dietary protein concentration is associated with the occurrence of anemia in fish fed high levels of pyridoxine certainly needs to be explored more. Nevertheless, the present result provides an important source of evidence that it is possible to retard weight gain and produce hematological anomalies at levels of B₆ supplementation that must be considered realistic if one considers the vitamin packages recommended in some garters for fish such as salmonids.

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References

- 1) J. A. Coates and J. E. Halver: Water soluble vitamin requirements of silver salmon. U. S. Fish Wildl. Serv. Bur., *Sport Fish Wildl. Spec. Sci. Rep. Wildl.*, **281**, 1-9 (1958).
- 2) J. E. Halver: Nutrition of salmonoid fishes, III. Water-soluble vitamin requirements of chinook salmon. *J. Nutr.*, **62**, 225-243 (1957).
- 3) A. M. Jr. Phillips and D. L. Livingston: The effect of season of the year and fish size on the pyridoxine requirement of brook trout, in: *The Nutrition of Trout*. Cortland Hatchery Rep. 32 N. Y. State Conserv. 29, 1965, pp. 15-19.
- 4) H. K. Dupree: Vitamins essential for the growth of channel catfish. U.S.D.I., B.S.F.W., Technical Paper, 1966, p. 12.
- 5) C. Ogino: B vitamin requirements of carp, *Cyprinus carpio*. I. Deficiency symptoms and requirements of vitamin B₆. *Nippon Suisan Gakkaishi*, **31**, 546-551 (1965).
- 6) H. Sakaguchi, F. Takeda, and K. Tange: Studies on vitamin requirements by yellowtail. 1. Vitamin B₆ and vitamin C deficiency symptoms. *Nippon Suisan Gakkaishi*, **35**, 1201-1206 (1969).
- 7) J. W. Adron, D. Knox, and C. B. Cowey: Studies on the nutrition of marine flatfish. The pyridoxine requirement of turbot (*Scophthalmus maximus*). *Br. J. Nutr.*, **40**, 261-268 (1978).
- 8) G. W. Kissil, C. B. Cowey, J. W. Adron, and R. H. Richards: Pyridoxine requirements of the gilthead bream, *Sparus aurata*. *Aquaculture*, **23**, 243-255 (1981).
- 9) J. W. Andrews and T. Murai: Pyridoxine requirements of channel catfish. *J. Nutr.*, **109**, 533-537 (1979).
- 10) T. Takeda and Y. Yone: Studies of nutrition of red sea bream. 2. Comparison of vitamin B₆ requirement level between fish fed a synthetic diet and fish fed beef liver during prefeeding period. *Rep. Fish Res. Lab., Kyushu Univ.*, **1**, 37-47 (1971).
- 11) B. Woodward: Dietary requirements of some water-soluble vitamins for young rainbow trout, in *Proceedings of the 25th Annual Nutrition Conference for Feed Manufacturers*, Toronto, Ontario, 1989, pp. 25-33.
- 12) A. M. Jr. Phillips, H. A. Podoliak, D. L. Livingston, R. F. Dumas, and R. W. Thoesen: Effect of dietary protein on the sensitivity of brook trout to a pyridoxine deficient deficiency. *N. Y. State Conserv. Dept. Fish Res. Bull.*, **23**, 67-71 (1959).
- 13) R. W. Hardy, J. E. Halver, and E. L. Brannon: Effect of dietary protein level on the pyridoxine requirement and disease resistance of chinook salmon, in "Finfish Nutrition and Fishfeed Technology" vol. II. (ed. by J. E. Halver and K. Tiews), Heeneman, Berlin, 1979, pp. 253-260.
- 14) Association of Official Analytical Chemists, in "Official Method of Analysis" (ed. by W. Horwite) 14th ed. Washington, D.C., USA,

- 1984, 1018 pp.
- 15) S. Y. Shiau and G. S. Suen: Estimation of the niacin requirements for tilapia fed diets containing glucose or dextrin. *J. Nutr.*, **122**, 2030–2036 (1992).
- 16) S. Y. Shiau and T. S. Hsu: L-Ascorbyl-2-sulfate has equal antiscorbutic activity as L-ascorbyl-2-monophosphate for tilapia, *Oreochromis niloticus* x *O. aureus*. *Aquaculture*, **133**, 147–157 (1995).
- 17) G. P. Tryfiates and S. Sattangi: Separation of vitamin B₆ compounds by paired ion high performance liquid chromatography. *J. Chromatogr.*, **227**, 181–186 (1982).
- 18) S. Y. Shiau, H. L. Yu, S. Hwa, S. Y. Chen, and S. I. Hsu: The influence of carboxymethylcellulose on growth, digestion, gastric emptying time and body composition of tilapia. *Aquaculture*, **70**, 345–354 (1988).
- 19) S. Y. Shiau and H. S. Liang: Carbohydrate utilization and digestibility by tilapia, *Oreochromis niloticus* x *O. aureus*, are affected by chromic oxide inclusion in the diet. *J. Nutr.*, **125**, 976–982 (1995).
- 20) S. Y. Shiau and M. J. Chen: Carbohydrate utilization by tilapia (*Oreochromis niloticus* x *O. aureus*) as influenced by different chromium sources. *J. Nutr.*, **123**, 1747–1753 (1993).
- 21) E. Casillas, J. Sundquist, and W. E. Ames: Optimization of assay conditions for, and the selected tissue distribution of, alanine aminotransferase and aspartate aminotransferase of English sole, *Parophrys vetulus girard*. *J. Fish. Biol.*, **21**, 197–204 (1982).
- 22) N. K. Agrawal and C. L. Mahajan: Haematological and haematopoietic studies in pyridoxine deficient fish, *Channa punctatus* Bloch. *J. Fish Biol.*, **22**, 91–103 (1983).
- 23) K. R. Robbins, H. W. Norton, and D. H. Baker: Estimation of nutrient requirements from growth data. *J. Nutr.*, **109**, 1710–1714 (1979).
- 24) K. W. Wang, T. Takeuchi, and T. Watanabe: Effects of dietary protein levels on growth of *Tilapia nilotica*. *Nippon Suisan Gakkaishi*, **51**, 133–140 (1985).
- 25) M. A. Mazid, Y. Tanaka, T. Katayama, M. A. Rahman, K. L. Simpson, and C. O. Chichester: Growth response of *Tilapia zilli* fingerlings fed isocaloric diets with variable protein levels. *Aquaculture*, **18**, 115–122 (1979).
- 26) K. Jauncey: The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapia (*Sarotherodon mossambicus*). *Aquaculture*, **27**, 97–109 (1982).
- 27) National Research Council, 1981, Nutrient Requirements of Domestic Animals. No. 16, Nutrition Requirements of Cold-water Fishes. National Academy Press, Washington, D.C., USA, 1981, p. 63.
- 28) J. E. Halver: The vitamins, in "Fish Nutrition 2nd edition", (ed by J. E. Halver), Academic Press, New York, N.Y., 1989, pp. 31–109.
- 29) B. Woodward: Dietary vitamin requirements of cultured young fish, with emphasis on quantitative estimates for salmonids. *Aquaculture*, **124**, 133–168 (1994).
- 30) C. B. Cowey: Use of synthetic diets and biochemical criteria in the assessment of nutrient requirement of fish. *J. Fish. Res. Board Can.*, **33**, 1040–1045 (1976).
- 31) C. E. Smith: Progress in sport fishery research. U.S. Fish Wildl. Serv. Bur. Sport Fish. Wildl. FGL, **54**: 52 (1967).
- 32) C. E. Smith, M. Brin, and J. E. Halver: Biochemical, physiological, and pathological changes in pyridoxine-deficient rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Board Can.*, **31**, 1893–1898 (1974).
- 33) M. L. Scott, M. C. Nesheim, and R. J. Young: The vitamin, in "Nutrition of the Chicken" (2nd ed.), M. L. Scott and Associates, Ithaca, N.Y., 1976, pp. 114–272.