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Effects of Dietary Vitamin B_6 Supplementation on Fillet Fatty Acid Composition and Fatty Acid Metabolism of Rainbow Trout Fed Vegetable Oil Based Diets

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ABSTRACT: Fish oil replacement in aquaculture feeds results in major modifications to the fatty acid makeup of cultured fish. Therefore, in vivo fatty acid biosynthesis has been a topic of considerable research interest. Evidence suggests that pyridoxine (vitamin B_6) plays a role in fatty acid metabolism, and in particular, the biosynthesis of LC-PUFA has been demonstrated in mammals. However, there is little information on the effects of dietary pyridoxine availability in fish fed diets lacking LC-PUFA. This study demonstrates a relationship between dietary pyridoxine supplementation and fatty acid metabolism in rainbow trout. In particular, the dietary pyridoxine level was shown to modulate and positively stimulate the activity of the fatty acid elongase and Δ-6 and Δ-5 desaturase enzymes, deduced by the whole-body fatty acid balance method. This activity was insufficient to compensate for a diet lacking in LC-PUFA but does highlight potential strategies to maximize this activity in cultured fish, especially when fish oil is replaced with vegetable oils.

KEYWORDS: *aquaculture, ALA, desaturase, DHA, elongase, EPA, fish oil replacement, pyridoxine, vitamin B_6*

INTRODUCTION

Due to economical and environmental constraints, the aquaculture industry is replacing fish oil with suitable alternatives in feed formulations. These alternative oils commonly contain polyunsaturated fatty acids (PUFA) with 18 carbon atoms (C_{18}), mainly represented by linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA; 18:3n-3) and variable amounts of saturated and monounsaturated fatty acids (SFA and MUFA, respectively). However, none of them contain long-chain polyunsaturated fatty acids (LC-PUFA). Consequently, their utilization in aquafeed results in the dilution in the content of the human health promoting n-3 LC-PUFA in cultured products.[1] Nevertheless, fish can produce n-3 LC-PUFA and n-6 LC-PUFA from dietary ALA and LA, respectively, via a series of alternating desaturase and elongase steps.[2] This metabolic capability has been reported to be insufficient to fully compensate for the reduction of dietary LC-PUFA, but has been shown to be more efficient in freshwater rainbow trout (*Oncorhynchus mykiss*) in comparison to other species.[3,4]

The activity of desaturase and elongase enzymes is known to be regulated by several nutritional and non-nutritional factors.[5] Among the nutritional factors, vitamin B_6 (pyridoxine), in addition to its main role in protein metabolism, has been speculated to play an important role as a cofactor or activator in the LC-PUFA biosynthetic pathway[6−8] and in general fatty acid metabolism.[9−11]

In vivo, the active form of pyridoxine is pyridoxal phosphate (PLP, pyridoxal-5′-phosphate, P5P), which is a cofactor for several enzymes involved in a variety of biochemical reactions, some of which still require elucidation.[12] In mammalians, previous investigations on pyridoxine have revealed close links between dietary pyridoxine content and the metabolism of fatty acids.[9,10,13] Cunnane et al.[9] showed that, in rats, pyridoxine deficiency causes an accumulation of LA, ALA, and γ-linolenic acid (GLA, 18:3n-6) in blood serum and speculated that both LA desaturation and GLA elongation might be impaired by pyridoxine deficiency. The same study also found an unrecognized effect influenced by pyridoxine on EFA transport between triglyceride (TG) and phospholipids (PL). A similar observation was made by Bordoni et al.[14] on EFA metabolism in rats fed pyridoxine-deficient diets. Likewise, in an epidemiological study in humans, it was reported that pyridoxine status was linked with low n-3 PUFA bioconversion, but n-6 PUFA bioconversion was unaffected.[15] In addition, the effects of pyridoxine-deficient diets on fatty acid metabolism in rats was assessed by Tsuge et al.,[6] who reported that it acted at the peroxisomal level. In particular, the activity of acyl-CoA oxidase (ACOX), the enzyme that catalyzes the first step in the peroxisomal β-oxidation of LC-PUFA, was shown to be significantly reduced.[6]

In fish, a previous study conducted on Atlantic salmon (*Salmo salar*) fed a nonsupplemented pyridoxine diet demonstrated reduced lipid content in muscle and reduced levels of n-3 PUFA and DHA in hepatic PL.[16] However, in the same study, it was reported that the hepatic desaturation and elongation of [1-^{14}C]ALA, following intraperitoneal injection of this radiolabeled fatty acid, was found to be unaffected by dietary

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pyridoxine. Interestingly, in another study aiming to determine the effects of dietary pyridoxine fortification on growth performance in rainbow trout fed a fish oil based diet, it was unexpectedly noted that the fatty acid composition of fish fillets was affected by dietary pyridoxine content.[17] This study clearly demonstrated that the percentage of n-3 LC-PUFA in trout fillets, in particular DHA, increased significantly with the increase of dietary pyridoxine content.[17] Importantly, in this study the diets contained an abundant amount of preformed n-3 LC-PUFA.

At this juncture it is clear that pyridoxine is a key micronutrient that can affect in vivo fatty acid metabolism, but its mechanisms of action and potential roles in improving LC-PUFA biosynthesis and deposition have not yet been fully elucidated. Moreover, given the promising results of increased n-3 LC-PUFA in the fillets of trout fed fish oil based diets fortified with pyridoxine,[17] and the known reduction of n-3 LC-PUFA in fish fed a vegetable oil based diet,[1] it would seem timely and pertinent to evaluate if dietary pyridoxine fortification can minimize this detrimental effect of fish oil replacement in aquafeed. The aim of the present study was therefore to explore the potential effects of dietary pyridoxine levels on fatty acid metabolism in fish fed diets lacking LC-PUFA. Specifically, the main objective of this study was to verify if dietary pyridoxine fortification could positively stimulate LC-PUFA biosynthesis and/or retention and deposition in rainbow trout when fed plant oil based diets containing an abundance of the fatty acid precursors, LA and ALA, and limited concentrations of LC-PUFA.

MATERIALS AND METHODS

Animals and Husbandry. Rainbow trout (30 g average initial mean weight) were obtained from DPI Victoria (Snobs Creek, VIC, Australia), transported to the Deakin University Aquaculture Research Facility (Warrnambool, VIC, Australia), and used in the present experiment. All procedures implemented over the course of this experiment were approved by the Deakin University Animal Welfare Committee (AWC ref A33/2010).

Prior to experimentation, fish were acclimatized to the new environmental conditions for 4 weeks while being fed a commercial diet twice daily at 2% of their body weight. The feeding experiment was conducted in a thermostatically controlled tank (1000 L capacity) recirculating system connected to a physical and biological filtration plant. The system was maintained on a 12 h light−12 h dark cycle at 14.0 ± 1.08 °C. Water quality parameters were measured every second day using Aquamerck test kits (Merck, Darmstadt, Germany) and maintained within optimal levels.

Following the acclimation period, 360 juveniles were individually weighed and randomly allocated in triplicate groups into 18 tanks (20 fish per tank; 3 tanks per treatment). At the commencement of the experiment, an additional sample of 10 fish was taken, euthanized in excess anesthetic, and stored at −20 °C until subsequent analysis. Fish were fed one of the four formulated diets twice daily to apparent satiation at 9:00 a.m. and 4:00 p.m. for a 60 day rearing period. Feed consumption was recorded weekly, and mortalities were recorded throughout the experimental period. Feces were collected during the last week of the feeding trial. At the end of the experiment, all fish were individually weighed, and eight fish from each experimental tank (24 fish per treatment) were randomly sampled, euthanized, and stored at −20 °C for further analysis.

Experimental Diets. Four isoproteic, isolipidic, semipurified experimental diets, varying only in their pyridoxine (vitamin B_6) content, were formulated. Fishmeal was included in the formulation to guarantee good palatability of the diet, but at very low concentration (9%) to minimize the inclusion of n-3 LC-PUFA derived from its residual oil. The added lipid source was a blend of canola (rapeseed) and linseed oils (70:30) (Table 1). The vitamin and mineral mix was purposely formulated, as described earlier,[18] with the exclusion of pyridoxine. Pyridoxine hydrochloride (PN, Sigma, St. Louis, MO, USA) was added to the dry ingredient mixture of the test diets after being properly mixed with dextrin to provide graded concentrations of 1.5, 3.8, 9.4, and 23.4 mg pyridoxine kg^{-1} diet. The experimental diets were manufactured following standard procedures implemented at Deakin University, as previously described by Brown.[19] The dry pellets were then transferred to sealed plastic bags and stored at −20 °C until used. The four experimental diets/treatments were designated T1−T4 in order of their pyridoxine content, which ranged from 1.5 to 23.4 mg kg^{-1}, respectively.

Chemical Analysis. The chemical composition of experimental diets and biological samples was analyzed in triplicate and conducted according to standard methods.[20] Briefly, moisture was determined by drying samples in an oven at 80 °C to constant weight. Protein content (N × 6.25) was determined using an automated Kjeltech 2300 (Foss Tecator, Geneva, Switzerland). Ash content was determined by incinerating samples (approximately 0.5 g) in a muffle furnace (Wit, C&L Tetlow, Melbourne, Australia) at 550 °C for 18 h. Lipid was determined by a chloroform/methanol (2:1) cold extraction. Nitrogen-free extract (NFE) was calculated by difference, and dietary energy content was calculated on the basis of 23.6, 39.5, and 17.2 kJ g^{-1} of protein, fat, and carbohydrate, respectively. Following the lipid extraction, fatty acids were esterified into methyl esters using an acid-catalyzed methylation method and analyzed by gas chromatography according to the methods previously used in the laboratory.[21] Briefly, 0.5 mL of C23:0 (Sigma-Aldrich, Inc.) was added to each sample as an internal standard. Fatty acid methyl esters were isolated and identified using a Shimadzu GC 17A (Shimadzu, Chiyoda-ku, Tokyo, Japan) equipped with an Omegawax 250 capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness; Supelco, Bellefonte, PA, USA), a flame ionization detector (FID), a Shimadzu AOC-20i autoinjector, and a split injection system (split ratio 50:1). Fatty acids were identified relative to known external standards (37-Component FAME Mix: PUFA No. I , marine source; PUFA No. II, animal source; and PUFA No. III, menhaden oil, all from Supleco). The resulting peaks were then corrected by the theoretical relative FID response factors and quantified relative to the internal standard.

Determination of Pyridoxine (Vitamin B_6). The analysis for pyridoxine content of the diets, fish whole bodies, and feces was implemented on blind samples by an accredited external laboratory (National Measurement Institute, Department of Innovation, Industry, Science and Research, Melbourne, VIC, Australia). Briefly, pyridoxine content was determined using reverse phase high-performance liquid chromatography (HPLC) as described by Bergaentzle et al.[22] Samples were homogenized and extracted in acetate buffer at pH 4.5, prior to using acid phosphatase and glyoxylic acid to convert various vitamin B_6 forms into pyridoxal form. The converted extract was then filtered, and an aliquot was reacted with borohydride to reduce pyridoxal to pyridoxine. Pyridoxine in the sample filtrate was determined by reverse phase HPLC on a C18 Nova-Pak column using fluorescence detection. Separation was made using a mobile phase of 0.0005 M hexane sulfonic acid (sodium salt)/0.05 M potassium dihydrogen phosphate in 8% acetonitrile/92% water, pH 2.5. Detection was performed by fluorescence using an excitation wavelength of 295 nm and emission wavelength of 390 nm.

Digestibility and Fatty Acid Metabolism Estimation. Chromic oxide in the diets and feces were estimated according to the method of Furukawa and Tsukahara.[23] Estimates of apparent digestibility coefficients (ADC) for dry matter, lipid, and total and individual fatty acids were calculated using standard formulas.[24]

The assessment of in vivo fish fatty acid metabolism in rainbow trout was performed using the whole-body fatty acid balance method, as described previously in detail.[25,26] Briefly, this method involves a series of computations that can be better described in two separate steps. In the first step the net appearance/disappearance of each individual fatty acid is deduced by means of a mass balance determined by the difference between total fatty acid gain (= final fatty acid content − initial fatty acid content) and the net fatty acid intake (= total fatty acid intake − fatty acid excretion in feces). Resulting data are then

Table 1. Ingredients and Proximate Composition of the Experimental Diets

	dietary treatments[a]			
	T1	T2	T3	T4
ingredients (g kg^{-1})				
fish meal[b]	90	90	90	90
defatted soybean meal[b]	287	287	287	287
wheat gluten[b]	4	4	4	4
whey protein[c]	135	135	135	135
lupin flour[d]	180	180	180	180
wheat flour[e]	6	6	6	6
gelatin[f]	28	28	28	28
CMC[g]	20	20	20	20
mineral mix[h]	40	40	40	40
vitamin mix[i]	30	30	30	30
choline[f]	5	5	5	5
Cr_2O_3[f]	2	2	2	2
canola oil[e]	120	120	120	120
linseed[j]	50	50	50	50
mg kg^{-1}				
pyridoxine hydrochloride[f] (vitamin B_6)	1.50	3.75	9.38	23.44
dextrin[f]	2998.50	2996.25	2990.62	2976.56
proximate composition (mg g^{-1})				
moisture	53.7	55.1	56.5	51.7
crude lipid	197.3	201.9	196.1	199.8
crude protein	396.6	396.6	396.7	403.0
ash	67.8	67.8	68.4	67.2
NFE[k]	256.1	278.6	282.3	278.3
energy[l] (kJ g^{-1})	21.6	22.1	22.0	22.2

[a]Diet abbreviations: T1, vitamin B_6, 2.4 mg/kg; T2, vitamin B_6, 4.6 mg/kg; T3, vitamin B_6, 7.8 mg/kg; T4, vitamin B_6, 19.0 mg/kg. [b]Ridley Agriproducts, Narangba, QLD, Australia. [c]Warrnambool cheese and butter factory, Warrnambool, VIC, Australia. [d]Irwin Valley, Palmyro, WA, Australia. [e]Black and Gold, Tooronga, VIC, Australia. [f]Sigma-Aldrich, Inc. St. Louis, MO, USA. [g]Carboxymethyl cellulose; BDH Laboratory Supplies, Poole, U.K. [h]Mineral mix supplemented (in g/kg diet): $Ca(C_6H_{10}O_6)\cdot 5H_2O$, 348.49; $Ca(H_2PO_4)_2\cdot H_2O$, 136; $MgSO_4\cdot 7H_2O$, 132; K_2HPO_4, 240; $NaH_2PO_4\cdot H_2O$, 88; NaCl, 45; $AlCl_3\cdot 6H_2O$, 0.15; KI, 0.15; $CuSO_4\cdot 5H_2O$, 0.5; $MnSO_4\cdot H_2O$, 0.7; $CoCl_2\cdot 6H_2O$, 1.0; $ZnSO_4\cdot 7H_2O$, 3.0; $NaSeO_3$, 0.01; $FeSO_4$, 5.0. [i]Pyridoxine-free vitamin mix (in g/kg diet): ascorbic acid, 50; DL-calcium pantothenate, 5; choline bitartrate, 100; inositol, 5; menadione, 2; niacin, 5; riboflavin, 3; thiamin hydrochloride, 0.5; DL-α-tocopherol acetate (250 IU/g), 8; vitamin A acetate (20000 IU/g), 5; biotin, 0.05; cholecalciferol (1 μg = 40 IU), 0.002; folic acid, 0.18; vitamin B_{12}, 0.002; α-cellulose, 816.266. [j]Sceney Chemical Pty., Ltd., Sunshine, VIC, Australia. [k]NFE (nitrogen-free extract): calculated by difference. [l]Calculated on the basis of 23.6, 39.5, and 17.2 kJ g^{-1} of protein, fat, and carbohydrate, respectively.

transformed from milligrams to micromoles of appeared/disappeared fatty acids per gram of body weight per day. The second step then involves a series of backward computations along all of the known fatty acid bioconversion pathways (n-3 PUFA, n-6 PUFA, and SFA + MUFA) so that the fate of each individual fatty acid toward bioconversion, oxidation, or deposition can be determined. Eventually, obtained data, described as apparent in vivo enzyme activity, can be reported in absolute terms (μmol g $fish^{-1}$ day^{-1}) or as a percentage of substrate availability.

Statistical Analysis. All data were reported as the mean ± the standard error of mean (n = 3; N = 12). Comparisons among treatments were analyzed by one-way analysis of variance (ANOVA) at a significance level of 0.05 following confirmation of normality and homogeneity of variance. When significant differences were detected by ANOVA, data were subjected to a Student−Newman−Keuls post hoc test for identifying homogeneous subsets. ANOVA was computed using SPSS ver. 17.0 (SPSS Inc., Chicago, IL, USA). All data were also analyzed by linear regression, relative to dietary pyridoxine content, and when appropriate, further curvilinear trends (Michaelis−Menten) were also computed. Regression analyses were computed using GraphPad Prism ver. 5.02 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Diet Composition. The analyzed pyridoxine (vitamin B_6) concentrations of the four experimental diets were 2.4, 4.6, 7.8, and 19.0 mg kg^{-1} of diet for T1, T2, T3, and T4, respectively. These values were slightly discrepant with the diet formulation, which were 1.5, 3.75, 9.38, and 23.44 mg kg^{-1}, respectively, indicating that some pyridoxine was present in the raw materials used for diet formulation (as in T1 and T2, more pyridoxine was measured than what was added) and that some of the pyridoxine in the two treatments with highest addition (T3 and T4) was lost during the diet manufacturing process. However, the recorded pyridoxine contents of the four experimental diets were adequately suited for the purpose of this study.

The proximate compositions of the four diets were similar across all four treatments, and the total lipid contents ranged from 196.1 to 201.9 mg kg^{-1} of dry diet (Table 1). Fatty acid analysis of different experimental diets reflected the objective of the formulations, and their fatty acid compositions were almost identical (Table 2). The percentages (on total fatty acids) of eicosapentaenoic acid (20:5n-3; EPA), docosahexaenoic acid (22:6n-3; DHA), and arachidonic acid (20:4n-6; ARA) in each experimental diet were minimal, and the concentrations of LA and ALA in all diets were constant (Table 2).

Growth Performance, Feed Utilization, and Biometry. During the entire feeding trial, all experimental diets were readily accepted by fish and mortality was very low (>1%) and independent of the dietary treatments. Throughout the 60 day

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Table 2. Fatty Acid Composition of Experimental Diets

	dietary treatments[a]			
fatty acid	T1	T2	T3	T4
14:0	4.2 (0.4)	4.3 (0.5)	4.0 (0.4)	4.5 (0.5)
16:0	54.8 (5.8)	56.4 (6.0)	54.5 (5.8)	55.0 (5.9)
16:1n-7	3.1 (0.3)	3.2 (0.3)	3.0 (0.3)	3.0 (0.3)
18:0	25.8 (2.7)	26.4 (2.8)	25.5 (2.7)	25.7 (2.7)
18:1n-9	422.1 (44.8)	418.3 (44.4)	419.9 (44.6)	418.9 (44.6)
18:1n-7	28.2 (3.0)	28.4 (3.0)	28.9 (3.1)	28.8 (3.1)
18:2n-6	196.1 (20.8)	196.6 (20.9)	198.5 (21.1)	196.8 (21.0)
18:3n-6	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
18:3n-3	180.7 (19.2)	181.3 (19.3)	181.1 (19.3)	181.0 (19.3)
18:4n-3	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
20:0	4.0 (0.4)	4.2 (0.4)	4.0 (0.4)	4.2 (0.5)
20:1[b]	6.8 (0.7)	7.5 (0.8)	6.9 (0.7)	7.0 (0.7)
20:2n-6	0.7 (0.1)	0.6 (0.1)	0.6 (0.1)	0.5 (0.1)
20:3n-6	0.2 (0.0)	0.2 (0.0)	0.2 (0.0)	0.1 (0.0)
20:4n-6	0.7 (0.1)	0.6 (0.1)	0.6 (0.1)	0.6 (0.1)
20:3n-3	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
20:4n-3	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
20:5n-3	2.7 (0.3)	2.7 (0.3)	2.9 (0.3)	2.8 (0.3)
22:0	2.8 (0.3)	2.9 (0.3)	2.7 (0.3)	2.9 (0.3)
22:1[c]	1.2 (0.1)	0.5 (0.0)	0.5 (0.1)	0.7 (0.1)
22:2n-6	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
22:4n-6	0.2 (0.0)	0.3 (0.1)	0.1 (0.0)	0.2 (0.0)
22:5n-3	2.0 (0.2)	1.6 (0.2)	1.2 (0.1)	1.5 (0.2)
22:6n-3	5.6 (0.6)	5.5 (0.6)	5.3 (0.6)	5.3 (0.6)
total	942.1 (100.0)	942.0 (100.0)	940.9 (100.0)	940.0 (100.0)
SFA	91.5 (9.7)	94.3 (10.0)	90.7 (9.6)	92.3 (9.8)
MUFA	461.3 (49.0)	457.8 (48.6)	459.2 (48.8)	458.5 (48.8)
PUFA	389.3 (41.3)	389.8 (41.4)	391.0 (41.5)	389.3 (41.4)
n-6 PUFA	198.1 (21.0)	198.5 (21.1)	200.3 (21.3)	198.6 (21.1)
n-6 LC-PUFA	1.9 (0.1)	1.8 (0.2)	1.7 (0.2)	1.6 (0.1)
n-3 PUFA	191.2 (20.3)	191.3 (20.3)	190.7 (20.3)	190.7 (20.3)
n-3 LC-PUFA	10.3 (1.1)	9.9 (1.0)	9.4 (1.0)	9.6 (1.0)

[a]Milligrams per gram lipid (w/w % of total fatty acids given in parentheses). See Table 1 for diet abbreviations. [b]Sum of 20:1 isomers. [c]Sum of 22:1 isomers.

experimental period, fish recorded a weight gain in excess of 4.2-fold their initial weight, regardless of the dietary treatment (Table 3), with no significant differences (ANOVA, $P > 0.05$) among treatments in relation to any of the recorded parameters relative to performance, feed utilization, and fish biometry. The values recorded for final mean weight, specific growth rate (SGR), and weight gain percent ranged from 155.8 to 166.8 g, from 2.34 to 2.46, and from 306.7 to 338%, respectively. The average feed consumption and food conversion ratio (FCR) values were approximately 160 g fish^{-1} and 1.25, respectively.

Proximate Composition and Pyridoxine Content. The proximate compositions of trout whole bodies and fillets at the beginning and end of the experiment are reported in Table 4. No statistically significant (ANOVA, $P > 0.05$) differences were recorded for ash, moisture, protein, or lipid contents in whole bodies or fish fillets among the four dietary treatments. A statistically significant linear regression was observed for fillet ash content ($P = 0.027$).

Pyridoxine (vitamin B_6) content in fish whole bodies showed a gradual increase with the increasing level of its dietary inclusion, ranging from 1.40 ± 0.50 mg/kg (T1) to 1.97 ± 0.18 mg/kg (T4), but no statistically significant differences could be observed among the four dietary treatments ($P > 0.05$, ANOVA and regression). The pyridoxine content in feces from all fish groups was below the minimum detection limit (0.2 mg/kg) of the methodology used.

Fillet and Whole-Body Fatty Acid Composition. The fatty acid compositions of total lipids of trout fillets and whole bodies are reported in Tables 5 and 6, respectively, and all values are reported as milligrams of fatty acid per gram of lipid. Regardless of the dietary treatment, the highest proportion of the SFA class was composed of 16:0 followed by 18:0 in trout fillet and whole-body samples. Similarly, the MUFA content was mainly represented by 18:1n-9 followed by 18:1n-7 and 20:1 in both tissues. Of the total PUFA, the amount of n-3 PUFA was almost identical to that of n-6 PUFA, in both fillets and whole bodies, with LA and ALA accounting for the majority, respectively. Irrespective of the dietary treatment and in both tissues, the LA content was much higher (~150 mg g^{-1}) than that of ALA (~97 mg g^{-1}).

In fillets (Table 5), ANOVA detected statistically significant differences ($P < 0.05$) for 18:1n-9 (T1 higher than T3), 20:1 (T1 higher than all other treatments), and total MUFA (T1 higher than T3). Interestingly, a statistically significant linear regression ($P = 0.038$) was recorded for docosapentaenoic acid (22:5n-3; DPA), with its content increasing from 4.27 to 5.85 mg g^{-1}, when moving from T1 to T4. Although not

Table 3. Growth, Feed Utilization, and Biometry Data of Rainbow Trout Fed Vitamin B_6 Supplemented Semipurified Diets at the End of the 60 Day Feeding Trial

	dietary treatments[a]				
	T1	T2	T3	T4	regression P value
initial mean wt (g)	38.61 ± 0.25	38.30 ± 0.52	38.02 ± 0.24	38.51 ± 0.50	ns
mean final wt (g)	161.12 ± 4.45	155.79 ± 3.22	166.82 ± 4.60	156.70 ± 4.34	ns
weight gain[b] (%)	317.45 ± 13.41	306.72 ± 5.48	338.60 ± 9.41	307.29 ± 15.33	ns
SGR[c] (% day^{-1})	2.38 ± 0.05	2.34 ± 0.02	2.46 ± 0.04	2.34 ± 0.06	ns
feed consumption (g/fish)	159.68 ± 2.62	163.97 ± 6.47	167.48 ± 0.63	166.05 ± 5.92	ns
FCR[d]	1.24 ± 0.03	1.32 ± 0.02	1.23 ± 0.05	1.33 ± 0.01	ns
PER[e] (% day^{-1})	1.93 ± 0.04	1.81 ± 0.03	1.94 ± 0.07	1.77 ± 0.02	ns
FDR[f] (% day^{-1})	4.16 ± 0.12	4.12 ± 0.05	4.13 ± 0.06	4.07 ± 0.06	ns
DP[g] (%)	83.75 ± 0.63	82.04 ± 0.96	84.06 ± 0.45	84.15 ± 0.24	ns
HSI[h] (%)	1.47 ± 0.07	1.46 ± 0.06	1.26 ± 0.06	1.51 ± 0.04	ns
FY[i] (%)	50.99 ± 1.32	49.57 ± 0.66	52.75 ± 1.56	52.44 ± 0.73	ns

[a]Data represent the mean ± SEM (n = 3, N = 12). No statistically significant differences were detected by ANOVA. See Table 1 for diet abbreviations [b]Weight gain % = (final weight − initial weight) × (initial weight)$^{-1}$ × 100. [c]Specific growth rate = [Ln(final weight) − Ln(initial weight)] × (number of days)$^{-1}$ × 100. [d]Feed conversion ratio = (dry feed fed) × (wet weight gain)$^{-1}$. [e]Protein efficiency ratio = (final weight − initial weight) × (mass of protein consumed)$^{-1}$. [f]Fat deposition rate = [Ln(final lipid) − Ln(initial lipid)] × (number of days)$^{-1}$. [g]Dress-out percentage = (CW × BW $^{-1}$) × 100, where CW is carcass gutted weight (g) and BW is body weight (g). [h]Hepatosomatic index percent = (LW × BW $^{-1}$) × 100, where LW is liver weight (g). [i]Fillet yield percentage = (FW × BW $^{-1}$) × 100, where FW is fillet weight (g).

Table 4. Proximate Composition of Fillets and Whole Bodies and Vitamin B_6 Concentration in Whole Bodies of Rainbow Trout Fed Vitamin B_6 Supplemented Experimental Diet over a 60 Day Period

	dietary treatments[a]				
	T1	T2	T3	T4	regression P value
fillet (%)					
protein	17.66 ± 0.35	17.72 ± 0.20	17.47 ± 0.21	17.53 ± 0.17	ns
moisture	69.70 ± 1.17	70.66 ± 0.34	72.16 ± 0.77	71.33 ± 0.69	ns
lipid	11.47 ± 0.83	10.45 ± 0.29	9.22 ± 0.76	10.00 ± 0.53	ns
ash	1.17 ± 0.01	1.17 ± 0.02	1.15 ± 0.01	1.14 ± 0.01	0.027
whole body (%)					
protein	15.33 ± 0.23	15.77 ± 0.10	16.11 ± 0.16	15.23 ± 0.43	ns
moisture	66.52 ± 0.38	66.31 ± 0.42	66.97 ± 0.28	67.19 ± 0.46	ns
lipid	16.34 ± 0.43	16.25 ± 0.36	15.20 ± 0.43	15.83 ± 0.29	ns
ash	1.81 ± 0.09	1.67 ± 0.05	1.72 ± 0.07	1.75 ± 0.05	ns
vitamin B_6 (mg kg^{-1})	1.40 ± 0.50	1.50 ± 0.30	1.70 ± 0.30	1.97 ± 0.18	ns

[a]Data represent the mean ± SEM (n = 3, N = 12). No statistically significant differences were detected by ANOVA. See Table 1 for diet abbreviations.

statically significant ($P > 0.05$), similar apparent trends were recorded for EPA, DHA, and, in general, total n-3 LC-PUFA.

In whole bodies (Table 6), the only statistically significant difference ($P < 0.05$) recorded by ANOVA was for 20:1, with T2 recording a lower content than all other treatments. The regression analysis recorded a statistically significant linear correlation between dietary pyridoxine content and 22:4n-6 and DPA whole-body content ($P = 0.009$ and $P = 0.012$, respectively). As reported for the fillet, apparent trends (not statistically significant, $P > 0.05$) of increased EPA, DHA, and total n-3 LC-PUFA contents were also evident in whole bodies of fish fed the four different experimental diets.

In whole bodies and fillets, the intermediate products of the n-3 and n-6 LC-PUFA bioconversion pathways, such as 18:4n-3 and 18:3n-6, showed an increasing trend with the increased dietary pyridoxine content from T1 to T3 and then slightly decreased in T4, although these trends were not significantly described by the regression analysis.

Fatty Acid Metabolism. Although the whole-body fatty acid balance method generated a large amount of data, describing the fate of all individual fatty acids toward β-oxidation, bioconversion, and deposition, no statistically significant differences were detected by ANOVA and, therefore, not all data were reported.

The key results of the whole-body fatty acid balance method, grouped by general enzymatic activity on the three main fatty acid pathways (SFA and MUFA, n-6 PUFA, and n-3 PUFA) are depicted in Table 7, and values are reported as apparent in vivo enzyme activity expressed as nanomoles of enzyme product produced per gram of body weight per day (nmol g^{-1} day^{-1}). Although not significantly different, T1 demonstrated a slightly higher fatty acid de novo production, lower β-oxidation, and higher elongation and Δ-9 desaturation of SFA and MUFA compared to the other three treatments. The β-oxidation of n-6 and n-3 PUFA recorded very similar values (~1700 nmol g^{-1} day^{-1}) and was not affected by dietary treatment. The elongation of n-3 PUFA was proportionally higher than that of n-6 PUFA (~800 vs ~130 nmol g^{-1} day^{-1}, respectively) and was not affected by dietary treatment. Similarly, the apparent in vivo Δ-6 and Δ-5 desaturase activities acting on n-3 PUFA were higher than that of n-6 PUFA (~700 and ~280 nmol g^{-1} day^{-1} vs ~800 and ~300 nmol g^{-1} day^{-1}, respectively) and were not

Table 5. Fatty Acid Composition (Milligrams per Gram of Lipid) of Rainbow Trout Fillets under Different Treatments at the End of the 60 Day Feeding Trial

	dietary treatments[a]				
fatty acid	T1	T2	T3	T4	regression P value[b]
	8.43 ± 0.18	8.18 ± 0.15	8.27 ± 0.38	8.77 ± 0.52	ns
16:0	95.86 ± 1.94	93.70 ± 1.69	92.38 ± 3.02	99.28 ± 2.44	ns
16:1n-7	11.30 ± 0.77	11.04 ± 1.10	10.23 ± 0.66	12.45 ± 0.68	ns
18:0	33.93 ± 0.21	33.56 ± 0.40	34.20 ± 0.93	34.05 ± 0.74	ns
18:1n-9	396.79 ± 2.60b	388.56 ± 2.23ab	382.46 ± 4.67a	388.80 ± 2.04ab	ns
18:1n-7	26.31 ± 0.08	25.96 ± 0.08	26.12 ± 0.65	26.63 ± 0.78	ns
18:2n-6	151.40 ± 2.49	152.62 ± 1.71	151.06 ± 2.58	148.52 ± 0.63	ns
18:3n-6	3.30 ± 0.19	3.49 ± 0.29	3.65 ± 0.10	3.60 ± 0.09	ns
18:3n-3	97.44 ± 2.88	98.38 ± 3.56	96.28 ± 3.41	94.35 ± 1.40	ns
18:4n-3	10.99 ± 0.62	11.94 ± 1.10	12.41 ± 0.23	12.33 ± 0.40	ns
20:0	2.32 ± 0.04	2.26 ± 0.06	2.27 ± 0.03	2.29 ± 0.01	ns
20:1[c]	13.62 ± 0.27b	12.35 ± 0.16a	12.60 ± 0.14a	12.85 ± 0.27a	ns
20:2n-6	5.76 ± 1.20	6.46 ± 0.25	6.47 ± 0.14	5.22 ± 1.06	ns
20:3n-6	0.65 ± 0.19	0.55 ± 0.07	0.57 ± 0.02	0.78 ± 0.22	ns
20:4n-6	3.08 ± 0.11	3.17 ± 0.11	3.57 ± 0.37	3.31 ± 0.20	ns
20:3n-3	3.98 ± 0.25	3.80 ± 0.34	3.86 ± 0.10	3.61 ± 0.05	ns
20:4n-3	4.08 ± 0.03	3.98 ± 0.16	4.36 ± 0.26	4.28 ± 0.13	ns
20:5n-3	7.22 ± 0.09	7.45 ± 0.40	8.32 ± 0.75	8.21 ± 0.43	ns
22:0	1.68 ± 0.04	1.68 ± 0.04	1.66 ± 0.03	1.67 ± 0.02	ns
22:1[d]	1.83 ± 0.13	1.51 ± 0.10	1.46 ± 0.15	1.46 ± 0.08	ns
22:2n-6	0.63 ± 0.04	0.64 ± 0.04	0.69 ± 0.02	0.58 ± 0.01	ns
22:4n-6	0.54 ± 0.02	0.54 ± 0.02	0.60 ± 0.05	0.61 ± 0.07	ns
22:5n-3	4.27 ± 0.06	4.34 ± 0.71	5.63 ± 0.62	5.85 ± 0.32	0.038
22:6n-3	35.90 ± 2.33	36.67 ± 0.70	41.61 ± 4.19	39.64 ± 3.28	ns
total	921.31 ± 8.72	912.84 ± 1.23	910.69 ± 1.63	919.13 ± 5.83	ns
SFA	142.23 ± 2.29	139.38 ± 1.66	138.77 ± 4.34	146.05 ± 3.46	ns
MUFA	449.85 ± 2.23b	439.42 ± 2.7ab3	432.87 ± 4.49a	442.19 ± 2.15ab	ns
PUFA	329.23 ± 7.66	334.04 ± 3.18	339.05 ± 2.28	330.88 ± 2.15	ns
n-6 PUFA	165.36 ± 2.82	167.47 ± 1.43	166.60 ± 1.92	162.61 ± 1.14	ns
n-6 LC-PUFA	10.66 ± 1.19	11.36 ± 0.15	11.89 ± 0.57	10.49 ± 0.85	ns
n-3 PUFA	163.87 ± 4.84	166.58 ± 1.79	172.45 ± 2.61	168.27 ± 2.72	ns
n-3 LC-PUFA	55.44 ± 2.57	56.25 ± 0.83	63.77 ± 5.15	61.60 ± 4.04	ns

[a]Data represent the mean ± standard error (n = 3; N = 12). Values in the same row with different letters are significantly different ($P < 0.05$; ANOVA and Student−Newman−Keuls post hoc test). See Table 1 for diet abbreviations. [b]P value of regression analysis is (relative to dietary content of pyridoxine) also reported at 5% level; ns = not significant. [c]Sum of 20:1 isomers. [d]Sum of 22:1 isomers.

affected by dietary treatment. However, regression analysis detected a statistically significant (P = 0.048) linear relationship for the apparent in vivo Δ-5 desaturase activity on n-6 PUFA, which was double in fish fed the diet with highest pyridoxine content (T4) compared to fish fed the diet with the lowest pyridoxine content (T1).

In Table 8, the results of fatty acid bioconversion along the n-6 LC-PUFA and n-3 LC-PUFA biosynthetic pathways are reported. No statistically significant differences were recorded by ANOVA, but the linear regression analysis demonstrated the existence of a statistically significant relationship for dietary pyridoxine content and apparent in vivo Δ-5 desaturase activity on n-6 PUFA (as above-reported) and for the elongation of 18:3n-6 to 20:3n-6 and 20:4n-6 (ARA) to 22:4n-6. All other individual fatty acid bioconversions showed an apparent trend of increased activity with the increase of pyridoxine dietary content, but the resultant slopes of these linear equations were not statistically different from zero. Therefore, data were plotted against dietary pyridoxine content and further analyzed by curvilinear regression (using the Michaelis−Menten equation for enzyme kinetics), and interesting trends were observed, clearly suggesting the existence of a direct effect of pyridoxine on the activity of these enzymes (Figures 1 and 2).

DISCUSSION

In the present study no pyridoxine-deficient treatment was present, and different dietary pyridoxine contents, varying from 2.4 to 19.0 mg kg^{-1}, did not affect overall growth performance, feed efficiency, and fish survival during the 60 day feeding trial in rainbow trout fed a vegetable oil based diet. This is in agreement with the results of a previous investigation on the same species in which fish oil based diets with various amounts of pyridoxine supplementation were fed[17] and in Atlantic salmon fed vegetable oil (soybean oil) and fish oil based diets supplemented with pyridoxine.[16,27,28] Similarly, the key fish biometrical parameters, such as hepatosomatic index, dress out percentage and fillet yield were not affected by the dietary pyridoxine content. This observation is in agreement with results (HSI values) previously reported for Atlantic salmon.[27] In contrast, a previous study by the same authors[16] demonstrated that Atlantic salmon fed pyridoxine-supplemented diets had significantly higher liver

Table 6. Fatty Acid Composition (Milligrams per Gram of Lipid) of Rainbow Trout Whole Bodies Fed the Four Experimental Diets Containing Different Pyridoxine Contents at the End of the 60 Day Feeding Trial

	dietary treatments[a]				
fatty acid	T1	T2	T3	T4	regression P value[b]
14:0	8.33 ± 0.30	8.42 ± 0.40	8.35 ± 0.10	8.80 ± 0.16	ns
16:0	87.36 ± 3.11	83.90 ± 1.40	83.37 ± 1.58	87.36 ± 0.97	ns
16:1n-7	11.16 ± 0.99	10.83 ± 0.87	10.57 ± 0.72	10.90 ± 0.60	ns
18:0	32.92 ± 0.51	32.93 ± 0.89	32.10 ± 0.76	34.41 ± 0.69	ns
18:1n-9	399.05 ± 5.42	383.79 ± 6.79	385.78 ± 2.94	386.61 ± 3.18	ns
18:1n-7	26.36 ± 0.89	25.45 ± 0.96	24.73 ± 0.39	24.50 ± 0.23	ns
18:2n-6	153.50 ± 1.09	151.05 ± 3.14	151.76 ± 1.95	150.61 ± 1.77	ns
18:3n-6	3.28 ± 0.30	3.63 ± 0.11	3.59 ± 0.40	3.31 ± 0.07	ns
18:3n-3	101.11 ± 1.46	96.84 ± 2.33	98.54 ± 2.21	96.52 ± 1.09	ns
18:4n-3	11.44 ± 0.81	13.49 ± 0.14	12.64 ± 1.27	11.62 ± 0.36	ns
20:0	2.43 ± 0.04	2.41 ± 0.02	2.41 ± 0.02	2.50 ± 0.08	ns
20:1[c]	13.56 ± 0.13b	12.75 ± 0.21a	13.37 ± 0.15b	13.52 ± 0.19b	ns
20:2n-6	6.77 ± 0.17	6.43 ± 0.24	7.07 ± 0.21	6.91 ± 0.25	ns
20:3n-6	0.49 ± 0.06	0.57 ± 0.03	0.51 ± 0.07	0.48 ± 0.02	ns
20:4n-6	3.08 ± 0.23	3.26 ± 0.11	3.32 ± 0.07	3.49 ± 0.25	ns
20:3n-3	4.38 ± 0.19	3.80 ± 0.16	4.48 ± 0.51	4.46 ± 0.18	ns
20:4n-3	4.33 ± 0.07	4.53 ± 0.08	4.55 ± 0.28	4.49 ± 0.11	ns
20:5n-3	7.93 ± 0.32	8.68 ± 0.92	8.14 ± 0.19	8.68 ± 0.39	ns
22:0	1.83 ± 0.07	1.91 ± 0.07	1.89 ± 0.02	1.95 ± 0.11	ns
22:1[d]	1.73 ± 0.05	1.72 ± 0.13	1.55 ± 0.21	1.65 ± 0.19	ns
22:2n-6	0.78 ± 0.03	0.81 ± 0.16	0.64 ± 0.14	0.71 ± 0.18	ns
22:4n-6	0.32 ± 0.21	0.44 ± 0.07	0.49 ± 0.17	1.01 ± 0.24	0.009
22:5n-3	5.47 ± 0.63	5.99 ± 0.80	7.42 ± 0.29	8.12 ± 0.67	0.012
22:6n-3	33.30 ± 1.56	36.39 ± 2.55	37.52 ± 0.53	37.71 ± 0.93	ns
total	920.89 ± 8.45	900.00 ± 8.24	904.81 ± 4.97	910.32 ± 5.31	ns
SFA	132.87 ± 3.76	129.56 ± 1.98	128.13 ± 2.20	135.01 ± 1.29	ns
MUFA	451.85 ± 6.40	434.54 ± 6.27	436.00 ± 3.23	437.18 ± 2.95	ns
PUFA	336.17 ± 1.68	335.90 ± 2.37	340.68 ± 3.63	338.13 ± 2.76	ns
n-6 PUFA	168.22 ± 0.64	166.18 ± 3.13	167.38 ± 1.80	166.53 ± 1.88	ns
n-6 LC-PUFA	11.43 ± 0.33	11.50 ± 0.08	12.03 ± 0.27	12.60 ± 0.82	ns
n-3 PUFA	167.96 ± 2.29	169.72 ± 1.42	173.30 ± 1.84	171.61 ± 1.20	ns
n-3 LC-PUFA	55.41 ± 2.48	59.39 ± 3.79	62.11 ± 0.90	63.46 ± 1.61	ns

[a]Data represent the mean ± standard error (n = 3; N = 12). Values in the same row with different letters are significantly different ($P < 0.05$; ANOVA and Student−Newman−Keuls post hoc test). See Table 1 for diet abbreviations. [b]P value of regression analysis (relative to dietary content of pyridoxine) is also reported at 5% level; ns = not significant. [c]Sum of 20:1 isomers. [d]Sum of 22:1 isomers.

weights and HSI values compared to those fed diets containing lower levels of pyridoxine.

The retention and deposition of pyridoxine into the whole bodies of trout were directly proportional to its dietary content, although the reported values were not significantly different from each other ($P > 0.05$). Similarly, other studies have reported significantly elevated levels of pyridoxine in the muscle tissue of rainbow trout[17] and Atlantic salmon[16] when fed increasing concentrations of this vitamin. In the latter study, the dietary pyridoxine supplementation ranged from 0 to 10 mg kg^{-1}, but the nonsupplemented basal diet contained 1.6 mg kg^{-1} of pyridoxine, which was roughly similar to the minimum dietary requirement for Atlantic salmon (2−3 mg kg^{-1}), previously determined by the same authors.[27] Another investigation carried out by the same authors on the same species showed reduced levels of tissue pyridoxine in Atlantic salmon fed a non-supplemented diet.[28] In line with the above studies, the present study on rainbow trout was composed of four pyridoxine-supplemented treatments, ranging from 2.4 (T1) to 19.0 (T4) mg kg^{1}, where the minimum level of dietary pyridoxine (T1) was similar to the defined minimum dietary requirement (2 mg kg^{1}) for rainbow trout.[29] Although not the objective of the present study and in consideration also that fish is not particularly rich in pyridoxine,[30] it was shown that the overall nutritional quality of cultured fish, and specifically its vitamin B_6 content, can be partially improved by appropriate dietary pyridoxine fortification.

The proximate composition analysis of trout tissues was unaffected by dietary pyridoxine content, and specifically, it was shown that the total lipid content in whole bodies and fillets of trout was not influenced by the dietary pyridoxine level. In contrast, dietary pyridoxine content has been previously demonstrated to have a significant impact on the total lipid content of Atlantic salmon fillets, resulting in higher concentrations in fish groups fed higher vitamin levels.[16] However, and in accordance with the present study, no significant differences were observed in relation to whole-body total lipid contents in Atlantic salmon fed different amounts of pyridoxine.[16]

The results of the fatty acid analysis of trout fillets and whole bodies did not show significant differences for the individual fatty acids, with the exception of some MUFA, that is, 18:1n-9 and 20:1, which were generally present in higher concentrations in the T1 treatment, which contained the lowest dietary

Table 7. Overall Fatty Acid Metabolism (as Total Apparent in Vivo Enzyme Activity, Nanomoles per Gram per Day) in Rainbow Trout Fed the Four Experimental Diets Containing Different Pyridoxine Contents, Deduced by the Whole-Body Fatty Acid Balance Method

	dietary treatments[a]				
	T1	T2	T3	T4	regression *P* value[b]
SFA and MUFA					
de novo production	228.2 ± 122.0	26.6 ± 26.6	34.9 ± 32.0	16.6 ± 8.8	ns
elongation	279.6 ± 123.5	66.9 ± 27.2	83.7 ± 40.8	67.4 ± 3.6	ns
β-oxidation	2507.2 ± 480.6	3444.4 ± 264.5	3485.8 ± 386.1	3484.0 ± 138.8	ns
Δ-9 desaturation	45.2 ± 23.4	23.3 ± 17.0	25.4 ± 12.9	22.5 ± 7.3	ns
n-6 PUFA					
elongation	125.4 ± 9.1	122.4 ± 7.0	125.4 ± 1.3	144.7 ± 16.1	ns
β-oxidation	1356.0 ± 131.2	1708.2 ± 62.1	1690.4 ± 110.9	1728.1 ± 40.6	ns
Δ-6 desaturation	76.1 ± 12.8	82.7 ± 1.3	81.1 ± 9.5	87.2 ± 5.3	ns
Δ-5 desaturation	23.3 ± 5.4	24.4 ± 1.5	26.5 ± 0.8	35.8 ± 6.2	0.026
n-3 PUFA					
elongation	776.0 ± 72.7	889.9 ± 129.4	928.8 ± 44.9	964.8 ± 51.2	ns
β-oxidation	1482.9 ± 98.6	1758.6 ± 107.5	1662.4 ± 110.4	1761.7 ± 25.7	ns
Δ-6 desaturation	659.6 ± 68.3	768.9 ± 88.2	757.3 ± 56.9	768.1 ± 33.8	ns
Δ-5 desaturation	221.5 ± 29.5	268.6 ± 56.0	281.3 ± 23.1	297.6 ± 19.2	ns

[a]Data represent the mean ± standard error (n = 3; N = 12). See Table 1 for diet abbreviations. [b]*P* value of regression analysis (relative to dietary content of pyridoxine) is also reported; ns = not significant.

Table 8. Apparent in Vivo Activity (Nanomoles per Gram per Day) of Key Enzymes in the LC-PUFA Biosynthetic Pathways in Rainbow Trout Fed the Four Experimental Diets Containing Different Pyridoxine Content Deduced by the Whole-Body Fatty Acid Balance Method

	dietary treatments[a]				
	T1	T2	T3	T4	regression *P* value[b]
n-6 LC-PUFA biosynthesis					
Δ-6 desaturase (18:2n-6 to 18:3n-6)	76.1 ± 12.8	82.7 ± 1.3	81.1 ± 9.5	87.2 ± 5.3	ns
elongase (18:3n-6 to 20:3n-6)	26.7 ± 6.4	28.9 ± 1.7	30.1 ± 2.0	39.6 ± 5.9	0.037
Δ-5 desaturase (20:3n-6 to 20:4n-6)	23.3 ± 5.4	24.4 ± 1.5	26.5 ± 0.8	35.8 ± 6.2	0.026
elongase (20:4n-6 to 22:4n-6)	2.2 ± 2.2	1.3 ± 1.0	3.7 ± 1.8	9.7 ± 3.0	0.008
n-3 LC-PUFA biosynthesis					
Δ-6 desaturase (18:3n-3 to 18:4n-3)	444.5 ± 50.8	522.7 ± 56.2	511.4 ± 46.4	515.7 ± 18.2	ns
elongase (18:4n-3 to 20:4n-3)	276.0 ± 32.0	325.1 ± 56.5	335.0 ± 21.6	351.9 ± 21.0	ns
Δ-5 desaturase (20:4n-3 to 20:5n-3)	221.5 ± 29.5	268.6 ± 56.0	281.3 ± 23.1	297.6 ± 19.2	ns
elongase (20:5n-3 to 22:5n-3)	222.3 ± 24.3	264.7 ± 42.5	288.0 ± 17.3	298.7 ± 12.5	ns
E + Δ-6 + CS[c] (22:5n-3 to 22:6n-3)	215.1 ± 17.6	246.2 ± 32.7	246.0 ± 10.6	252.5 ± 15.6	ns

[a]Data represent the mean ± standard error (n = 3; N = 12). See Table 1 for diet abbreviations. [b]*P* value of regression analysis (relative to dietary content of pyridoxine) is also reported; ns = not significant. [c]E + Δ-6 + CS = elongase (22:5n-3 to 24:5n-3) + Δ-6 desaturase (24:5n-3 to 24:6n-3) + chain shortening (24:6n-3 to 22:6n-3).

pyridoxine content. The results of the in vivo fatty acid metabolism did not reveal any statistically significant modification to the overall nonessential fatty acid metabolism. However, it was interesting to note that the fish fed the T1 diet demonstrated a higher (not significant, $P > 0.05$) de novo production of SFA, lower *β*-oxidation of SFA and MUFA, and higher Δ-9 desaturation activity, compared to the other three groups, suggesting the existence of a direct relationship between pyridoxine availability and SFA and MUFA metabolism. This observation has not been made previously, and in consideration of the known roles of pyridoxine in reducing plasma cholesterol and low-density lipoprotein[31] and the multitude of roles of pyridoxine (as PLP) in mitochondria activity,[32] the roles of pyridoxine in SFA and MUFA metabolism clearly warrant further investigation.

A significant linear trend was observed for DPA content in fillet and whole-body samples. Although DPA is not commonly considered as one of the main human health promoting n-3 LC-PUFA, as the vast majority of studies have primarily focused on EPA and DHA, this fatty acid has recently been recognized to play a series of important metabolic functions and exhibit health-promoting properties.[33] In the present study, all animals were fed almost identical amounts of DPA, as was the case for all other LC-PUFA, and thus it is possible that this trend toward increased DPA accumulation in synchrony with the increase in dietary pyridoxine concentrations could be attributable to potential positive effects of pyridoxine on the elongation of EPA or a negative effect on the further bioconversion or *β*-oxidation of DPA. Similarly, the 22:4n-6 content in trout whole-body samples demonstrated a significant linear trend, suggesting that dietary pyridoxine may also positively affect the elongation of ARA or reduce the further bioconversion or *β*-oxidation of this fatty acid. Other key LC-PUFA, such as EPA, DHA, and ARA in

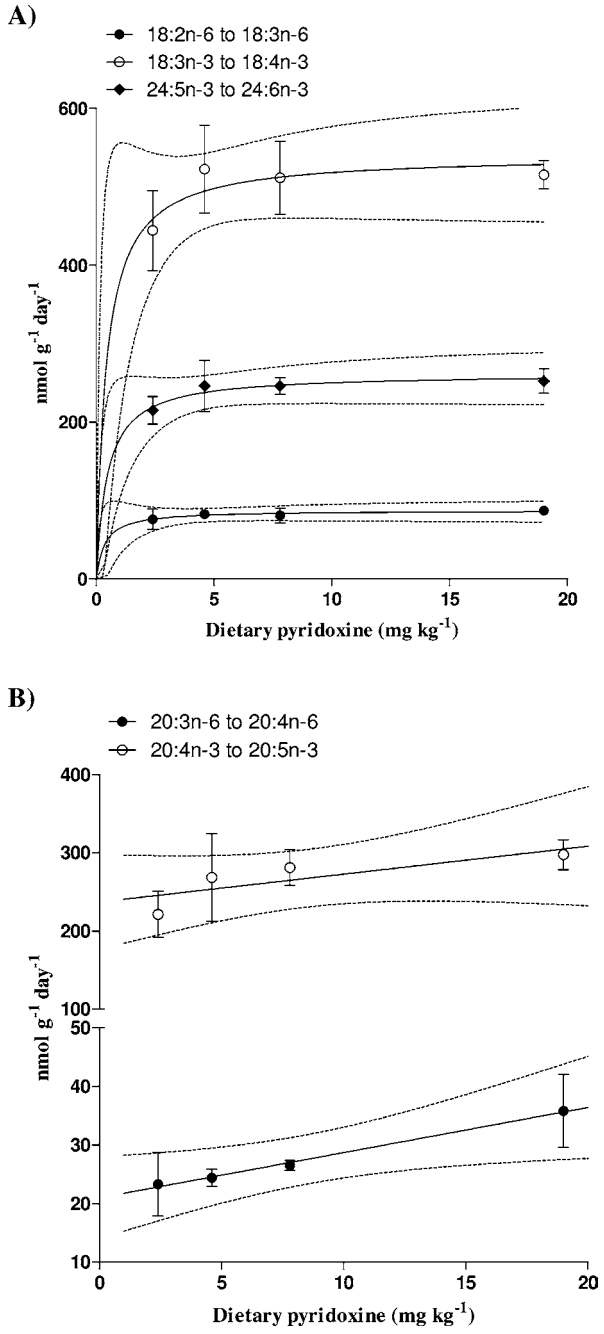

Figure 1. Apparent in vivo desaturase activity (nmol g^{-1} day^{-1}) in rainbow trout fed the four experimental diets containing different pyridoxine contents, deduced by the whole-body fatty acid balance method: (A) Δ-6 desaturase activity acting on 18:2n-6, regression equation $Y = 87.54X \times (0.3560 + X)^{-1}$, normality of residuals $P > 0.1$; 18:3n-3, regression equation $Y = 540.2X \times (0.4247 + X)^{-1}$, normality of residuals $P > 0.1$; and 24:5n-3 regression equation $Y = 261.9X \times (0.4659 + X)^{-1}$, normality of residuals $P > 0$; (B) Δ-5 desaturase activity acting on 20:3n-6, regression equation $Y = 0.7712X + 20.98$, $R^2 = 0.40$, $P = 0.0263$; 20:4n-3, regression equation $Y = 3.570X + 237.1$, $R^2 = 0.16$, $P > 0.05$.

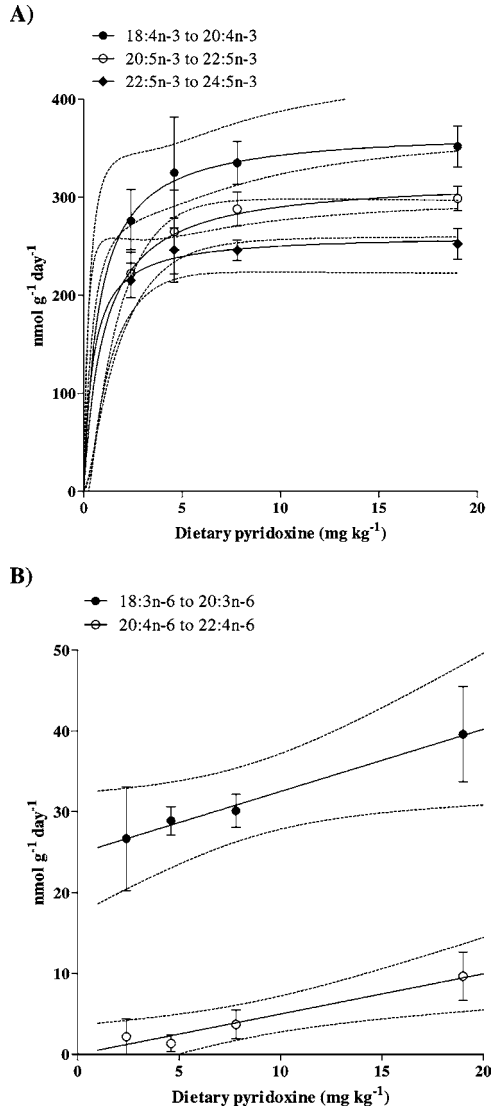

Figure 2. Apparent in vivo elongase activity (nmol g^{-1} day^{-1}) in rainbow trout fed the four experimental diets containing different pyridoxine contents, deduced by the whole-body fatty acid balance method: (A) elongase acting on n-3 PUFA 18:4n-3, regression equation $Y = 368.8X \times (0.7560 + X)^{-1}$, normality of residuals $P = 0.095$; 20:5n-3, regression equation $Y = 319.2X \times (0.9978 + X)^{-1}$, normality of residuals $P > 0.1$; and 22:5n-3 regression equation $Y = 261.9X \times (0.4659 + X)^{-1}$, normality of residuals $P > 0$; (B) elognase acting on n-6 PUFA 18:3n-6, regression equation $Y = 0.7697X + 24.82$, $R^2 = 0.37$, $P = 0.0366$, and 20:4n-6, regression equation $Y = 0.4970X + 0.0213$, $R^2 = 0.52$, $P = 0.0085$.

trout fillet and whole body, showed apparent increasing trends (although not statistically significant $P > 0.05$) with the increasing dietary availability of pyridoxine. Accordingly, a previous study conducted on the same species (but with fish oil based diets) demonstrated a significantly higher content of LC-PUFA in fillets, DHA in particular, in experimental groups supplemented with pyridoxine in comparison to the deficient group.[17] In agreement with the results of present study, previous investigations conducted using rats reported higher levels of ARA in pyridoxine-supplemented groups compared to the non-supplemented group.[9,14,34] Similarly, the results of the previous study on Atlantic salmon[16] reported significantly higher levels of DHA and total n-3 PUFA in hepatic phospholipids of Atlantic salmon fed pyridoxine-supplemented diets.

The results of the whole-body fatty acid balance method, employed to determine the fatty acid metabolism in rainbow trout during the present investigation, revealed a series of interesting trends of fatty acid enzyme activity relative to dietary pyridoxine supplementation. The activity of Δ-6 desaturase on its

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three substrates (18:2n-6, 18:3n-3, and 24:5n-3) was increased by the dietary pyridoxine content, peaking at the inclusion level of 4.6 mg kg^{-1}. On the other hand, Δ-5 desaturase activity on 20:3n-6 and 20:4n-3 (to produce ARA and EPA, respectively) showed a direct linear relationship with dietary pyridoxine content. Similar trends were also recorded for elongase activity on the various substrates, with the most evident effect on the elongation of 18:3n-6 and 20:4n-6, which were properly described by a linear relationship ($P < 0.05$), clearly suggesting that pyridoxine has a direct positive effect on this enzymatic activity. Accordingly, in a study carried out on rats by Bertrandt et al.,[34] it was suggested that dietary supplementation with pyridoxine might stimulate pathways of PUFA synthesis. Similarly, Tsuge et al.[6] clearly showed that the activity of Δ-6 desaturase was significantly lower (∼64%) in rats fed a pyridoxine-deficient diet compared to the pair-fed control group. A direct effect of pyridoxine status on fatty acid elongation in microsomes and mitochondria in rat brains has been shown.[35] Accordingly, impaired fatty acid desaturation and elongation was apparent in rats receiving pyridoxine-deficient diets.[9]

However, in Atlantic salmon,[16] it was reported that the desaturation and elongation of [1-^{14}C]18:3n-3 in liver following intraperitoneal injection was not affected by dietary pyridoxine level. The discrepancy between this study and the results of the present study are likely attributable to the differing pyridoxine administration methods and the vastly different experimental durations.

In conclusion, whereas the actual mechanism by which pyridoxine affects the activity of enzymes involved in the LC-PUFA biosynthetic pathway is not yet fully elucidated, the present study further confirms that dietary pyridoxine level directly modulates and positively stimulates LC-PUFA biosynthesis. Overfortification with pyridoxine, above the minimum dietary requirement, has no significant impact on growth performance of rainbow trout, but can be responsible for improved apparent in vivo LC-PUFA biosynthesis. Therefore, it is possible to suggest that dietary pyridoxine levels higher than the minimum dietary requirement should be included in aquafeed formulations, especially when fish oil is replaced with alternative oils, to maximize the LC-PUFA biosynthetic capability of cultured fish. However, it is also important to emphasize that the actual modification of the final fatty acid makeup of fish tissues in response to pyridoxine fortification is trivial. Thus, whereas the results of the present study could contribute toward expanding the knowledge of fatty acid metabolism in fish, and tentatively improve the efficiency of n-3 LC-PUFA utilization in aquaculture, the problem of substituting fish oil in aquafeed formulations and the resultant detrimental modification of the nutritional qualities of culture products is still far from being solved.

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Notes

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