

Effect of Dietary Inositol on Growth, Feed Utilization and Blood Biochemical Parameters for Juvenile Barramundi (*Lates calcarifer* Bloch)

Shi-Qiang Diao, Zhong Huang, Shui-Sheng Chen, Jin Niu,
Zhuo-Jia Li, Xian Ding and Hei-Zhao Lin
South China Sea Fisheries Research Institute of CAFS,
231 Xingang Road Western, Guangzhou 510300, PR, China

Abstract: Problem statement: The utilization of inositol was detected for growth performance and serum biochemical parameters of juvenile barramundi (*Lates calcarifer*). **Approach:** A 56 day feeding trial was conducted to evaluate the effect of inositol on growth, feed utilization and serum biochemical parameters for juvenile barramundi *Lates calcarifer* (initial size 5.51 ± 0.07 g). Six experimental fish meal-based isonitrogenous (42% crude protein) and isolipidic (10% crude lipid) diets containing levels of inositol (350, 364, 458, 507, 720, 1050 mg kg⁻¹ diet) were formulated. **Results:** Fish fed diet containing inositol 507 mg kg⁻¹ diet had the significantly highest weight gain (WG, %) among all the groups ($p < 0.05$) and had significantly lower Feed Conversion Ratio (FCR) than fish fed the diet containing inositol 458 mg kg⁻¹ diet ($p < 0.05$). Survival and Hepatosomatic Index (HSI) were not significantly affected by graded levels of dietary inositol ($p > 0.05$). However, significantly lower Viscerasomatic Index (VSI) was found in fish fed diet without supplemental inositol ($p < 0.05$). Dietary inositol levels did not affect whole body moisture, crude protein and lipid contents ($p > 0.05$). Total protein and triacylglycerol in serum increased with increasing dietary inositol levels up to 507 mg kg⁻¹ diet ($p < 0.05$). The significantly lowest blood urea nitrogen was found in fish fed dietary inositol 458 mg kg⁻¹ diet among all groups except for 720 mg kg⁻¹ diet. Total Cholesterol (TC) of fish fed dietary inositol 507-1050 mg kg⁻¹ diet was higher than those of fish fed dietary inositol 350-458 mg kg⁻¹ diet. **Conclusion:** Results of the present investigation demonstrated significant improvement of growth and feed utilization of juvenile barramundi can be achieved by inositol supplementation at 507 mg kg⁻¹ diet.

Key words: *Lates calcarifer* bloch, inositol, growth, blood biochemical index

INTRODUCTION

Barramundi (*Lates calcarifer*) is native to the Indo-Pacific region (Glencross, 2006) and an economically important species in Southeast Asian countries (Tantikittia *et al.*, 2005). It is a carnivorous species which can be reared in marine water, brackish water, or freshwater (Harpaz *et al.*, 2005). Glencross (2006) reviewed barramundi nutrition research included the requirements for most nutrients, energy demand and ingredient utilization. Recent research indicated that lupin protein (Katersky *et al.*, 2009) and defatted soybean protein (Tantikittia *et al.*, 2005) were suitable protein sources for barramundi feeds. Williams *et al.* (2006) also indicated that a dietary n-3 HUFA was required for rapid growth and efficient feed conversion of juvenile barramundi. In our laboratory, research has focused on determining effects of dietary *Bacillus licheniformis* (Yuan *et al.*, 2009) and Chinese herbal

medicines (Lu *et al.*, 2009) on blood biochemical indices in cultured *Lates calarifer*.

Inositol is widely distributed in plants and animals (Peres *et al.*, 2004), which is classified as a vitamin-like nutrient (Shiau and Su, 2005) and is an essential dietary ingredient for most aquatic animals (Michael and Koshio, 2008). The requirement of dietary inositol has been reported by Li *et al.* (2001), Shiau and Su (2005) and Wen *et al.* (2007) in various aquatic animals. Dietary inositol also affected blood chemistry in fish (Waagbo *et al.*, 1998; Wen *et al.* 2007). Certain dietary vitamins have been identified as essential for barramundi; however, it is difficult to define the requirement for all essential vitamins because of poor acceptance of a purified diet by barramundi (Glencross, 2006).

The purpose of this study was to evaluate the influence of the dietary levels of inositol on growth performance, feed utilization and serum biochemical parameters in juvenile barramundi.

Corresponding Author: Hei-Zhao Lin, South China Sea Fisheries Research Institute of CAFS, 231 Xingang Road Western, Guangzhou 510300, PR, China

MATERIALS AND METHODS

Experimental diets: Diets were formulated to be grossly isonitrogenous (crude protein, 43%) and isolipidic (crude lipid, 12%). The experimental diet formulation and proximate are shown in Table 1. Inositol (Sigma) was added to the test diets at a concentration of 0, 50, 100, 200, 400 and 800 g kg⁻¹ diet. All ingredients were mechanically mixed, pressure-pelleted using a laboratory pelleting machine through a 2.5 mm die. The pellets were air-dried at room temperature to a moisture content of about 10% and stored in a freezer at -20°C until used.

Experimental fish and feeding: The trial was conducted at an experimental station of South China Sea Fisheries Research Institute of CAFS (Sanya, Hainan). Barramundi were obtained from a private commercial hatchery and acclimated to the laboratory conditions for 2 weeks. At the end of the acclimation period, a total of 540 fish (mean initial weight 5.51±0.07 g) were randomly distributed into each of 18 500 L cylindrical plastic tanks. Each experimental diet was randomly assigned to triplicate tanks. Tanks were supplied with filtered seawater with a flow rate of approximately 2 L min⁻¹ in a flow-through system with aeration. The fish were fed with the respective diet to apparent satiation twice a day at 0800 and 1700 h. During the trial, the water temperature was maintained at 29.0±2.0°C and salinity was 28±0.3 g L⁻¹. Dissolved oxygen ranged from 5.10-6.93 mg L⁻¹. The

experimental units were under a natural light and dark cycle. The experiment lasted for 56 days.

Sampling: At the conclusion of the 8-week period, the fish were deprived for 1 day prior to sampling, then counted and batch weighed to determine survival, Final Body Weight (FBW), Weight Gain (WG), Feed Conversion Ratio (FCR). Five fish from each tank were randomly collected, killed for the determination of the Hepatosomatic Index (HSI) and Viscerasomatic Index (VSI). Blood was taken by puncturing the caudal veins immediately after catching.

Chemical analysis: Crude protein was determined by the Kjeldahl method using a Kjeltac 2200 Auto Distillation Unit (Foss Tecator AB, Switzerland). Crude lipid was determined by the ether-extraction method using a Soxtec Avanti 2050 (Foss Tecator AB, Switzerland). Moisture and ash were determined using the standard methods (AOAC, 1984). The inositol concentration in the diets was determined using an enzymatic assay as described by Ashizawa *et al.* (2000).

The standard methods on human were used to determine biochemical parameters (Rehulka, 2000). The serum was collected by centrifuging blood samples at 3000 rpm for 10 min. The biochemical indices of the serum were determined within 24 h of storage at 4°C. A Beckman LX20 automatic biochemical analyzer (Harbor Blvd., Fullerton, USA) was used for the determinations. These included Total Protein (TP), Blood Urea Nitrogen (BUN), Total Cholesterol (TC) and Triacylglycerol (TG).

Table 1: Composition and nutrients content of diets (g kg⁻¹)

Ingredient	Diet1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish meal	360.0	360.00	360.00	360.00	360.0	360.0
Soybean meal	270.0	27.00	27.00	27.00	270.0	270.0
Corn	100.0	10.00	10.00	10.00	100.0	100.0
Fermented soybean meal	100.0	10.00	10.00	10.00	100.0	100.0
Fish oil	70.0	70.00	7.00	7.00	70.0	70.0
Vitamin mix (inositol free)	1.0	1.00	0.10	0.10	1.0	1.0
Mineral mix	5.0	5.00	0.50	0.50	5.0	5.0
Vitamin C stable	0.5	0.50	0.05	0.05	0.5	0.5
Choline chloride	0.5	0.50	0.05	0.05	0.5	0.5
Starch	93.0	92.95	92.90	92.80	92.6	92.2
Inositol	0.0	0.05	0.10	0.20	0.4	0.8
Nutrients content (g kg⁻¹)						
Moisture	95.0	95.00	104.00	96.40	98.6	105.0
Crude protein	429.0	431.00	417.00	423.00	427.0	406.0
Crude lipid	102.0	101.00	93.40	98.20	91.7	100.0
Ash	95.0	97.00	96.00	93.00	94.0	93.0
Inositol (mg kg ⁻¹)	350.0	364.00	458.00	507.00	720.0	1050.0

¹: Vitamin mix (g kg⁻¹): Retinyl acetate 2.5; cholecalciferol 6.25; all-rac-a-tocopheryl acetate 75; menadione 2.5; vitamin 0.25; riboflavin 1.0; Dcalcium pantothenate 5.0; Pyridoxine HCL 0.75g; Cyanocobalamin 2.5; Niacin 2.5; Folic acid 0.25; Biotine 2.5; cellulose 899; ²: Mineral mix (g kg⁻¹): KCl 90; KI 0.04; CaHPO₄·2H₂O 500; NaCl 40; CuSO₄·5H₂O 3.0; ZnSO₄·7H₂O 4.0; CoSO₄·7H₂O 0.02; FeSO₄·7H₂O 20; MnSO₄·H₂O 3.0; CaCO₃ 215; MgSO₄·7H₂O 124; Cellulose 0.94

Statistical analysis: The data were subjected to one-way Analysis Of Variance (ANOVA). If significant ($p < 0.05$) differences were found, Duncan's multiple range test was used to rank the groups using the SPSS program Version 13.0 for Windows (SPSS Inc., Michigan Avenue, Chicago, IL, USA).

RESULTS

Survival and growth parameters: The fish growth performance of fish fed various dietary inositol was shown in Table 2. Weight gain was the significantly highest for barramundi fed the diet containing inositol 507 mg kg⁻¹ diet among all the groups ($p < 0.05$). FCR of fish fed the diet containing inositol 507 mg kg⁻¹ diet was significantly lower than the fish fed the diet containing inositol 458 mg kg⁻¹ diet; however, no significant difference was found compared to the other groups. Dietary inositol did not significantly affect survival ($p > 0.05$). Hepatosomatic index was not significantly affected by graded levels of dietary inositol ($p > 0.05$). However, significantly lower viscerasomatic index was found in the fish fed diet without supplemental inositol ($p < 0.05$).

The proximate composition of the fish whole body:

The whole-body composition of fish fed diets containing graded levels of inositol is presented in Table 3. The moisture content of the whole body averaged 71.0%; the crude protein and lipid contents averaged 17.5 and 7.0%, respectively. The moisture, crude protein and lipid contents of the fish whole body was not affected by inositol levels ($p > 0.05$).

Serum biochemical parameters:

Serum biochemical parameters for barramundi fed graded levels of inositol are shown in Table 4. Serum Total Protein (TP) increased with increasing dietary inositol levels up to 507 mg kg⁻¹ diet ($p < 0.05$). Triacylglycerol (TG) also increased with increasing dietary inositol levels up to 507-720 mg kg⁻¹ diet ($p < 0.05$). The lowest Blood Urea Nitrogen (BUN) was found in the fish fed dietary inositol 458 mg kg⁻¹ diet and was also significantly different compared to 350, 364, 507 and 1050 mg kg⁻¹ diet ($p < 0.05$). Total Cholesterol (TC) of fish fed dietary inositol 507-1050 mg kg⁻¹ diet was higher than those of fish fed dietary inositol 350-458 mg kg⁻¹ diet, but no significant difference was observed ($p > 0.05$).

Table 2: Effect of different dietary inositol levels on growth parameters of *Lates calcarifer*

Parameters	Dietary inositol levels (mg kg ⁻¹ diet)					
	350	364	458	507	720	1050
Initial weight (g)	5.51±0.11	5.54±0.05	5.52±0.13	5.53±0.07	5.47±0.03	5.51±0.06
Final weight (g)	35.77±2.42 ^a	36.29±3.97 ^a	36.60±0.49 ^a	44.11±2.00 ^b	35.71±1.37 ^a	38.31±0.98 ^a
Weight gain (%)	548.60±35.82 ^a	555.19±76.18 ^a	563.23±7.62 ^a	698.21±39.39 ^b	552.96±22.39 ^a	595.58±10.93 ^a
Survival (%)	88.89±8.39	90.00±6.67	90.00±3.33	87.78±6.94	88.89±1.92	90.00±0.00
FCR	1.04±0.09 ^{ab}	1.03±0.05 ^{ab}	1.08±0.01 ^b	0.98±0.01 ^a	1.03±0.04 ^{ab}	0.96±0.00 ^a
HIS	2.03±0.10	2.22±0.12	2.21±0.13	2.29±0.11	2.09±0.14	2.19±0.13
VIS	9.66±0.08 ^a	10.23±0.1 ^b	10.43±0.19 ^{bc}	10.08±0.19 ^b	10.24±0.25 ^b	10.54±0.27 ^c

¹: Values in the same row with different superscripts are significantly different ($p < 0.05$). Data are expressed as mean values ± SD (n = 3); ²: Weight gain = 100 × (final body weight - initial body weight)/(initial body weight); ³: Feed Conversion Ratio (FCR) = Weight gain (g) dry⁻¹ feed (g); ⁴: Hepatosomatic index (HIS) = 100 × liver weight/body weight; ⁵: Viscerasomatic index (VSI) = 100 × viscera weight/body weight

Table 3: Whole body composition of *Lates calcarifer* fed diets containing graded levels of inositol

Parameters	Dietary inositol levels (mg kg ⁻¹ diet)					
	350	364	458	507	720	1050
Moisture	71.59±0.84	70.50±0.55	71.61±0.49	71.24±0.88	70.46±0.89	70.87±0.29
Crude protein	17.40±0.47	17.55±0.14	17.52±0.07	17.41±0.00	17.59±0.10	17.46±0.12
Crude lipid	6.74±0.11	7.27±0.26	6.68±0.24	7.08±0.51	7.28±0.41	6.93±0.24

Values in the same row with different superscripts are significantly different ($p < 0.05$). Data are expressed as mean values ± SD (n = 3)

Table 4: Effect of on serum biochemical parameters of *Lates calcarifer* fed diets containing graded levels of inositol

Parameters	Dietary inositol levels (mg kg ⁻¹ diet)					
	350	364	458	507	720	1050
TP (g L ⁻¹)	48.00±1.74 ^a	49.82±1.06 ^{ab}	52.26±2.53 ^b	55.32±0.22 ^c	52.09±0.67 ^b	48.22±0.94 ^a
BUN (mmol L ⁻¹)	3.80±0.10 ^{bc}	3.83±0.81 ^{bc}	2.60±0.50 ^a	3.55±0.15 ^{bc}	3.20±0.10 ^{ab}	4.30±0.60 ^c
TC (mmol L ⁻¹)	6.76±0.17 ^a	6.66±0.28 ^a	6.61±0.48 ^a	7.76±0.34 ^c	7.56±0.37 ^{bc}	7.07±0.27 ^{ab}
TG (mmol L ⁻¹)	1.96±0.13 ^{ab}	1.59±0.30 ^a	1.88±0.36 ^{ab}	2.02±0.18 ^{ab}	2.12±0.32 ^{ab}	2.24±0.42 ^b

Values in the same row with different superscripts are significantly different ($p < 0.05$). Data are expressed as mean values ± SD (n = 3). TP: Total Protein; BUN: Blood Urea Nitrogen; TC: Total Cholesterol; TG: Triacylglycerol

DISCUSSION

The importance of dietary inositol for normal growth of juvenile *Lates calcarifer* maybe demonstrated in the present study. Although dietary inositol did not significantly affect survival ($p>0.05$), weight gain was the significantly highest for barramundi fed the diet containing inositol 507 mg kg⁻¹ diet among all the groups ($p<0.05$). Glencross (2006) reviewed that no differences in growth, feed conversion or survival were observed when barramundi were fed practical diets added inositol. On the other hand, inositol was required for its normal growth using semi-purified diets. Weight gain increased for fish fed dietary inositol up to the requirement level. The optimal dietary inositol requirement for the maximum growth of juvenile Jian carp (*Cyprinus carpio*) was estimated to be 518.0 mg kg⁻¹ diet (Jiang *et al.*, 2009); juvenile tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) required 400 mg kg⁻¹ diet (Shiau and Su, 2005); juvenile flounder *Paralichthys olivaceus* required 800-1200 mg kg⁻¹ diet (Li *et al.*, 2001) and *Lateclabrax japonicus* required 500 mg kg⁻¹ diet (Zhong and Zhang, 2001); however, the suitable requirement for dietary inositol of growing grass carp (*Ctrnopharyngodon idella*) was approximately 166-214 mg kg⁻¹ diet (Wen *et al.*, 2007). Waagbo *et al.* (1998) reported that no real effect of dietary inositol supplementation on growth and mortality in Atlantic salmon (*Salmo salar*) feeding practical diets. Moreover, juveniles Nile tilapia (*Oreochromis niloticus*) (Peres *et al.*, 2004) and sunshine bass (*Morone chrysops*♀ × *Morone saxatilis*♂) (Deng *et al.*, 2002) fed purified diets did not require an exogenous source of inositol for normal growth and feed utilization. Dietary inositol did not significantly affect survival ($p>0.05$). This maybe practical diet contain sufficient levels of inositol to meet various metabolic needs of fish because this vitamin is widely distributed in common feed ingredients (Peres *et al.*, 2004).

Hepatosomatic weight were higher at high inositol levels compared with the unsupplemented diet maybe due to inositol increased the promotion of liver development (Jiang *et al.*, 2009). In the present study, the results showed that HSI of barramundi was not affected by dietary levels of inositol. This result demonstrated that high inositol levels had no potential to affect the health of fish. It was also found that dietary inositol did not affect HSI of in Atlantic salmon (Waagbo *et al.*, 1998), sunshine bass (Deng *et al.*, 2002) and Nile tilapia (Peres *et al.*, 2004).

The significantly higher accumulation of lipid in liver and muscle of fish could be an indication of

inositol deficiency (Peres *et al.*, 2004). In the present study, no significant difference was found in the moisture, crude protein and lipid contents of the fish whole body. This is also consistent with studies on Atlantic salmon (Waagbo *et al.*, 1998), grass carp (Wen *et al.*, 2007) and Kuruma shrimp (*Marsupenaeus japonicus*) (Michael and Koshio, 2008). However, in Jian carp (Jiang *et al.*, 2009), whole body moisture was not affected by inositol levels ($p>0.05$), but body protein increased significantly with increasing dietary inositol levels up to 384.2 mg kg⁻¹ diet ($p<0.05$) and body fat was the highest for fish fed diets containing 838.8 and 990.3 mg kg⁻¹ diet and the lowest for fish fed the unsupplemented diet ($p<0.05$).

Good state of health for fish is the basic precondition for successful fish culture. Monitoring the physiological state of fish has become an integral part of the routine examination of fish health (Rehulka, 2000). Apparently any adverse effects were not found on the state of the fish health in this study. However, differences were observed in serum parameters of cultured barramundi among dietary groups. Dietary herbs (Lu *et al.*, 2009) and *B. licheniformis* (Yuan *et al.*, 2009) could change some blood hematological and blood biochemical indices of the fish. Serum TP levels can be used as a diagnostic tool and a valuable test for evaluating the general physiological state in fish (Pedro *et al.*, 2005). In the present study, TP increased with increasing dietary inositol levels up to 507 mg kg⁻¹ diet ($p<0.05$). Significantly low levels of total plasma protein were reported for some infected fish (Benli and Yildiz, 2004; Yildiz and Aydin, 2006; Rehulka and Minarik, 2007); meanwhile, clinical chemistry analyses in blood plasma indicated the decreased levels of total protein (Rehulka and Minarik, 2007). The significantly lowest Blood Urea Nitrogen (BUN) was found in the fish fed dietary inositol 458 mg kg⁻¹ diet among all the groups except for the 720 mg kg⁻¹ diet. The increase of BUN may reflect kidney dysfunction (Liu *et al.*, 2007) and its increased levels generally signal a higher rate of conversion of nitrogen compounds (Rehulka and Minarik, 2003). The TC of fish fed dietary inositol 507-1050 mg kg⁻¹ diet was higher than those of the fish fed dietary inositol 350-458 mg kg⁻¹ diet, but no significant difference was observed ($p>0.05$). In grass carp (Wen *et al.*, 2007), TC was significantly lowest for fish fed the unsupplemented diet ($p<0.05$). However, the total protein and cholesterol concentrations in plasma were not significantly affected by dietary inositol (Waagbo *et al.*, 1998). TG increased with increasing dietary inositol levels up to 507-720 mg kg⁻¹ diet ($p<0.05$) in this study. This result did not agree with Waagbo *et al.* (1998) for Atlantic salmon

and Wen *et al.* (2007) for *Ctrnopharyngodon idella*, which plasma TG was negatively correlated to dietary inositol supplementation.

CONCLUSION

Results of the present investigation demonstrate significant improvement of growth and feed utilization of juvenile barramundi can be achieved by inositol supplementation at 507 mg kg⁻¹ diet.

REFERENCES

- AOAC., 1984. Official Methods of Analysis. 14th Edn., Association of Official Analytical Chemists, Arlington, VA., pp: 114.
- Ashizawa, N., M. Yoshida and T. Aotsuka, 2000. An enzymatic assay for myo-inositol in tissue samples. *J. Biochem. Biophys. Methods*, 44: 89-94. DOI: 10.1016/S0165-022X(00)00069-5
- Benli, A.C.K. and H.Y. Yildiz, 2004. Blood parameters in Nile tilapia (*Oreochromis niloticus* L.) spontaneously infected with *Edwardsiella tarda*. *Aquac. Res.*, 35: 1388-1390. DOI: 10.1111/j.1365-2109.2004.01158.x
- Deng, D.F., G.I. Hemre and R.P. Wilson, 2002. Juvenile sunshine bass (*Morone chrysops*♀×*Morone saxatilis*♂) do not require dietary myo-inositol. *Aquaculture*, 213: 387-393. DOI: 10.1016/S0044-8486(02)00119-9
- Glencross, B., 2006. The nutritional management of barramundi, *Lates calcarifer*-a review. *Aquac. Nutr.*, 12: 291-309. DOI: 10.1111/j.1365-2095.2006.00410.x
- Harpaz, S., Y. Hakim, T. Slosman and O.T. Erolodogan, 2005. Effects of adding salt to the diet of Asian sea bass *Lates calcarifer* reared in fresh or salt water recirculating tanks, on growth and brush border enzyme activity. *Aquaculture*, 248: 315-324. DOI: 10.1016/j.aquaculture.2005.03.007
- Jiang, W.D., L. Feng, Y. Liu, J. Jiang and X.Q. Zhou, 2009. Growth, digestive capacity and intestinal microflora of juvenile Jian carp (*Cyprinus carpio* var. Jian) fed graded levels of dietary inositol. *Aquac. Res.*, 40: 955-962. DOI: 10.1111/j.1365-2109.2009.02191.x
- Katersky, R.S. and C.G. Carter, 2009. Growth and protein synthesis of barramundi, *Lates calcarifer*, fed lupin as a partial protein replacement. *Comparit. Biochem. Physiol.-Part A: Mol. Integrat. Physiol.*, 152: 513-517. DOI: 10.1016/j.cbpa.2008.12.017
- Li, A.J., D.B. Zhang, W.Q. Wei and X.J. Zhang, 2001. A study on the nutritional requirement for juvenile flounder *Paralichthys olivaceus*. *J. Zhejiang Ocean Univ. (Nat. Sci.)*, 20: 6-10. http://d.wanfangdata.com.cn/Periodical_zjhyxyxb-zr2001z1002.aspx
- Liu, S.M., X.N. Zang, B. Liu, X.C. Zhang and A.K. Kiu *et al.*, 2007. Effect of growth hormone transgenic *Synechocystis* on growth, feed efficiency, muscle composition, haematology and histology of turbot (*Scophthalmus maximus* L.). *Aquac. Res.*, 38: 1283-1292. DOI: 10.1111/j.1365-2109.2007.01796.x
- Lu, X., H.Z. Lin, Z.J. Li, F.H. Yuan and Q.B. Yang, 2009. Effect of dietary Chinese herbal medicines on hematological and blood biochemical indices in cultured seabass *Lates calarifer*. *J. Dalian Fish. Univ.*, 24: 279-282. http://en.cnki.com.cn/Article_en/CJFDTOTAL-DLSC200903019.htm
- Michael, F.R. and S. Koshio, 2008. Biochemical studies on the interactive effects of dietary choline and inositol in juvenile Kuruma shrimp, *Marsupenaeus japonicus* Bate. *Aquaculture*, 285: 179-183. DOI: 10.1016/j.aquaculture.2008.08.006
- Pedro, N., A.I. Guijarro, M.A. López-Patino, R. Martinez-Alvarez and M.J. Delgado, 2005. Daily and seasonal variations in haematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. *Aquac. Res.*, 36: 1185-1196. DOI: 10.1111/j.1365-2109.2005.01338.x
- Peres, H., C. Lim and P.K. Klesius, 2004. Growth, chemical composition and resistance to *Streptococcus iniae* challenge of juvenile Nile tilapia (*Oreochromis niloticus*) fed graded levels of dietary inositol. *Aquaculture*, 235: 423-432. DOI: 10.1016/j.aquaculture.2003.09.021
- Rehulka, J., 2000. Influence of astaxanthin on growth rate, condition and some blood indices of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, 190: 27-47. DOI: 10.1016/S0044-8486(00)00383-5
- Rehulka, J. and B. Minarik, 2007. Blood parameters in brook trout *Salvelinus fontinalis* (Mitchill, 1815), affected by columnaris disease. *Aquac. Res.*, 38: 1182-1197. DOI: 10.1111/j.1365-2109.2007.01786.x
- Rehulka, J. and B. Minarik, 2003. Effect of lecithin on the haematological and condition indices of the rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquac. Res.*, 34: 617-627. DOI: 10.1046/j.1365-2109.2003.00855.x

- Shiau, S.Y. and S.L. Su, 2005. Juvenile tilapia (*Oreochromis niloticus*×*Oreochromis aureus*) requires dietary myo-inositol for maximal growth. *Aquaculture*, 243: 273-277. DOI: 10.1016/j.aquaculture.2004.10.002
- Tantikittia, C., W. Sangpong and S. Chiavareesajjaa, 2005. Effects of defatted soybean protein levels on growth performance and nitrogen and phosphorus excretion in Asian seabass (*Lates calcarifer*). *Aquaculture*, 248: 41-50. DOI: 10.1016/j.aquaculture.2005.04.027
- Waagbo, R., K. Sandnes and O. Lie, 1998. Effects of inositol supplementation on growth, chemical composition and blood chemistry in Atlantic salmon. *Salmo salar* L., fry. *Aquac. Nutr.*, 4: 53-59. DOI: 10.1046/j.1365-2095.1998.00043.x
- Wen, H., Z.Y. Zhao, M. Jiang, A.L. Liu and F. Wu *et al.*, 2007. Dietary myo-inositol requirement for grass carp, *Ctrnopharyngodon idella* fingerling. *J. Fish. Sci. China*, 14: 794-800. http://en.cnki.com.cn/Article_en/CJFDTOTAL-ZSCK200705013.htm
- Williams, K.C., C.G. Barlow, L. Rodgers and C. Agcopra, 2006. Dietary composition manipulation to enhance the performance of juvenile barramundi (*Lates calcarifer* Bloch) reared in cool water. *Aquac. Res.*, 37: 914-927. DOI: 10.1111/j.1365-2109.2006.01513.x
- Yildiz, H. and S. Aydin, 2006. Pathological effects of *Arcobacter cryaerophilus* Infection in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Acta Veterinaria Hungarica*, 54: 191-199. DOI: 10.1556/AVet.54.2006.2.6
- Yuan, F.H., H.Z. Lin, Z.J. Li, X. Lu and Q.B. Yang, 2009. Effects of dietary *Bacillus licheniformis* on blood physiological-biochemical indices in cultured *Lates calarifer*. *South China Fish. Sci.*, 5: 45-50. <http://www.cqvip.com/qk/60642A/200902/29941297.html>
- Zhong, W.R. and X.H. Zhang, 2001. Studies on the requirements of *Lateclabrax japonicus* for vitamins at various growth stages. *J. Zhejiang Ocean Univ. (Nat. Sci.)*, 20: 99-102. <http://www.cqvip.com/qk/97875A/200903/>