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Research Article

The pantothenic acid requirement in juvenile spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869)

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ABSTRACT. An 18-week feeding trial was conducted to estimate the optimum dietary pantothenic acid (PA) requirement for juvenile of *Lutjanus guttatus*. Seven isoproteic and isocaloric practical diets were formulated containing graded levels of pantothenic acid (PA) 2.57, 31.97, 47.91, 63.79, 76.83, 103.15 and 121.91 mg kg^{-1}. Triplicate groups of 15 fish, with an initial weight of 10.2 ± 1.4 g, were cultured in 21 glass-fiber tanks and received each diet at random three times daily to apparent satiety. Results showed that the weight gain of fish fed diet 2.57 mg kg^{-1} PA was significantly less than the weight gain of fish fed diets containing greater quantities of PA. However, no significant differences were observed in the weight gain of the fish fed diets containing between 31.97 to 121.91 mg kg^{-1} PA. External signs of deficiency in the fish of diet (2.57 mg kg^{-1} PA) included caudal fin erosion, desquamation of the skin, hemorrhages in the body and fins, lethargy and 80% mortality rate were recorded. Hematological parameters indicated that there was no clear reduction of hemoglobin with PA in the diets. The dietary PA requirement to attain maximum growth rate, good survival, and the absence of external injuries range between 40.10 and 44.29 mg kg^{-1}.

Keywords: *Lutjanus guttatus*, pantothenic acid, requirement, deficiency signs.

INTRODUCTION

Pantothenic acid (PA), also known as vitamin B_5, is a water-soluble vitamin composed of pantoic acid and beta-alanine. PA is necessary for the synthesis of coenzyme A (CoA), which is essential for cell function and energy production, the synthesis of the neurotransmitter acetylcholine, acetylation of aromatic amines and the synthesis of cholesterol (Kaplan & Lipmann, 1948; Depeint *et al.*, 2006). The function of CoA is to transport acyl groups in the enzymatic reactions of oxidation and the synthesis of fatty acids, carbohydrates, and proteins; it is involved in approximately 70 other enzymatic reactions (Webster & Lim, 2002). Also, it is important to note the role of CoA in heme synthesis (prosthetic group of hemoglobin, myoglobin, and the cytochromes) (Depeint *et al.*, 2006).

The dietary PA requirement has been established as between 10 and 80 mg kg^{-1} for several freshwater fish species including rainbow trout (*Oncorhynchus mykiss*) (McLaren *et al.*, 1947; Cho & Woodward, 1990); common carp (*Cyprinus carpio*) (Ogino, 1967); channel catfish (*Ictalurus punctatus*) (Murai & Andrews, 1979; Wilson *et al.*, 1983); blue tilapia (*Oreochromis aureus*) (Roem *et al.*, 1991; Soliman & Wilson, 1992); Mayan cichlid (*Cichlasoma urophthalmus*) (Chavez *et al.*, 1990); and Jian carp (*C. carpio* var. Jian) (Wen *et al.*, 2009, 2010). However, the PA requirements for euryhaline or marine species have only been reported for three species: the Asian seabass (*Lates calcarifes*)

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15 mg kg^{-1} (Boonyaratpalin & Wanakowat, 1993); yellowtail (*Seriola quinqueradiata*) 13.5 mg kg^{-1} (Shimeno, 1991), and Malabar grouper (*Epinephelus malabaricus*) 11 mg kg^{-1} (Lin & Shiau, 2012). All these papers also reported mortality in fish fed deficient diets, so it is important to determine the requirement of pantothenic acid in their diets.

Spotted rose snapper (*Lutjanus guttatus*) has a wide distribution from Mexico to Peru (Allen, 1995) where it reaches high prices (around US$7.00 kg^{-1}) (Ibarra-Castro & Duncan, 2007). *L. gutattus* eggs and larvae are now massively cultured at the Centro de Investigación en Alimentación y Desarrollo (CIAD), from broodstock that undergoes natural maturation and spawns in captivity (Alvarez-Lajonchère & Puello-Cruz, 2011; Ibarra-Castro *et al.*, 2012, 2013). However, no specific diet covers the requirements of this species and the problems of juvenile's health related to the use of trout food.

Due to the importance of this fish species in Latin America, it is necessary to study its particular requirements. Abdo *et al.* (2010) evaluated the effect of different dietary protein and lipid levels on growth, feed efficiency and survival of juvenile snapper *Lutjanus guttatus* and found that the highest weight gained, and better feed conversion ratio were obtained in fish fed diets with 45 and 50% protein and the three lipids levels included in the experiment (9, 12, 15%). Survival, feed consumption, and condition factor were not affected by the treatments. There are also advances in the tolerance of vegetable ingredients and other by-products for this fish species. Silva-Carrillo *et al.* (2012) in a study to evaluate the replacement of fish meal with soybean meal in diets for juveniles spotted rose snapper found that this ingredient is acceptable to supply 20% of protein, but higher dietary supplement reduces the levels of performance. Hernández *et al.* (2014) found that up to 50% of the fish meal protein can be replaced by by-product meal food grade, without negative effects on health and growth performance on juvenile *L. gutattus*. There is also the need to determine the needs of micronutrients such as vitamin and minerals. Chávez-Sanchez *et al.* (2014) determined that vitamin C requirement for *L. guttatus* juveniles was estimated to be 29 mg kg^{-1} of ascorbic acid (AA) based on weight gain, and specific growth rate, but was more than 250 mg kg^{-1} of AA to eliminate clinical signs and histopathological lesions. This study aimed to estimate the PA requirement in juveniles of *L. guttatus*.

MATERIALS AND METHODS

Experimental diets

Seven isoproteic and isocaloric diets (50% protein and 15% lipids) were prepared using a basal diet (Silva-Carrillo *et al.*, 2012) (Table 1) with different levels (D0, D20, D40, D60, D80, D100, D120 mg kg^{-1}) of PA 98% pure. An extra 10% of PA was added to reduce loss during the food preparation process (Slinger *et al.*, 1979). The dry ingredients were thoroughly mixed, followed by the oils, and finally, the amount of water required for compaction in a meat mill was added. The diets were dehydrated in a forced-air oven at 40°C for 12 h, then crushed, labeled and stored at -4°C.

Fish and experimental procedure

The *L. guttatus* juveniles were obtained from the CIAD pilot plant of marine fish production. The seven diets were assessed in triplicate using a total of 21 experimental fiberglass tanks with a capacity of 300 L each. Fifteen fish (10.2 ± 1.4 g) were randomly stocked in each tank with aeration and 2 L min^{-1} in a flow-through saltwater tank system. The tanks were siphoned daily to prevent the accumulation of feces that might affect the water quality. During the study, the average and standard deviation of water quality parameters were: temperature 23.9 ± 1.6°C, dissolved oxygen 6 ± 0.5 mg L^{-1}, salinity 34.6 ± 0.4 g L^{-1}, and pH 8.1 ± 0.3.

The experimental diets were provided by hand to apparent satiety three times a day (8:00; 12:00; 16:00 h), recording the daily feed consumption by tank, as well as any observations regarding external signs of disease or nutritional deficiency, and mortality. The experimental period was 18 weeks.

Growth and feeding performance

Every 15 days, all fish in each tank were anesthetized with 2-phenoxyethanol (Sigma®, St. Louis, MO, USA) at a concentration of 0.3 mL L^{-1}. The fish were counted and were individually weighed, using a digital scale (accuracy of 0.01 g). Total length was also measured for each fish. The growth and feed efficiency of the fish were assessed by calculating the weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival:

$$MWG\ (g) = [\text{final mean weight gain (g)} - \text{mean initial weight (g)}]$$

$$SGR = [(\ln \text{final weight} - \ln \text{initial weight}) / \text{number of days}] \times 100$$

$$FCR = \text{feed intake (g)} / MWG\ (g)$$

Chemical analyses

Experimental diets and fish carcasses at the end of the experiment were subjected to proximate analyses under standard methods (AOAC, 2000). Crude protein content (total nitrogen × 6.25) was determined using a LECO FP-528 nitrogen analyzer (Instrument Corpora-

Table 1. Experimental diets formulation and chemical proximate analysis. *Vitamin premix (mg kg^{-1} diet): riboflavin, 8.75; pantothenic acid, 0; niacin, 10; vitamin B12, 1; choline chloride, 1,538.46; biotin, 10; thiamine, 1.08; pyridoxine, 7.31; inositol, 153.84; folic acid, 4.08; vitamin C, 0; vitamin A, 0.75; vitamin E, 30; vitamin D, 0.06; vitamin K, 16.5. **Mineral premix (mg kg^{-1}): copper sulphate, 12; iodine, 11; iron sulphate, 375; manganese oxide, 20.96; zinc sulphate, 41.66; sodium selenite, 30.

	D-Calcium pantothenate added (mg kg^{-1})						
	0	20	40	60	80	100	120
	PA determined by laboratory procedure (mg kg^{-1})						
Ingredients (%)	2.57 ± 0.80	31.97 ± 0.43	47.91 ± 0.16	63.79 ± 1.09	76.93 ± 7.53	103.15 ± 8.07	121.91 ± 4.02
Fish meal	57.25	57.25	57.25	57.25	57.25	57.25	57.25
Soybean meal	6.35	6.35	6.35	6.35	6.35	6.35	6.35
Krill meal	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Squid meal	4.06	4.06	4.06	4.06	4.06	4.06	4.06
Fish oil	2.45	2.45	2.45	2.45	2.45	2.45	2.45
Soy oil	7.09	7	7	7	7	7	7.09
Dextrine	14.56	14.56	14.56	14.56	14.56	14.56	14.56
Vitamin premix*	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Mineral premix**	0.23	0.23	0.23	0.23	0.23	0.23	0.23
Vitamin C	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Alginate	3	3	3	3	3	3	3
Carotenoids	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Soy Lecithin	1.5	1.5	1.5	1.5	1.5	1.5	1.5
	Proximate analysis						
Moisture	6.3 ± 0.1	6.5 ± 0.17	5.2 ± 0.1	7.3 ± 0.3	4.99 ± 0.04	4.94 ± 0.1	5.7 ± 0.2
Lipids	15.9 ± 0.2	16.10 ± 0.1	16.10 ± 0.1	16.01 ± 0.1	16.21 ± 0.2	15.96 ± 0.01	16.71 ± 0.2
Proteins	53.6 ± 0.1	50.4 ± 0.1	48.34 ± 0.1	53.5 ± 0.1	53.26 ± 0.1	53.65 ± 0.1	51.13 ± 0.05
Ash	11.7 ± 0.1	12.53 ± 0.1	13.4 ± 0.01	10.31 ± 0.1	10.4 ± 0.02	10.15 ± 0.004	12.93 ± 0.2

tion St. Joseph, MI, USA). Crude fat concentrations were determined using a micro Foss Soxtec Avanti 2050 Automatic System (Foss Soxtec, Hoganäs, Sweden) after extraction with petroleum ether. Incineration of the samples determined ash content in a muffle furnace at 550°C (Fisher Scientific International Inc., Pittsburgh, PA, USA).

The PA contents of the diets and liver of the fish were determined using high-performance liquid chromatography (HPLC) following the modified methods proposed by Romera *et al.* (1996) and Wang *et al.* (2004).

Hematology and blood chemistry analysis

At the end of the experiment, ten fish per treatment was, and blood was extracted from the caudal vein with 1 mL tuberculin syringes. The leukocyte and erythrocyte counts were obtained using the method proposed by Natt & Herrick (1952), while hematocrit was evaluated following Del Río-Zaragoza *et al.* (2008). The hemoglobin concentration was determined using a colorimetric kit (RANDOX), based on the cyanmethemoglobin method.

The total protein and triglyceride contents were determined from blood serum. Total protein was determined with the Biuret method using a colorimetric kit (BioSystems). The triglycerides were measured with a kit for triglycerides-LS quantification (MexLab Group), and the readings were performed in a spectrophotometer at 545 and 520 nm respectively.

Statistical analysis

Tests of normality and homoscedasticity were performed on data obtained for growth, survival, food intake, food efficiency, and hematology and blood chemistry parameters for the different treatments. A one-way ANOVA was performed with a significance level of 95% to estimate the requirement of d-calcium pantothenate for maximum growth and PA storage in the liver by juveniles of *L. guttatus*. The differences among the means were compared through an analysis of multiple comparisons using Duncan´s new multiple range test and for non-parametric data an arcsine transformation was carried out.

RESULTS

Clinical signs

After 18 weeks of feeding the experimental diets, fish fed diets from D2O (31.97 mg PA kg^{-1} diet) to D120

Table 2. Growth and feed efficiency of *L. guttatus* juveniles fed with different amounts of pantothenic acid mg kg^{-1} (mean ± SD). MWG: mean individual weight gain. WG: weight gain. SGR: specific growth rate. FCR: feed conversion ratio. Values in the same line with different superscript are not significantly different ($P < 0.05$).

PA (mg kg^{-1})	Initial weight (g)	Final weight (g)	WG (g)	SGR (% day^{-1})	FCR	Survival %
2.57	10.07 ± 1.37^{a}	73.75 ± 18.42^{a}	63.68 ± 6.65^{a}	1.58 ± 0.08^{a}	2.08 ± 0.46^{a}	33 ± 6.67
31.97	9.95 ± 1.19^{a}	93 ± 18.52^{ab}	72.98 ± 0.36^{ab}	1.68 ± 0.02^{ab}	1.53 ± 0.27^{b}	82 ± 15.4
47.91	10.49 ± 1.5^{a}	94.86 ± 19.20^{b}	84.54 ± 1.99^{b}	1.76 ± 0.05^{b}	1.46 ± 0.0^{b}	82 ± 7.7
63.79	10.26 ± 1.36^{a}	88.12 ± 18.86^{b}	77.86 ± 2.89^{b}	1.71 ± 0.04^{b}	1.49 ± 0.07^{b}	84 ± 3.85
76.83	10.01 ± 1.36^{a}	87.95 ± 20.27^{b}	72.14 ± 10.05^{b}	1.72 ± 0.1^{b}	1.41 ± 0.30^{b}	80 ± 6.67
103.15	10.12 ± 1.33^{a}	88.88 ± 17.50^{b}	72.88 ± 10.55^{b}	1.72 ± 0.1^{b}	1.35 ± 0.25^{b}	84 ± 10.18
121.91	10.25 ± 1.44^{a}	89.27 ± 19.41^{b}	79.02 ± 3.0^{b}	1.72 ± 0.02^{b}	1.51 ± 0.09^{b}	91 ± 7.7

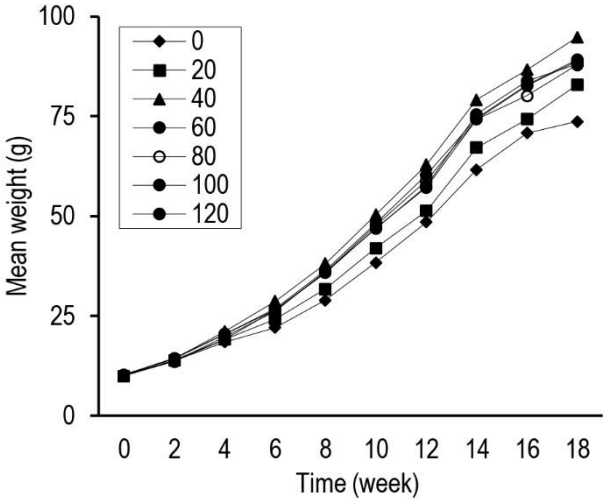


Figure 1. Mean growth of *L. guttatus* juveniles fed with different levels of pantothenic acid.

(121.91 mg PA kg^{-1} diet), did not show external signs of deficiency or severe mortality. However, after the fourth experimental week, the fish fed diet D0 (2.57 mg PA kg^{-1} diet) started to show clinical signs of calcium pantothenate deficiency, such as lethargy, body hemorrhages, and skin desquamation, as well as epithelial detachment, bleeding and complete loss of the caudal fin preventing swimming. The latter is causing diminished dietary intake and growth resulting in mortalities.

Mortality

Mortalities began in the first week in fish fed the diet D0 containing 2.57 mg kg^{-1} PA, and by the end of the experiment, these fish had a survival rate of around 33%. In contrast, the fish fed diets D20 to D120 (31.97 to 121.91 mg kg^{-1} PA) showed a survival rate of over 80% (Table 2).

Fish performance

The growth rate of *L. guttatus* improved gradually while PA in the diets increased (Fig. 1). Fish fed diet D0 (2.57 mg kg^{-1}) showed significantly lower values ($P < 0.05$) in final weight, weight gain, growth rate, specific growth rate (SGR) and feed conversion ratio in comparison with the fish fed experimental diets D20-D120. In contrast, fish fed diet D40 (47.91 mg kg^{-1} PA) had the highest values of all the parameters mentioned, but these were not significantly different from those obtained in fish fed the other diets, except for D0 (Table 2).

Pantothenic acid requirement

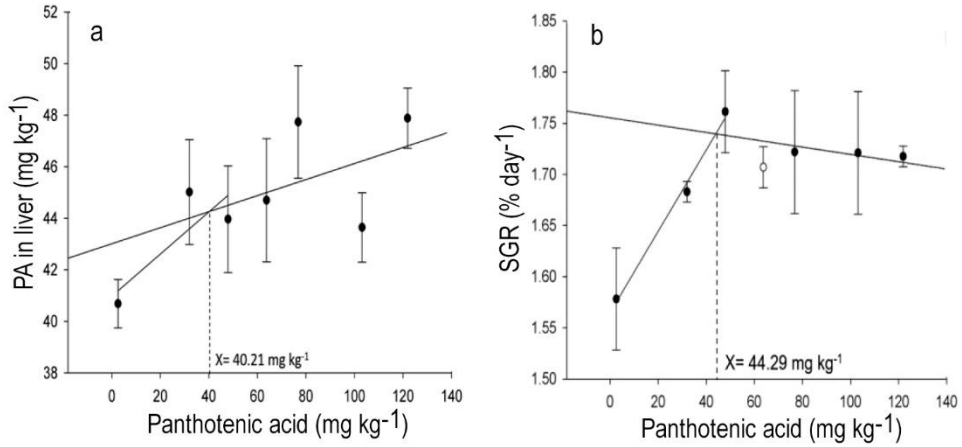
Relating the PA to SGR, the PA level for maximum growth rate was estimated at 44.29 mg kg^{-1} (Fig. 2a). The graph shows that when there is more PA in the diet, fish growth increases until no more PA is necessary for this function. Fish acquired the maximum storage of PA in the liver fed 40.10 mg kg^{-1} PA diet (Fig. 2b). The quantity of dietary PA is similar to that calculated as necessary for fish to attain maximum growth.

Pantothenic acid and lipids in the carcass of experimental fish

L. guttatus has a positive correlation between PA and lipid content in the experimental fish (>PA in diets >lipids in carcass) (Fig. 3). Lipid content in the fish carcass was significantly higher in fish fed diet D40 (47.91 mg kg^{-1} PA) to D120 (121.91) in comparison with diets D0 (2.57 mg kg^{-1} PA) and D20 (31.97 mg kg^{-1} PA).

Hematology and blood chemistry analysis

The deficiency of PA depletes the concentration of CoA, which reduces the conversion to succinyl-CoA synthetase and glycine as the first step in the synthesis, subsequently reducing the concentration of hemoglobin in the blood (Depeint *et al.*, 2006). In the present study, there was no clear reduction of hemoglobin, which again indicates that the amount of PA present in each of the diets was sufficient for the synthesis of proteins such as hemoglobin during the experimental period (Table 3). By the other hand, Soliman & Wilson (1992)



Figures 2. The pantothenic acid requirement concerning a) PA and b) SGR content in the liver of *L. guttatus* juveniles fed different levels of the vitamin.

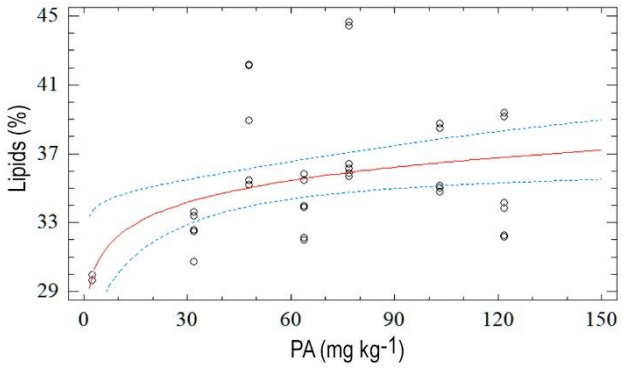


Figure 3. The relation between pantothenic acid and lipid content in the carcass of the fish.

reported that hematocrit and hemoglobin levels were significantly lower in *O. aureus* fed the pantothenic acid-free diet than in fish fed 10, 20 and 40 mg calcium d-pantothenate kg^{-1} diet. Also, Lin *et al.* (2012) reported erythrocyte count, hematocrit and hemoglobin concentration higher in fish fed diets with ≥5 mg PA kg diet than fish the control diet.

DISCUSSION

Clinical signs and mortality

Calcium pantothenate deficiency causes skin and fin erosion, hemorrhages and mortality as clinical signs in fish, as has been observed in *Salmo gaidnieri* (Tunison *et al.*, 1944), *S. fontinalis* (McLaren *et al.*, 1947; Phillips & Brockway, 1957), *C. carpio* (Ogino, 1967; Wen *et al.*, 2009); *Ictalurus punctatus* (Murai & Andrews, 1979; Wilson *et al.*, 1983; Robinson *et al.*, 2006), *Paralichthys olivaceus* (Arai *et al.*, 1972); *Sparus aurata* (Morris *et al.*, 1995); *Seriola quinqueradiata* (Hosokawa *et al.*, 1992); *Epinephelus malabaricus* (Lin & Shiau, 2012) and in *L. gutattus* (this study) only fish fed diet D0 showed theses characteristic signs. About mortality, in *S. aurata*, mortality was observed after the 13th week, rising from 1.7% to 13.3% in seven days when fish were fed PA-deficient diets (Morris *et al.*, 1995). Lin & Shiau (2012) recorded survival of 44% in *E. malabaricus* after eight weeks of feeding with a diet containing 0 mg PA kg^{-1} diet. In *Lates calcarifer*, no survival was observed within six weeks using semi-purified diets containing 0 mg kg^{-1} of CP (Boonyaratpalin *et al.*, 1994). Chavez *et al.* (1990) found in *Cichlasoma urophthalmus* that 100% mortality was reached at days 30, 40 and 63 in fish that were fed diets containing 40, 60 and 80 mg kg^{-1} of calcium pantothenate (CP) respectively, while no mortality was observed when fish were fed diets containing 120 and 320 mg kg^{-1} of CP. In our study with *L. gutattus* over 80% mortality was observed in fish fed diets containing 2.57 mg kg^{-1} PA and only 20% mortality was observed when fish were fed diets D20 to D120 (31.97 to 121.91 mg kg^{-1} PA) (Table 2). The mortality and deficiency signs in all previously mentioned fish species indicate that at least low levels of PA in fish diets are essential for fish survival.

Fish performance

In the Mayan cichlid *C. urophthalmus*, the best performance regarding weight gain, individual food intake, and specific growth rate was obtained in fish fed the diet with 160 mg kg^{-1} of PA (Chávez *et al.*, 1990). Wen *et al.* (2009) observed that PA improves SGR, the protein productive value, protein efficiency ratio and lipid production value of *C. carpio* var. Jian fed semi-purified diets supplemented with seven levels of PA; the feed efficiency of fish fed the control diet was significantly lower than any other group. Lin & Shiau

Table 3. Hematological parameters of *L. gutattus* juveniles fed with different levels of d-calcium pantothenate. Values are mean ± SE of three replicates. Values in the same line with different superscripts are significantly different ($P < 0.05$).

	Haematocrit (%)	Hemoglobin	Erythrocytes ($x10^6$ mm^3)	Leucocytes	Protein (g L^{-1})	Triglyceride (mg dL^{-1})
D0	59 ± 7.47^{a}	7.01 ± 2.02^{ab}	3.585 ± 0.75^{ab}	14688 ± 2541^{b}	46.71 ± 4.04^{a}	398.97 ± 64.42^{b}
D20	58 ± 3.66^{a}	7.71 ± 1.14^{a}	3.722 ± 0.06^{a}	17263 ± 3040^{a}	49.86 ± 6.16^{a}	509.09 ± 200.76^{ab}
D40	57 ± 3.15^{a}	7.02 ± 1.30^{ab}	3.077 ± 0.62^{abc}	15530 ± 2322^{ab}	50.63 ± 5.33^{a}	501.86 ± 163.91^{ab}
D60	58 ± 1.62^{a}	5.86 ± 0.70^{b}	3.345 ± 0.78^{abc}	14480 ± 1564^{b}	50.09 ± 5.76^{a}	627.36 ± 179.96^{a}
D80	60 ± 8.47^{a}	6.21 ± 0.90^{ab}	2.909 ± 0.69^{bc}	12256 ± 1699^{c}	53.62 ± 7.14^{a}	612.04 ± 278.70^{ab}
D100	61 ± 6.01a	6.27 ± 3.36^{b}	2.760 ± 0.55^{c}	12145 ± 1920c	49.49 ± 9.49^{a}	438.38 ± 182.40^{ab}
D120	59 ± 8.44^{a}	6.22 ± 1.14^{ab}	2.930 ± 0.33^{bc}	11406 ± 885^{c}	51.53 ± 5.15^{a}	563.31 ± 250.91^{ab}

(2012) found that the weight gain and feed efficiency of *E. malabaricus* fed diets supplemented with ≥10 mg PA kg^{-1} were significantly higher than for fish fed the PA deficient diet ($P < 0.05$). In this study, the growth rate of *L. guttatus* improved gradually while PA in the diets increased except in fish fed diet D0 (2.57 mg kg^{-1}), and there was a significant difference in all nutritional parameters analyzed in fish fed this diet (D0) with fish fed diets with higher PA. Pantothenic acid had been recognized as a growth determinant of universal occurrence since its initial investigation (William *et al*., 1933) where was demonstrated that PA is an important constituent of two coenzymes, coenzyme A (CoA) and the acyl carrier protein ACP.

Pantothenic acid content in diets and liver

The liver is the central organ of metabolism and has a high affinity for pantothenic acid (Pietrzik & Horning, 1980; Matsumoto *et al.*, 1994). PA is stored as CoA in highest concentrations in multiple different organs in the following order: liver, adrenal glands, kidney, brain, testes, eggs yolk and fresh vegetables (Dike, 1965; Pauling, 1970; Friedich, 1988). Matsumoto *et al.* (1994) observed significantly lower concentrations of pantothenic acid in the liver of deficient fish (*O. mykiss*, 150 g initial weight), compared to the initial values but only after 28 weeks of feeding. Lin & Shiau (2012), who studied *E. malabaricus* (15 g initial weight), found that hepatic PA concentration was highest in fish fed diets with ≥10 mg kg^{-1} PA, followed by 5 mg kg^{-1} PA diet, and lowest in fish fed the control diet, after eight weeks of feeding. Pantothenic acid concentration in the liver of 10 g *L. gutattus* was not significantly different among treatments after 18 weeks of feeding (5 g less and ten weeks less than *E. malabaricus*). However, it is expected that in our case, with more experimental time and a concurrent increase in the weight of the fish, the amount of PA in the liver of *L. guttatus* will reduce in fish fed the PA deficient diet and, in contrast, will increase in the fish fed PA sufficient diets.

PA and lipid in the carcass

CoA activates fatty acids before they can be synthesized into triglycerides. Shiau & Hsu (1999) mentioned that these characteristics explain the higher lipid concentration in the hepatopancreas of the shrimp *Penaeus monodon* when fed diets with lower levels of PA (>lipid hepatopancreas <PA in diet). However, this is not the case in blue tilapia, where the level of PA in the diet did not affect body composition, including lipids (Soliman & Wilson, 1992). The lipid content in tilapia flesh is lower in comparison with other fish, so it is possible that in this fish the PA deficiency is not expressed in the same way as in shrimp.

Contrary to this, lipids on the *C. carpio* carcass were negatively related to the graded levels of PA in the diet (<lipid in carcass >PA in diet) (Wen *et al.*, 2009), possibly because carp accumulate more fat in the axial muscle. In comparison, in *Salvelinus namaycush* (Poston & Paige, 1982) and *L. gutattus*, there was a positive correlation between PA and lipid content in the experimental fish (>lipids in carcass >PA in diets) (Fig. 3). Dietary lipid and fatty acids can have three primary fates in fish, they can be incorporated into cell membranes and hence the flesh of the fish, they can be oxidized to provide energy, or lipoid can be deposited in adipose or other tissues for energy storage (Leaver *et al.*, 2008). The increase of PA in *L. gutattus* indicates that it is used to deposit lipids to further mobilize it for growth and energy as shown in Figure 3.

Pantothenic acid requirement

In this study, the dietary PA requirement was estimated to be 44.29 and 40.10 mg kg^{-1} in relation to SGR and PA in the liver respectively. Therefore, it is not unusual for the nutritional requirement of this vitamin to vary with its role. For *E. malabaricus*, Lin & Shiau (2012) estimated that 10.7 mg kg^{-1} PA is necessary in the diet for optimum weight gain, whereas requirements of 11.9 and 10.4 mg kg^{-1} PA were proposed in order to attain maximum PA and Coenzyme A concentration in the

Table 4. The pantothenic acid requirement in different fish species, using different parameters (mg kg^{-1}).

Species	*Ictalurus punctatus*	*Ictalurus punctatus*	*Seriola quinqueradiata*	*Oreaochromis aureus*	*Cyprinus carpio* var. Jian	*Cyprinus carpio* var. Jian	*Epinephlus malabaricus*	*Lutjanus gutattus*
Max growth	10	15	13.5 - 35.9	≥5	23		11	44.29
Enhance intestine enzyme activities					23			
To eliminate deficiency signs	10	15						31.97
To reduce gill lesions		40						
Increase protein and weight in intestine and HP					≥ 25.6			
Feed efficiency	10	15					10	31.97
Lipid content in the carcass								47.91
Higher survival, red cell blood count, hematocrit and hemoglobin concentration				≥10		>25.6	≥5	
Hepatic PA concentration							≥ 10	40.10
Increase serum immunology	65					56.1 and 65.9		
Protein utilization				> 10				
Reference	Murai & Andrews (1979)	Wilson *et al.* (1983)	Aoki (1984)	Soliman & Wilson (1992)	Wen *et al.* (2009)	Wen *et al.* (2010)	Lin *et al.* (2012)	This study

liver. Comparably, Shimeno (1991) estimated that 13.5 mg kg^{-1} PA is required in the diet of *S. quinqueradiata* to achieve optimum weight gain, whereas approximately 35.9 mg kg^{-1} PA is necessary for maximum PA concentration in the liver.

The variability in the PA requirements estimated for different freshwater, euryhaline and marine fish species can be observed in Table 4. This information shows that to determine the particular requirement of PA for each fish species, not only weight gain and a lack of clinical deficiency signs need to be taken into account, but additional parameters also need to be included. For example, maximum PA or CoA concentration in the liver and other sensitive tissues, including gills or erythrocytes, should also be considered.

Histological studies should be performed to determine whether there are pathological changes due to PA deficiency and whether the CoA precursor is sufficiently abundant for incorporation into 4% of its known enzymatic reactions, including the role of CoA in heme synthesis, lipid metabolism or as a prosthetic group in the TCA cycle (Depeint *et al.*, 2006).

Hematology and blood chemistry analysis

The deficiency of PA depletes the concentration of CoA, which reduces the conversion to succinyl-CoA synthetase and glycine as the first step in the synthesis, subsequently reducing the concentration of hemoglobin in the blood (Depeint *et al.*, 2006). In the present study, there was no clear reduction of hemoglobin, which again indicates that the amount of PA present in each of the diets was sufficient for the synthesis of proteins such as hemoglobin during the experimental period. By the other hand, Soliman & Wilson (1992) reported that hematocrit and hemoglobin levels were significantly lower in *O. aureus* fed the pantothenic acid-free diet than in fish fed 10, 20 and 40 mg calcium d-pantothenate kg^{-1} diet. Also, Lin *et al.* (2012) reported erythrocyte count, hematocrit and hemoglobin concentration higher in fish fed diets with ≥5 mg PA kg diet than fish the control diet.

An increase in the leucocyte count of fish fed diets with low levels of PA was observed. Since leucocytes are involved in the regulation of the immunological function of the organism, and increase in number as a protective response of the fish during the trial (Wedemeyer & McLeay, 1981; Das *et al*., 2006; Del Río-Zaragoza *et al*., 2008).

CONCLUSIONS

The present study findings indicate that in order for *L. guttatus* juveniles to attain maximum growth rate, good

survival, the absence of external injuries, hepatic concentration in the liver, and the vitamin requirement of PA ranges between 31.27 to 47.91 mg kg^{-1} overall. The determination of PA requirements indicates higher amounts compared to the other mentioned species and therefore, considering the variability of results that outcome from the different parameters analyzed, further studies should be undertaken.

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