www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 16: 339-345 (2016) DOI: 10.4194/1303-2712-v16_2_14

RESEARCH PAPER

Effects of Dietary Vitamin A on Growth Performance, Blood Biochemical Indices and Body Composition of Juvenile Grass Carp (*Ctenopharyngodon Idellus*)

Fan Wu[1], Wenhuan Zhu[1], Mengmei Liu[1], Chen Chen[1], Jiaojiao Chen[1], Qingsong Tan[1,*]

[1] Ministry of Agriculture, Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Fisheries college, Key Laboratory of Freshwater Animal Breeding, Huazhong Agricultural University, Wuhan 430070, China

* Corresponding Author: Tel.: 86.27 87282113;
E-mail: qstan@hotmail.com

Received 24 November 2015
Accepted 30 March 2016

Abstract

A 12-week growth study was conducted to investigate the effects of dietary vitamin A (VA) on growth performance, blood biochemical indices and body composition of grass carp. Six isonitrogenous and isoenergetic semi-purified diets were compounded with different VA level at 68, 328, 599, 1327, 2584 and 4769 IU/kg diet, respectively. The specific growth rate (SGR) increased significantly (P<0.05) with dietary VA level, and the highest SGR value was at 4769 IU/kg group. However, the feed conversion ratio (FCR), feeding rate (FR) and survival rate (SR) of juvenile grass carp were not significantly affected by dietary VA level (P>0.05). As the VA level increased, hepatosomatic index (HSI) increased significantly, peaked at the 1327 IU/kg group (P<0.05), and then showed a decreasing trend. Serum superoxide dismutase (SOD) activity in the control group was significantly lower than those in other groups and then increased with dietary VA level. Whereas, serum alkaline phosphatase (ALP) activity first increased and then decreased with dietary VA level, reaching the highest point at 1327 IU/kg group. No significant difference was observed on the whole body composition among treatments (P>0.05). In conclusion, appropriate dietary vitamin A supplementation could be helpful for the growth and antioxidant capacity of grass carp.

Keywords: Grass carp; vitamin A; growth; blood biochemical indices; body composition.

Introduction

Vitamin A (VA) shares a variety of biochemical functions, including maintaining normal vision, controlling the differentiation and proliferation of cells (Moren *et al*., 2004; Hemre *et al*., 2004), participating in mucus production and bone growth (Saleh *et al*., 1995), regulation of glucose concentrations and lipid metabolism of the whole body (Mohamed *et al*., 2003; Hernandez *et al*., 2005), affecting fish immune responses (Rhee *et al*., 2012; Furuita *et al*.,2001; Thompson *et al*., 1995). Stunted growth and development of fish have been reported when insufficient or excessive VA intake occured, especially in juvenile fish (Rønnestad *et al*., 1998). VA deficiency can lead to photophobia, exophthalmos, cataract, retinal degeneration, growth delay, fin hemorrhage, lack of pelvic fins, skeletal deformities and even increased mortality (Hu *et al*., 2006, Moren *et al*., 2004, Hemre *et al*., 2004, Mazurais *et al*., 2009, Yang *et al*., 2008). Whereas when feeding too much VA, fish also would show growth retardation, increased mortality, vertebral deformities and fin necrosis phenomenon (Dedi *et al*., 1995, Takeuchi *et al*., 1998, Mohamed et a1., 2003).

The superoxide dismutase (SOD) is a scavenger of active oxygen radicals, and acts as a key component of the cellular antioxidant that protects cells and the extracellular matrix from the harmful effects of radicals (Afonso *et al*., 2007). Serum total SOD activity was even considered as one of the cancer related biomarkers (Maruyama *et al*., 2009). The antioxidant potential of VA in nutrition had been carefully reviewed (Palace *et al*., 1999). However, few studies have been performed to clarify the connection between vitamin A intake levels and the antioxidant defense systems, which attracts the reasearch interest of modern studies (Cha *et al*., 2016). The alkaline phosphatase (ALP) is a kind of lysosomal enzyme, participating in the skeleton mineralization of the aquatic animals and performing membrane transport activities (Öner *et al*., 2008), which could be used as an indicator of bone development (Skillington *et al*., 2002). Skeletal deformities caused by dietary VA deficiency have been mentioned above. However, there is no information about the relationship between dietary VA level and serum ALP activity in fish.

Grass carp is an important and increasingly popular inland aquaculture species in China. And it has been introduced to over 40 other countries, because of its euryphagy, rapid growth, high yield and delicious taste. There have been many researches about different levels of vitamin effect on its growth and health statues. However, there is little information about the effects of dietary VA on growth performance and serum SOD and ALP activities in grass carp. Therefore, the purpose of this study was to confirm the importance and indispensability of dietary VA to the growth and health of juvenile grass carp.

Materials and Methods

Diets were compounded with defatted fish meal, casein and gelatin as dietary protein source，α-starch and corn starch as carbohydrate source, and linseed oil, soybean oil and palm oil as lipid source. And retinyl acetate (Kingdomway Group, Xiamen, China) was utilized as VA source. The dietary VA concentrations of the six experimental diets were 68 (0), 328 (300), 599 (600), 1327 (1200), 2584 (2400), 4769 (4800) IU/kg diet, respectively. And the basal diet without addition of retinyl acetate was considered as the control group. Diets were processed into 1.5 mm diameter pellets, dried at room temperature to < 10% moisture, ground and sieved to appropriate size before being stored at −20 °C. The formulation and proximate composition of the basal diet are presented in Table 1.

[Table 1 The formulation and proximate composition of the basal diet for juvenile grass carp]

Juvenile grass carp were obtained from a fish farm in Xiantao City (China), and then transported to an indoor system at the Nahu Fish Culture Experimental Station of Huazhong Agricultural University. Fish were fed to apparent satiation with the control diet twice a day at 08:00 and 16:00 for two weeks. After the acclimation, fish were starved for 24h to measure the initial body weight. Then fish (initial body weight: 5.0g) were selected and randomly distributed into eighteen fibre glass tanks (water volume: 150L) with 30 fish each tank. Each experiment diet was fed to three randomly assigned tanks. The fish were fed to apparent satiation twice daily (08:00 and 16:00) during the whole 12-week feeding trial. The amount of feed consumed by the fish in each tank was recorded daily. Uneaten feed was collected by siphoning 0.5 h after feeding and dried at 60 °C to correct the feed intake. Each tank was a part of a semi flow-through system. The water exchange in each tank was kept at a flow-rate of dechlorided tap water 1.5 L/min. Dissolved oxygen (DO) value was above 5 mg/L, the water temperature ranged from 23 to 29 °C, the ammonia content was 0.33±0.01mg/L and the pH was about 7.7 during the experimental period. Animal treatments in the present study were conducted under principles of good laboratory animal care, and were approved by the Ethical Committee for Laboratory Animals Care and Use of Huazhong Agricultural University.

At the end of the 12-week feeding trial, all fish were starved for 24h, then anaesthetised with tricaine methanesulfonate (MS-222) at 75 mg/L water and batch-weighed. Five fish from each tank were randomly selected for blood sampling. Blood were withdrawn from caudal vein by syringes and then were centrifuged (3,500 rpm, 10 min) at 4 °C. Serum samples were collected, stored at −80 °C for various assays. After being bled, hepatopancreas was dissected out on ice and weighed individually to calculate hepatosomatic index (HSI). Besides, other five fish from each tank were randomly selected, minced, pooled and stored at -80 °C for whole-body proximate composition assay.

Crude protein (N × 6.25) was determined following the Kjeldahl method after an acid digestion

Table 1. The formulation and proximate composition of the basal diet for juvenile grass carp for 12 weeks

Ingredients	%	Ingredients	%
Defatted fish meal	5.00	choline chloride (50%)	1.00
casein	24.66	Ethoxy quinoline（30%）	0.05
gelatin	9.00	cellulose	4.60
α-starch	24.0	Vitamin A-free vitamin premix[b]	1.00
corn starch	19.16	Vitamin A premix[c]	3.00
linseed oil	1.47	Total	100.00
soybean oil	1.38	*Proximate composition (% dry basis)*	
palm oil	1.20	Crude fat	6.65
DL-Met（99%）	0.18	Crude ash	7.42
Mineral mixture[a]	2.00	Moisture	10.98
$Ca(H_2PO_4)$	2.30	Crude protein	28.72

a. Per kilogram of mineral premix containing: $MgSO_4.7H_20$ (9.86% Mg), 350.91g; $FeSO_4.7H_2O$ (19.7% Fe), 38.07g; CuSO4.5H2O (25.00% Cu), 0.60g; $ZnSO_4.7H_2O$ (22.5% Zn), 6.67g; $MnSO_4.H_2O$ (31.8% Mn), 2.04g; KI (3.8% I), 1.45g; $NaSeO_3$ (1% Se), 2.50g; corn starch，597.76g.

b. Per kilogram of vitamin A -free vitamin premix containing：VD_3 (500,000 IU/g), 0.480g; DL-a tocopherol acetate (50%), 20.00g; VK_3(23%)，0.22g; VB_{12}（1%）,0.1g; D- biotin（2%）, 5g; Folic acid（96%）, 0.52g; B_1(90%), 0.13g; V_c acetate (93%), 7.16g; B_3 (99%), 2.58g; inositol (99%), 52.33g; B_5 (98%), 3.07g; B_2 (80%), 0.99g; B_6 (81%), 0.75g; corn starch, 906.67g.

c. Vitamin A premixes were compounded by adding retinol acetate according to the designed vitamin A content in the diet to replace the equal quantity of cornstarch for the control diet.

using Kjelmet Auto Analyzer (Hanon K9860). Crude lipid was evaluated by the ether-extraction method using Soxhlet extractor. Crude ash was measured using a muffle furnace at 550 °C for 5 h. Moisture was determined by oven drying at 105 °C for 6 h. Energy content of the diets was measured by a Parr 6200 calorimeter equipped with a Parr 1108 Oxygen Bomb and a Parr 6510 water handling system (Parr Instrument Company, Moline, IL, USA).

The diets were digested with a 0.4% alcalase solution (2.4 AU ml^{-1}) to enhance the extraction of fat-soluble components. Total vitamin A contents in the diets were then measured as ROH using high performance liquid chromatography (HPLC) method described by Nöll (1996).

The activities of serum superoxide dismutase (SOD) and alkaline phosphatase (ALP) were determined through an automatic biochemical analyzer (Abbott Aeroset, Abbott Laboratories, Abbott Park, IL, USA) in the Zhongnan Hospital of Wuhan University. Pyorgallol autoxidation method (Marklund and Marklund, 1974) was used to determine the activity of SOD by using commercial kit (Fujian Fuyuan biological technology Co., LTD, Fujian, China). The activity of ALP was determined by using Alkaline Phosphatase Test Kit (4, Nitrophenyl phosphate disodium salt, 2-Amino-2-methyl—1-propanol Method), which was purchased from Ningbo Ruyuan biological technology co., LTD (Ningbo, China).

All data were expressed as mean±S.E. The effects of diet treatments were assessed by one-way ANOVA and differences between group means were compared by post-hoc Duncan's test using SPSS/PC (SPSS, 19) computer program. Differences between treatments were considered significantly when $P<0.05$.

Results

Grass carp in the control showed normal behavior and appearance for about 45 days. Thereafter, sluggish movement, exophthalmia, and vertebral deformity were observed in the control. At the end of the trial, when sampling, vertebral deformity, this VA deficiency symptom, as presented in Figure 1, was observed in fish fed with diets containing both 68 and 328 IU/kg groups. The incidence of this deformity at 68 and 328 IU/kg groups were 10% and 3%, respectively. However, this deficiency symptom wasn't observed in fish fed VA level at and above 599 IU/kg.

The growth performance, feed utilization, survival rate and HSI of fish fed with different levels of VA diets at the end of the growth trial are shown in Table 2. There were no significant differences in survival rate among groups in spite of the obvious deficiency symptoms in fish of both 68 and 328 IU/kg groups. The supplementation of VA to the diet significantly（$P<0.05$）improved the specific growth rate (SGR) of juvenile grass carp when the VA concentration was 2584 or 4769 IU/kg. SGR increased as dietary VA level increased (Table 2) and the highest SGR value was 1.49 %/d at 4769 IU/kg group. Although feed conversion ratio (FCR) and feeding rate (FR) of juvenile grass carp were not significantly ($P>0.05$) different in this study, FCR tended to decrease as dietary VA level increased. HSI increased first and then decreased, and the highest HSI was observed at 1327 IU/kg group.

[Table 2 Growth performance of juvenile grass carp fed with different levels of VA after 12 weeks]

As shown in Table 3, the activities of serum SOD and ALP were significantly affected by the dietary VA level ($P<0.05$). The SOD activity increased with the dietary VA concentration. However, the activity of serum ALP first increased and then decreased ($P<0.05$) as dietary VA level increased, and the highest ALP activity was observed at 1327 IU/kg group.

[Table 3 Effects of dietary VA concentrations on serum SOD, ALP activities of juvenile grass carp at the end of the growth trial]

As shown in Table 4, no significant differences were observed in the contents of crude protein, crude lipid, crude ash and moisture of the whole body among six groups ($P>0.05$).

[Table 4 Effects of dietary VA concentrations on whole body proximate composition of juvenile grass carp at the end of the growth trial]

Discussion

In the present study, the necessity of dietary VA for normal growth of juvenile grass carp was confirmed, which was similar with the results reported in greasy grouper (Mohamed *et al*. 2003), Atlantic halibut (Moren *et al*. 2004), hybrid tilapia (Hu *et al*. 2006), Japanese flounder (Hernandez *et al*. 2007), European sea bass larvae (Mazurais *et al*. 2009) and sea bass (Georga *et al*. 2011). There were many VA deficiency symptoms reported in fish, such as depressed feed consumption (Saleh *et al*. 1995), hemorrhages in eyes, fins and skin (Moren *et al*. 2004), cataract (Moren *et al*. 2004, Hemre *et al*. 2004), photophobia (Hu *et al*. 2006), bone deformities (Yang *et al*. 2008) and lack of pelvic fins (Mazurais *et al*. 2009). In this study, vertebral deformities were visibly observed in grass carp, and the incidence of this deformity in 68 IU/kg group was much higher than that in 328 IU/kg group, which may suggest the indispensability of VA to the skeletal development. Similar results have been reported in European sea bass (Georga *et al*., 2011). Although fish fed with diets containing VA at 599 and 1327 IU/kg showed no nutritional deficiency symptoms, the SGR of these two groups were both significantly lower than that of the group fed VA 4769 IU/kg diet. So we confirm that even through without observing vitamin A deficiency symptoms in grass carp, the vitamin A

Figure 1. Pictures of vertebral deformity when sampling from fish in the control group. Vertebral deformity would be visibly observed and distinguished from the normal fish.

Table 2. Growth performance of juvenile grass carp fed at different levels of vitamin A after 12 weeks feeding trial

Treatment（IU VA /kg)[a]	IBW (g)[b]	SGR (%/d)[c]	FCR [d]	FR (%)[e]	SR (%)	HSI (%)[f]
68	4.66±0.16	0.98±0.12[a]	3.22±0.93	2.92±0.54	91.1±1.11	2.50±0.03[a]
328	5.15±0.14	1.05±0.08[ab]	3.01±0.34	2.96±0.26	93.3±1.93	2.80±0.39[ab]
599	5.15±0.04	1.07±0.09[ab]	2.62±0.11	2.64±0.11	94.5±2.2	3.04±0.18[ab]
1327	5.11±0.27	1.11±0.03[ab]	2.81±0.23	2.96±0.31	92.2±1.1	3.23±0.02[b]
2584	4.64±0.12	1.29±0.09[bc]	2.25±0.06	2.68±0.11	95.0±1.7	3.10±0.08[ab]
4769	5.04±0.06	1.49±0.09[c]	2.39±0.25	3.18±0.17	91.1±1.1	2.62±0.06[ab]

Values are presented as means±S.E.M. Values within the same row with different letters are significantly different (*P*<0.05).
[a] : International units of Vitamin A per kg diet.
[b] IBW: Initial Body Weight.
[c] SGR: specific growth rate(%)=100×[In(FBW)-In(IBW)]/days, FBW: final body weight.
[d] FCR: feed conversion ratio =feed intake/ wet weight gain.
[e] FR: feeding rate (% BW/d)=(100×total feed intake)/[days×(initial BW + final BW)/2].
[f] HSI: hepatosomatic index=(hepatopancreas weight/BW)×100.

Table 3. Effects of dietary vitamin A concentrations on serum SOD, ALP activities of juvenile grass carp at the end of the growth trial

Treatment（IU VA/ kg)	SOD (U/ml)	ALP (U/L)
68	42.24±2.05[a]	239.20±5.79[a]
328	48.28±1.72[b]	285.10±7.85[b]
599	51.50±1.28[bc]	319.87±10.05[cd]
1327	52.42±0.86[bcd]	340.23±5.84[d]
2584	54.70±1.05[cd]	313.50±11.40[c]
4769	56.90±1.52[d]	271.20±4.58[b]

Values are presented as means±S.E. Values within the same row with different superscript letters were significantly different (P<0.05).

Table 4. Effects of dietary vitamin A concentrations on whole body proximate composition of juvenile grass carp at the end of the growth trial

Treatment（IU VA/ kg)	Moisture (%）	Crude ash (%）	Crude fat (%）	Crude protein (%）
68	70.25±0.71	3.01±0.02	10.37±0.90	15.65±0.87
328	69.61±0.8	2.97±0.04	10.57±0.86	15.50±0.20
599	71.61±0.91	2.99±0.07	9.38±0.45	15.71±0.16
1327	70.66±0.41	3.05±0.04	9.69±0.41	15.68±0.16
2584	71.13±0.89	2.96±0.04	9.57±0.09	15.69±0.57
4769	70.07±0.31	3.09±0.12	10.31±0.36	15.72±0.11

Values are presented as means±S.E. Values within a column with different superscript letters were significantly different（P<0.05）

supplementation dose might be insufficient, because of the low SGR value. In this study, 599 and 1327 IU/kg could be considered as suboptimal but asymptomatic dietary vitamin A concentration.

No diet related mortality was observed among groups in this study, which suggests that VA deficient diet could not increase the fish mortality. This result was similar with the results reported by Hernandez (2005). However, Mohamed *et al*. (2003), Hemre *et al*. (2004) and Yang *et al*. (2008) draw the opposite results that VA deficiency in the diet increased mortality. The different results may be involved in the size of the experimental fish and the feeding period of the diet (Moren *et al*. 2004). The smaller size of the initial fish and the longer of the feeding period, more likely it is to appear increasing mortality.

The present study showed that SGR increased significantly ($P<0.05$) with increasing VA level, which indicated that dietary VA level could promote growth performance of grass carp. Because of differences of fish species, development stage, initial body weight, dietary composition and experimental conditions and other interacting nutrients, the dietary optimal supplementation dose could be different. And based on the SGR data, the supplementation dose in this study should be no less than 4769 IU/kg, which is similar to the requirement values reported for hybrid tilapia (Hu *et al*. 2006), and greasy grouper (Mohamed *et al*. 2003), which have requirements between 2000 and 5000 IU/kg. In our study, different VA levels showed no significant effect on the FCR and FR, as reported in Atlantic salmon by Thompson *et al*. (1994). This indicated that VA may not improve the palatability of the feed to promote growth. However, the FCR in this study showed some decreasing tendency as dietary VA increased, which suggests that VA level would change the metabolic pathways to improve the fish growth and feed utilization. And the regulatory mechanism of VA to promote fish growth still remains unclear and further intensive studies about how VA promote growth of juvenile grass carp need to be conducted.

Diets containing different levels of VA showed no significant difference on the whole body composition of grass carp in this study. Similar results were reported by Thompson *et al*. (1995). However, contradictory results were reported in greasy grouper (Mohamed *et al*. 2003), which indicated a significant decrease of body fat as the dietary VA level increased. Biometric indices of particular organs or tissues relative to total body mass (such as HSI) were considered as indices of change in nutritional and energy status. Interestingly, HSI increased first and then decreased with the increase of VA supplemental level and the highest value was observed at 1327 IU/kg group in our study. Hepatopancreas is an important organ for lipid metabolism and storage. VA is involved in the metabolism of fat (Mohamed *et al*. 2003; Hernandez *et al*. 2005). The increased HSI in grass carp fed with higher VA level diet may be due to the active metabolism and deposition of fat.

Serum biochemical indices are usually used to evaluate the health status in fish. SOD is a kind of homodimeric enzyme involved in detoxification processes, catalyzing the dismutation of the superoxide anion radical into oxygen and hydrogen peroxide (Bannister *et al*. 1987). The higher SOD activity suggests the stronger ability of radical clearance (Muñoz *et al*. 2000). In the present study, the serum SOD activity increased in accordance with the fish SGR as VA level increased, and the highest value of serum SOD activity occurred in the 4769 IU/kg group, which suggested that the optimal dietary VA level would be helpful for the antioxidant defense system against oxidative stress. ALP is a kind of lysosomal enzyme, which extensively exists in cells from various tissues, especially in the hepatopancreas and bone cells (osteoblasts). Total serum ALP can be considered as an indirect index of osteoblast activity and it is commonly used in diagnosing and monitoring bone formation rate (Skillington *et al*., 2002). In this study, the serum ALP activity first increased and then decreased with increasing dietary VA level. The increase of serum ALP activity may result from increased osteoblastic activity, which suggests that proper incorporation of VA in the diet would be benefit for bone formation. However, the increase of serum ALP activity may result from cholestasis and chronic disease. In rainbow trout, significantly higher plasma alkaline phosphatase activity was found in the fish fed diet with vitamin A 2,704,000 IU kg^{-1} than those fed with vitamin A 4000 IU kg^{-1} (The Retina Society Terminology Committee, 1983). Moreover, it was suggested that VA can modify fish skeletogenesis indirectly, via its effects on the levels of thyroid hormone (Fernández *et al*. 2009). The effect of VA on bone metabolism deserves further investigation.

In conclusion, the preliminary study indicated that appropriate VA could be helpful for the growth performance and antioxidant defense system. And dietary VA levels had no significant effects on the whole body composition of grass carp. VA is required for normal growth of juvenile grass carp and the regulatory mechanism of VA to promote fish growth still remains unclear, which needs further studies.

Acknowledgment

This work was financially supported by the Special Fund for Agro-scientific Research in the Public Interest (201003020).

References

Afonso, V., Champy, R., Mitrovic, D., Collin, P. and Lomri, A. 2007. Reactive oxygen species and superoxide dismutases: role in joint diseases. *Joint Bone Spine*, 74, 324–329. doi:10.1016/j.jbspin.2007.02.002

Bannister, J. V., Bannister, W. H., and Rotilio, G. 1987. Aspects of the structure, function, and applications of superoxide dismutas. *Critical Reviews in Biochemistry* 22(2): 111-180. doi:10.3109/10409238709083738

Cha, J.H., Yu, Q.M., Seo, J.S., 2016. Vitamin A supplementation modifies the antioxidant system in rats. *Nutrition Research and Practice*, 10(1), 26-32. doi:10.4162/nrp.2016.10.1.26

The Retina Society Terminology Committee, 1983. The classification of retinal detachment with proliferative vitreoretinopathy. *Ophthalmology*, 90, 121-125. doi: 10.1016/S0161-6420(83)34588-7

Dedi, J., Takeuchi, T., Seikai, T., and Watanabe, T. 1995. Hypervitaminosis and safe levels of vitamin A for larval flounder *Paralichthys olivaceus* fed Artemia nauplii. *Aquaculture*, 133(2), 135-146. doi:10.1016/0044-8486(95)00015-T

Fernández, I., Pimentel, M. S., Ortiz-Delgado, J. B., Hontoria, F., Sarasquete, C., Estévez, A., Infanteand, Z. J. L., and Gisbert, E. 2009. Effects of dietary vitamin A on Senegalese sole (*Solea senegalensis*) skeletogenesis and larval quality. *Aquaculture*, 295(3), 250-265. doi:10.1016/j.aquaculture.2009.06.046

Furuita, H., Tanaka, H., Yamamoto, T., Shiraishi, M., and Takeuchi, T. 2001. Effects of high dose of vitamin A on reproduction and egg quality of Japanese flounder *Paralichthys olivaceus*. *Fisheries science*, 67(4), 606-613. doi: 10.1046/j.1444-2906.2001.00296.x

Georga, I., Glynatsi, N., Baltzois, A., Karamanos, D., Mazurais, D., Darias, M. J., Cahu, C. L., Zambonino-Infante J. L. and Koumoundouros, G. 2011. Effect of vitamin A on the skeletal morphogenesis of European sea bass, *Dicentrarchus labrax (Linnaeus, 1758)*. *Aquaculture Research*, 42(5), 684-692. doi:10.1111/j.1365-2109.2010.02676.x

Hemre, G. I., Deng, D. F., Wilson, R. P., and Berntssen, M. H. 2004. Vitamin A metabolism and early biological responses in juvenile sunshine bass (*Morone chrysops* × *M. saxatilis*) fed graded levels of vitamin A. *Aquaculture*, 235(1), 645-658. doi:10.1016/j.aquaculture.2004.01.031

Hernandez, L. H. H., Teshima, S. I., Ishikawa, M., Alam, S., Koshio, S., and Tanaka, Y. 2005. Dietary vitamin A requirements of juvenile Japanese flounder *Paralichthys olivaceus*. *Aquaculture Nutrition*, 11(1), 3-9. doi: 10.1111/j.1365-2095.2004.00317.x

Hernandez, L. H. H., Teshima, S. I., Koshio, S., Ishikawa, M., Tanaka, Y., and Alam, M. S. 2007. Effects of vitamin A on growth, serum anti-bacterial activity and transaminase activities in the juvenile Japanese flounder, *Paralichthys olivaceus*. *Aquaculture*, 262(2), 444-450. doi:10.1016/j.aquaculture.2006.10.012

Hu, C. J., Chen, S. M., Pan, C. H., and Huang, C. H. 2006. Effects of dietary vitamin A or β-carotene concentrations on growth of juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquaculture*, 253(1), 602-607. doi:10.1016/j.aquaculture.2005.09.003

Öner, M., Atli, G., Canli, M., 2008. Changes in serum Biochemical parameters of fresh water fish Oreochromis Niloticus following prolonged Metal (Ag Cd Cr Cu Zn) Exposures. Environmental Toxicology and Chemistry, 27(2):360-6. doi: 10.1897/07-281R.1

Palace, V.P., Khaper, N., Qin, Q., Singal, P.K., 1999. Antioxidant potentials of vitamin A and carotenoids and their relevance to heart disease. *Free Radical Biology and Medicne*, 26, 746–761. doi:10.1016/S0891-5849(98)00266-4

Marklund, S., Marklund, G., 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry*, 47(3), 469-74. doi: 10.1111/j.1432-1033.1974.tb03714.x

Maruyama, K., Iso, H., Ito., Y., Watanabe, Y., Inaba, Y., Ta jima K., Nakachi, K., Tamakoshi, A. 2009. Associations of food and nutrient intakes with serum IGF-I, IGF-II, IGFBP-3, TGF-β1, total SOD activity and sFas levels among middle-aged Japanese: the Japan Collaborative Cohort study. *Asian Pacific Journal of Cancer Prevention*, 10 *(*Suppl*)* 7–22. doi:10.1158/1940-6207.CAPR-11-0407.

Mazurais, D., Glynatsi, N., Darias, M. J., Christodoulopoulou, S., Cahu, C. L., Zambonino-Infante, J. L., and Koumoundouros, G. 2009. Optimal levels of dietary vitamin A for reduced deformity incidence during development of European sea bass larvae (*Dicentrarchus labrax*) depend on malformation type. *Aquaculture*, 294(3), 262-270. doi:10.1016/j.aquaculture.2009.06.008

Mohamed, J. S., Sivaram, V., Roy, T. C., Marian, M. P., Murugadass, S., and Hussain, M. R. 2003. Dietary vitamin A requirement of juvenile greasy grouper (*Epinephelus tauvina*). *Aquaculture*, 219(1), 693-701. doi:10.1016/S0044-8486(02)00665-8

Moren, M., Opstad, I., Berntssen, M. H. G., Zambonino-Infante, J. L., and Hamre, K. 2004. An optimum level of vitamin A supplements for Atlantic halibut (*Hippoglossus hippoglossus L.*) juveniles. *Aquaculture*, 235(1), 587-599. doi:10.1016/j.aquaculture.2004.01.030

Muñoz, M., Cedeño, R., Rodríguez, J., van der Knaap, W. P., Mialhe, E., and Bachère, E. 2000. Measurement of reactive oxygen intermediate production in haemocytes of the penaeid shrimp, *Penaeus vannamei*. *Aquaculture*, 191(1), 89-107. doi:10.1016/S0044-8486(00)00420-8

Nöll, G. N. (1996). High-performance liquid chromatographic analysis of retinal and retinol isomers. Journal of Chromatography A, 721(2), 247-259. doi:10.1016/0021-9673(95)00789-X

Rhee, E. J., Nallamshetty, S., and Plutzky, J. 2012. Retinoid metabolism and its effects on the vasculature. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, 1821(1), 230-240. doi:10.1016/j.bbalip.2011.07.001

Rønnestad, I., Hemre, G. I., Finn, R. N., and Lie, Ø. 1998. Alternate sources and dynamics of vitamin A and its incorporation into the eyes during the early endotrophic and exotrophic larval stages of Atlantic halibut (*Hippoglossus hippoglossus L.*). *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 119(3), 787-793. doi:10.1016/S1095-6433(98)01017-4

Saleh, G., Eleraky, W., and Gropp, J. M. 1995. A short note on the effects of vitamin A hypervitaminosis and hypovitaminosis on health and growth of *Tilapia nilotica* (*Oreochromis niloticus*). *Journal of Applied Ichthyology*, 11(3-4), 382-385. doi: 10.1111/j.1439-0426.1995.tb00046.x

Skillington, J., Choy, L., Derynck, R. 2002. Bone morphogenetic protein and retinoic acid signaling cooperate to induce osteoblast differentiation of preadipocytes. *The Journal of cell biology*, 159, 135-146. doi/10.1083/jcb.200204060

Takeuchi, T., Dedi, J., Haga, Y., Seikai, T., and Watanabe, T. 1998. Effect of vitamin A compounds on bone deformity in larval Japanese flounder (*Paralichthys* *olivaceus*). *Aquaculture*, 169(3), 155-165. doi:10.1016/S0044-8486(98)00373-1

Thompson, I., Choubert, G., Houlihan, D. F., and Secombes, C. J. 1995. The effect of dietary vitamin A and astaxanthin on the immunocompetence of rainbow trout. *Aquaculture*, 133(2), 91-102. doi:10.1016/0044-8486(95)00024-V

Thompson, I., Fletcher, T. C., Houlihan, D. F., and Secombes, C. J. 1994. The effect of dietary vitamin A on the immunocompetence of Atlantic salmon (*Salmo salar L.*). *Fish Physiology and Biochemistry*, 12(6), 513-523. doi:10.1007/BF00004453

Yang, Q. H., Zhou, X. Q., Jiang, J., and Liu, Y. 2008. Effect of dietary vitamin A deficiency on growth performance, feed utilization and immune responses of juvenile Jian carp (*Cyprinus carpio var. Jian*). *Aquaculture Research*, 39(8), 902-906. doi: 10.1111/j.1365-2109.2008.01945.x