The Open Access Israeli Journal of Aquaculture – Bamidgeh

As from **January 2010** The Israeli Journal of Aquaculture - Bamidgeh (IJA) will be published exclusively as **an on-line Open Access (OA)** quarterly accessible by all AquacultureHub (http://www.aquaculturehub.org) members and registered individuals and institutions. Please visit our website (http://siamb.org.il) for free registration form, further information and instructions.

This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor to obtain alternative sources of income to support this policy for as long as possible.

Published under auspices of

The Society of Israeli Aquaculture and Marine Biotechnology (SIAMB),

University of Hawai'i at Mānoa Library

&

University of Hawai'i at Mānoa Aquaculture Program

in association with

AquacultureHub

http://www.aquaculturehub.org

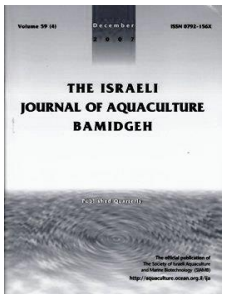
ISSN 0792 - 156X

PUBLISHER:

Israeli Journal of Aquaculture - BAMIGDEH - Kibbutz Ein Hamifratz, Mobile Post 25210, ISRAEL

Phone: + 972 52 3965809

http://siamb.org.il



The Effect of Dietary Lipid Levels on Growth Performance, Lipid Deposition, and Antioxidant Status of Juvenile Turbot, *Scophthalmus maximus*, Fed Isonitrogenous and Isoenergetics Diets

Hongjuan Zhang[1], Jingwu Sun[2], Haiyan Liu[1*], Wenqing Zhao[1], Zhencai Yang[1]

[1] Laboratory of Aquatic Nutrition and Ecology, College of Life Science, Hebei Normal University, Shijiazhuang, Hebei 050024, China.
[2] Shijiazhuang University, Shijiazhuang, Hebei 050035, China

(Received 21.7.2014, Accepted 6.10.2014)

Key words: turbot, feed, lipid level, antioxidant status

Abstract

The purpose of this study was to investigate the effects of different lipid (fish oil/soybean oil=1:1, w/w) levels in diets with 45% crude protein on growth performance, body composition, and antioxidant status of turbot (Scophthalmus maximus L.). 2040 turbot (39±0.2g) were randomly divided into 4 groups with 3 replicates and fed isonitrogenous and isoenergetic diets with lipid levels of 6.0%(L6.0), 8.5%(L8.5), 11.0%(L11.0), and 13.5%(L13.5) for 56 days. Hepatic lipase (HL) and superoxide dismutase (SOD) activity were evaluated, as well as lipid peroxidation measured as malondialdehyde (MDA). Results showed that no significant difference was observed in feeding rate (FR), protein efficiency ratio (PER) and protein retention, while dietary lipid levels caused a significant increase ($P<0.05$) in specific growth rate (SGR) The L13.5 group showed the highest lipid and energy retention and this was significantly higher than in other groups; lipid content in whole body and liver increased significantly with increased dietary lipid ($P<0.05$); the HL and SOD activity, and MDA in the liver all increased significantly ($P<0.05$)with dietary lipid supplementation in L13.5. In general, high dietary lipid levels enhanced growth rate of turbot, but the increment of growth was due mainly to excessive lipid deposition. Oxidation stress was observed in fish fed the diet containing 13.5% lipid. The optimal level of dietary lipid for good growth and antioxidant status in turbot was found to be 11%.

* Corresponding author. Haiyan Liu; Tel/Fax: +86-0311-80787598; E-mail address: liuhaiyan@hebtu.edu.cn

Introduction

Turbot (*Scophthalmus maximus* L.) is a valuable commercial species of flatfish native mainly to the Atlantic coast of Europe. Due to its delicate flavor and rapid growth, turbot culture has increased rapidly in China since its introduction in 1992 (Shi et al., 2004). China has become the main provider of commercially farmed turbot in the world. Production of turbot has reached an annual level of 50,000–60,000 tons in recent years. This is about seven times the total production of turbot in Europe (FAO, 2010). However, turbot culture in China relies on trash fish as the main source of feed, thus there is a growing need to develop alternative high quality commercial feeds.

Generally, carnivorous fish utilize dietary protein and lipid more effectively and efficiently than carbohydrates (NRC, 2011). In addition to being the source of fat-soluble vitamins, lipids also provide essential components for the formation of cell and tissue membranes (Sargent, 1995). Furthermore, high dietary lipid levels can promote the protein-sparing effect, and also reduce the excretion of ammonia which causes water pollution (Yigit et al., 2002). As a carnivorous marine species, turbot require higher crude protein levels in their diet (Lee et al., 2003). Optimal dietary lipid levels of turbot reported in previous literature have been controversial and ranged from 8-23% (Sevgili et al., 2012; Niu et al., 2013). The ability to utilize lipids as a protein sparing effect for turbot is also disputable (Sevgili et al., 2014).

In previous studies, most research has focused more on the growth performance of fish and less on the correlation between dietary macronutrient levels and fish health. High levels of dietary lipids increase susceptibility to autoxidation and tissue lipid peroxidation. These may also adversely affect the immune response and disease resistance of fish (Dias et al., 2004). Animal cells require a proper balance between oxidants and antioxidants for their survival, and the antioxidant levels affect the health of the fish (Liu et al., 2008).

This study was undertaken to investigate the influence of different lipid levels in diets with constant protein and energy level, on growth, lipid deposition, and antioxidant status in turbot.

Materials and Methods

Experimental diets. White fish meal, squid liver powder, and plant protein mixture were used as protein sources, and fish oil and soybean oil (1:1) as main lipid sources. Four isonitrogenous (45% crude protein) and isoenergetic (17.80 MJ/KG) experimental practical diets were formulated to contain 6.0% (L6.0), 8.5% (L8.5), 11.0% (L11.0), and 13.5% (L13.5) lipid levels. The gradation of lipid was obtained by regulating α-starch and zeolite powder content of the diets. Detailed information about feed components and chemical composition of the experimental diets are shown in Table 1.

Table 1. Ingredients and proximate composition of experimental diets

	Diets			
Ingredients(*%*)	*L6.0*	*L8.5*	*L11*	*L13.5*
White fish meal	48.00	48.00	48.00	48.00
Squid liver meal	4.10	4.10	4.10	4.10
Plant protein premix[1]	18.60	18.60	18.60	18.60
α-starch	21.18	15.12	9.06	3.00
Soybean oil	0.00	1.34	2.69	4.04
Fish oil	0.00	1.34	2.69	4.04
Amino acid premix[2]	0.94	0.94	0.94	0.94
Stone powder	0.48	0.48	0.48	0.48
Zeolite powder	0.00	3.38	6.74	10.10
Choline chloride (50%)	0.20	0.20	0.20	0.20
Calcium Hydrogen Phosphate	2.60	2.60	2.60	2.60
Sodium alginate	0.85	0.85	0.85	0.85
Ethoxy quinoline	0.05	0.05	0.05	0.05
Vitamin premix[3]	2.00	2.00	2.00	2.00
Mineral premix[4]	1.00	1.00	1.00	1.00
Analyzed nutrients compositions (% dry matter basis)				
Crude protein	44.23	44.90	44.70	44.73
Crude lipid	6.22	8.56	11.01	13.60
Crude ash	16.42	18.8	21.07	23.43
Gross energy (MJ/KG)	17.55	17.67	17.67	17.44

[1] Plant protein premix (g/100g diet): Extruded soybean, 6.00; Fermented soybean meal, 3.00; Brewer's yeast, 3.00; Wheat gluten meal, 6.60.

[2] Amino acid premix (g/100g diet): lysine, 0.18; methionine, 0.44; threonine, 0.02. Taurine, 0.3;

[3] Vitamin premix (mg/kg diet): thiamin, 25; riboflavin, 45; pyridoxine HCl, 20; vitamin B_{12}, 0.1; vitamin K3, 10; inositol, 800; pantothenic acid, 60; niacin acid, 200; folic acid, 20; biotin, 1.20; retinol acetate, 32; cholecalciferol, 5; alpha-tocopherol, 120; ascorbic acid, 2500; wheat middling, 16.16 g/kg diet.

[4] Mineral premix: (mg/kg diet): $CuSO_4 \cdot 5H_2O$, 10; $FeSO_4 \cdot H_2O$, 80; $ZnSO_4 \cdot H_2O$, 50; $MnSO_4 \cdot H_2O$, 60; $MgSO_4 \cdot 7H_2O$, 1200; NaF, 2; KI, 0.8; $CoCl_2 \cdot 6H_2O$ (1%), 50; zeolite, 8.55 g /kg diet.

All ingredients were ground into fine powder and sieved through 175-μm mesh. The powder was weighed and made into soft pellets (diameter: 2 mm) using an EL-260 feed machine (Friendship machinery Corp., Weihai, China). The determined dietary lipid was 6.22%, 8.56%, 11.01% and 13.6% respectively. All pellets were held at -20 °C until use.

Fish and experimental conditions. Juvenile turbot were obtained from Tianhe farm in Qinhuangdao, China. The entire experiment was conducted under natural photoperiodic conditions. The fish were acclimated to experimental conditions for 2 weeks, during which fish were fed the L6.0 diet. When the experiment began, the fish were fasted for 24h and weighed. Healthy fish of similar size were selected and distributed randomly into 12 fiberglass tanks (2m×1m×1m) filled with 1000 l water, 170 fish per tank. Water flow rate was 2 l/min. Water temperature ranged from 13-15 °C, the dissolved oxygen, ammonia-N, and salinity was 5.61 ± 0.04mg/l, 0.19 ± 0.07 mg/l, and 25 ±1.5‰ respectively throughout the experiment.

Throughout the 56 day experiment each experimental diet was allocated to triplicate tanks distributed randomly. For 8 weeks fish were hand-fed their respective diets twice daily at 0800 and 1600 to apparent satiation. Satiation was achieved by allowing fish to eat until feeding activity stopped and no leftover feed remained in the tank. Feed consumption was recorded daily.

Growth performance. At the end of the experiment, all fish were fasted for 24 hours and then weighed. Growth and feed utilization variables, such as feeding rate (FR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), and lipid retention (LR) were calculated as follows (See table 2).

SR (%) = 100 × (number of final surviving fish/number of initial fish)

FR (%/d) = feed intake× 100/((FBW+IBW) /2×days)

FCR = feed intake/(FBW-IBW)

SGR (%/d)= (lnFBW -lnIBW) × 100/days

PER = weight gain× 100/ protein intake

Table 2. The growth performances of juvenile turbot fed with the test diets (M±SD)

	L6.0	*L8.5*	*L11*	*L13.5*
IBW(g)	38.86±0.02	39.00±0.10	39.09±0.07	38.98±0.12
FBW(g)	59.17±0.78^{a}	59.52±0.22^{a}	61.65±1.23^{ab}	64.02±1.49^{b}
SR(%)[1]	97.45 ±1.7	97.83±0.91	96.66 ±2.77	97.04 ±2.05
FR(%/BW/d)[2]	0.68±0.02	0.63±0.01	0.65±0.01	0.66±0.01
FCR[3]	0.94±0.02 ^{a}	0.87±0.02^{ab}	0.84 ±0.02^{ab}	0.79 ±0.04^{b}
SGR(%/d)[4]	0.73±0.02^{a}	0.73±0.02^{a}	0.78±0.04^{ab}	0.86±0.03^{b}
PER(%)[5]	240.54±7.72	257.50±11.17	267.97±22.14	285.49±25.02

Values in the same row with different superscripts are significantly different (*P<0.05*).

[1]SR (%) = 100 × (number of final surviving fish/number of initial fish)

[2]FR (%/d) = feed intake× 100/((FBW+IBW) /2×days)

[3]FCR = feed intake/(FBW-IBW)

[4]SGR (%/d)= (lnFBW -lnIBW) × 100/days

[5]PER = weight gain× 100/ protein intake

IBW = initial body weight; FBW = final body weight; SR = survival rate; FR = feeding rate; FCR = feed conversion ratio; SGR = specific growth rate; PER = protein efficiency ratio.

Sampling and chemical analysis. At the end of the trial, three fish from each tank were sampled and stored at -20 ^{o}C for whole body composition analysis. The other nine fish from the same tank were dissected and the liver removed for analysis of lipid and malondialdehyde (MDA) content, and hepatic lipase (HL) and superoxide dismutase (SOD) activity.

Proximate composition analyses of diets, whole body, and livers were conducted following standard laboratory procedures (AOAC 2006). Dry matter was analyzed by drying the samples to constant weight at 105 °C. Crude protein (CP) was determined using a KjeltecTM 2300 Unit (Foss, Hillerød, Denmark) by the Kjeldahl method, and CP content was calculated by multiplying nitrogen by 6.25. Crude lipid was analysed with a Soxtex System 1043(Foss). Ash was analyzed by combustion in a muffle furnace (Zhonghuan, Tianjin, China) at 550 °C for 16 h. Gross energy was determined using Parr 1281 Automatic Bomb Calorimeter (Parr, Moline, IL, USA).

SOD activity, MDA content, and HL activity in the liver were assayed using commercial kits (No. A001, No. A003 and No. A054; Nanjing Jiancheng Bioengineering

Institute, Nanjing, China) by a spectrophotometry (TU-1901; Purkinje General Ltd., Beijing, China). Total protein concentration was measured with bovine serum albumin as the standard.

Statistical analysis. Results are presented as mean ± SD. STATISTICA 10.0 software (Statsoft, Inc., Tulsa, OK, USA) was used for statistical analysis. The Shapiro-Wilk test was used to check all data for normality, and the homogeneity of variances was checked using Levene's test. The data of different treatments were subjected to one-way analysis of variance (ANOVA); if there was an overall significant effect, Duncan's multiple range test was applied to compare the means between individual treatments. Differences were considered to be significant at $P < 0.05$.

Results

Data on growth performance and feed utilization (see Table 2). During the *w*hole experimental period, the survival rates in all groups were more than 96% with no significant difference between them. There was no significant difference in feeding rate (FR) among all groups (*P>0.05*), but significant differences were observed in specific growth rate when dietary lipid level increased (*P<0.05*). Fish in group L13.5 had the greatest growth performance, followed by group L11.0. The feed conversion ratio (FCR) of fish in group L13.5 was significantly lower than in groups L6.0 and L8.5 (*P<0.05*), while there was no significant difference in group L11.0. The protein efficiency ratio (PER) was not statistically different between all groups (*P>0.05*).

The retention of dietary protein, lipid and energy are shown in Table 3.

Table 3. Lipid, protein and energy utilization of turbot fed experimental diets with different lipid levels (M±SD)

	L6.0	*L8.5*	*L11*	*L13.5*
Lipid				
Intake(g/kg BW/d)	0.42±0.01^{a}	0.54±0.01^{b}	0.71±0.01^{c}	0.90±0.02^{d}
Gain(g/kg BW /d)	0.22±0.06^{a}	0.25±0.02^{a}	0.31±0.08^{a}	0.67±0.08^{b}
Retention (%)	52.01±12.56^{a}	47.15±4.72^{a}	50.93±6.71^{a}	73.84±7.48^{b}
Protein				
Intake(g/kg BW /d)	2.97±0.09	2.82±0.06	2.88±0.04	2.95±0.06
Gain(g/kg BW /d)	1.16±0.03	1.14±0.01	1.20±0.06	1.28±0.04
Retention (%)	38.87±0.68	40.62±1.00	41.80±1.84	43.46±2.13
Energy				
Intake(g/kg BW/d)	516.16±13.65	479.54±12.49	487.82±7.43	480.49±13.84
Gain(g/ kg BW/d)	144.44±14.37^{a}	148.89±6.09^{a}	169.81±9.82^{a}	242.64±11.24^{b}
Retention (%)	27.91±2.37^{a}	31.15±2.02^{a}	34.80±1.90^{a}	50.53±2.27^{b}

Values in the same row with different superscripts are significantly different (*P<0.05*).
BW: body weight; Nutrient intake = (feed intake×%nutrient) / ((FBW+IBW) /2×days); Nutrient gain = (FBW × final body nutrient) - (IBW × initial body nutrient) / (FBW+IBW) /2×days); Nutrient retention = nutrient gain× 100/nutrient intake

It was obvious that lipid intake increased significantly in relation to the supplemented dietary lipid among all groups, while the protein and energy intake did not differ significantly (*P>0.05*). Lipid and energy retention was significantly greater (*P<0.05*) in the L13.5 group than in the other groups. Protein retention was similar in all groups.

Proximate analysis of whole body and liver composition of juvenile turbot at the end of the feeding trial are given in Table 4. There were no significant differences in whole body protein, moisture, and ash contents, in fish fed the different lipid levels (*P>0.05*). Whole body and liver lipid contents increased significantly in relation to the dietary lipid level (*P<0.05*).

Table 4. Effect of different lipid levels on body composition (in % of wet weight) and liver (in % of dry weight) of juvenile turbot (M ± SD)

	L6.0	*L8.5*	*L11*	*L13.5*
Moisture (%)	77.09±0.82	76.92±0.57	75.89±0.92	75.56±0.20
Crude lipid (%)	3.01±0.23^{a}	3.05±0.10^{a}	3.58±0.29^{a}	4.86±0.41^{b}
Crude protein (%)	14.93±0.47	14.80±0.19	14.77±0.29	14.66±0.10
Crude ash (%)	3.94±0.13	3.86±0.15	4.00±0.17	3.82±0.09
Liver lipid level	33.49±2.41^{a}	36.26±0.74^{a}	42.5±2.33^{b}	47.22±1.28^{b}

Values in the same row with different superscripts are significantly different (*P<0.05*).

HL and SOD activity, and MDA content in juvenile turbot liver were all affected by dietary lipid levels (Table 5). The HLand SOD activity, and MDA content, were all significantly higher in L13.5 group (*P<0.05*).

Table 5. Effects of dietary lipid levels on HL、SOD activities and MDA contents in turbot's liver (M±SD)

	L6.0	*L8.5*	*L11*	*L13.5*
HL(U/g.prot)	6.54±0.75[a]	7.18±1.24[a]	7.04±4.09[a]	10.51±2.89[b]
SOD(U/mg.prot)	4.21±0.5[a]	6.03±1.27[ab]	5.84±0.68[ab]	7.5±1.92[b]
MDA(nmol/mg.prot)	10.02±2.16[a]	11.55±2.85[ab]	14.27±0.85[ab]	22.25±3.59[b]

HL= Hepatic lipase; SOD = superoxide dismutase; MDA = malondialdehyde.
Values in the same row with different superscripts are significantly different (*P<0.05*).

Discussion

Contrary to other publications, the voluntary feeding intake in this study was constant in all treatments, but the feeding rate of turbot decreased in relation to dietary energy content as reported in other publications (Bromley et al., 1980; Regost et al., 2001; Leknes et al., 2012). This phenomenon in turbot is the same as in other fish and higher vertebrates, the voluntary feed intake appeared to depend on the energy content of the diets (Kissileff and Van Itallie, 1982; Boujard and MeÂdale, 1994).

Growth performance and feed utilization of turbot increased in relation to increasing lipid levels; best growth performance was achieved in the L13.5 group. There are a few reports which support these results. The best growth and feed conversion ratio was observed in juvenile turbot (54g) fed 13% lipid as opposed to those fed 10%, 16% and 19% lipid (Sevgili et al. 2014). The weight gain of turbot (47g) increased in fish fed diets containing lipid at 16.8% compared to 6.9% (Cho et al. 2005). The dietary lipid level of 20% was thought to be better for the growth of juvenile turbot (27g) (Ma et al. 2001). However, a limit to the growth-promoting effect of high dietary lipid was indicated in a study by Regost et al. (2001), where growth performance of large turbot (660g) was higher in fish fed diets containing10% lipid compared to those fed 15%, 20% and 25% lipid. There were no significant differences between 15%-16% and 23%-25% dietary lipid levels in growth for large turbot (580g, 202g, and 111g) (Sæther and Jobling, 2001; Leknes et al., 2012; and Niu et al., 2013). The precise amount of lipid required for satisfactory growth depends on fish species, fish size, dietary protein level, and carbohydrate level (NRC, 2011). All of the previous studies indicate that a different growth response to dietary lipid between juvenile turbots and large turbots exists where small juvenile turbots require more dietary lipid than larger ones. However, growth performance at all stages of maturation did not increase when the dietary lipid level was above 15%.

Protein-sparing by dietary lipids has been shown to occur in a majority of carnivorous fish however these findings do not always apply to turbots (Regost et al., 2001; Sæther and jobling, 2001; Sevgili et al., 2014). In some cases, a protein sparing effect was observed at lower dietary protein levels and feeding rates in turbot (Nijhof, 1993; Cho et al., 2005). In this study, the dietary protein (45%) was similar to Sæther and Jobling (2001) and was relatively lower compared with others (Regost et al., 2001 and Cho et al. 2005). Although growth increased in relation to lipid levels, the PER was not significantly affected. It seems therefore that turbots have a limited ability to use higher levels of dietary lipid. This is further seen in protein and lipid retention. Protein retention did not significantly increase with increased dietary lipid, while lipid retention increased significantly, especially in L13.5 group. This indicates that increased turbot growth in this study was achieved mainly from increased lipid deposition.

Studies have shown that high lipid levels in diets can cause deposition of excessive lipid in fish tissue (Andersen and Alsted, 1993). In the present study, the effect of dietary lipid levels on the chemical composition of body and liver was significant only for lipid content, and there was an inverse correlation between body moisture and lipid content. These results agree with reports on turbot by Andersen and Alsted (1993), Sæther and Jobling (2001), Cho et al. (2005) and Sevgili et al. (2014). The increase of lipid deposition resulted in the increase of body and liver lipid content of turbot. Lipid deposition (total content, sterols, and tocopherols) occurs mainly at the periphery of fin roots, and under the skin (Andersen and Alsted, 1993; Regost et al., 2001; Aubourg et al., 2007). Since the abdomen of turbot is small compared with other fish species, turbot has limited ability to store lipid in the body. On the one hand, the deposition of

excessive dietary lipid in fish flesh would be a major issue relating to carcass and product quality (Hillestad et al., 1998); on the other hand, excessive lipid deposition in the liver can also lead to fatty liver syndrome which may be associated with increased lipid peroxidation, impaired liver function, and necrosis (Craig et al., 1999).

The liver is the main site of various key metabolic pathways, and also the most frequently studied tissue with regard to oxidative stress (Bagnyukova et al., 2005). In this study, hepatic lipase (HL), and superoxide dismutase (SOD) activity, and malondialdehyde (MDA) content in the liver were significantly enhanced when lipid levels were highest. In this study, the enhancement of HL did not prevent excess deposition of lipid in the liver. SOD is a free radical-scavenging enzyme providing the first defense against oxidative stress, removal of superoxide (O^{2-}) and prevention of formation of other reactive oxygen species (ROS) generated in aerobic animals during normal cellular metabolism (McCord and Fridovich, 1969). Hepatic SOD activity reflects the antioxidant response of fish to some environmental stressors (Lushchak and Bagnyukova, 2006；Liu et al., 2008). MDA can be used as a biomarker of liver damage, stress, and welfare in fish (Sanz et al., 2012). The highest liver MDA content (nearly double) was observed in the L13.5 group. This demonstrates that an imbalance exists between the generation and removal of ROS at high lipid levels where high dietary lipid levels seemed to have caused oxidative stress in the turbot liver.

The nutrient requirements in fish have generally been based on growth performance and deficiency symptoms (Lim and Webster, 2001). Thorough knowledge of the metabolic potential of fish relative to their physiological condition and body health is needed as a prerequisite for the establishment of optimal nutrient requirements. In the present study, the growth performance of turbot was enhanced in relation to dietary lipid levels that ranged from 6.0% to 13.5%; the best dietary lipid level for optimum growth was 13.5% however the increment of growth mainly derived from the excess deposition of lipid in body and dietary lipid levels above 11% resulted in oxidative stress.

Acknowledgements

This study was supported by the Hebei Science and Technology (12220501D, C2013205016) project; the special Fund for Hebei Characteristics of Seafood Innovation Team of Modern Agro-industry Technology Research System; National Natural Science Foundation of China Project No. 31272315.

References

Andersen N.G. and N.S. Alsted, 1993. Growth and body composition of turbot (*Scophthalmus maximus*) in relation to different lipid/protein ratios in the diet. In: Kaushik S.J., Luquet P. (Eds.), Fish Nutrition in Practice, Biarritz, France, INRA Editions. Les Colloques NO. 61, pp. 479-491.

Aubourg S.P., Losada V. and R. Prego, 2007. Distribution of lipids and trace minerals in different muscle sites of farmed and wild turbot (*Psetta maxima*). *Int. J. Food Sci. Technol.*, 42**:**1456-1464.

Bagnyukova T.V., Storey K.B. and V.I. Lushchak, 2005. Adaptive response of antioxidant enzymes to catalase inhibition by aminotriazole in goldfish liver and kidney. *Comp. Biochem. Physiol.* B, 142:335-341.

Boujard T. and F. MeÂdale, 1994. Regulation of voluntary feed intake in juvenile rainbow trout fed by hand or by self-feeders with diets containing two different protein/energy ratios. *Aquatic Living Resources*, 7:211-215.

Bromley P. J., 1980. Effect of dietary protein, lipid and energy content on the growth of turbot (*Scophthalmus maximus* L.). *Aquaculture*,19:359-369.

Cho S.H., Lee S.M., Lee S.M. and J.H. Lee, 2005. Effect of dietary protein and lipid levels on growth and body composition of juvenile turbot (*Scophthalmus maximus* L.) reared under optimum salinity and temperature conditions. *Aquacult. Nutr.*, 11**:**235-240.

Craig S.R., Washburn B.S. and D.M. III Gatlin, 1999. Effects of dietary lipids on body composition and liver function in juvenile red drum, *Sciaenops ocellatus*. *Fish Physiol. Biochem.* 21: 249-255.

Dias J., De Coen W., Gomes E., Rees J., Soares F., Dinis M. and P. Sorgeloos, 2004. Effect of dietary non-protein energy levels on condition and oxidative status of Senegalese (*Solea senegalensis*) juveniles. *Aquaculture*, 231:417-433.

Hillestad M., Johnsen F., Austsreng E. and T. Asgard, 1998. Long-term effects of dietary fat level and feeding rate on growth, feed utilization and carcass quality of Atlantic salmon. *Aquacult. Nutr*., 4:89-97.
FAO, 2010. The State of the World Fisheries and Aquaculture 2010. Fisheries and Aquaculture Dept, Food & Agriculture Organization of the United Nations (FAO), Rome, Italy.
Kissileff H.R. and T.B. Van Itallie, 1982. Physiology of the control of food intake. *Ann. Rev. Nutr.*, 2:371-418.
Lee J.K., Cho S.H., Park S.U., Kim K.D. and S.M. Lee, 2003. Dietary protein requirement for young turbot (*Scophthalmus maximus* L.). *Aquacult. Nutr.*, 9:283-286.
Leknes E., Imsland A. K., Gústavsson A., Gunnarsson S., Thorarensen H. and J. Árnason, 2012. Optimum feed formulation for turbot, *Scophthalmus maximus* (Rafinesque, 1810) in the grow-out phase. *Aquaculture*, 344-349:114-119.
Lim C. and C.D. Webster, 2001. *Nutrition and Fish Health*. Food Products Press, Binghamton, New York.
Liu H., Xie S., Zhu X., Lei W., Han D. and Y. Yang, 2008. Effects of dietary ascorbic acid supplementation on the growth performance, immune and stress response in juvenile *Leiocassis longirostris* Günther exposed to ammonia. *Aquacult. Res*., 39:1628-1638.
Lushchak V.I. and T.V. Bagnyukova, 2006. Effects of different environmental oxygen levels on free radical processes in fish. *Comp. Biochem. Physiol.* B 144:283-289.
Ma A.J., Chen S.Q., Lei J.L., Chen D.G., Yu D.X., Wang L.C. and S.H. Zhang, 2001. Influences of the main energy matter in feed on the growth of young turbot (*Scophthalmus Maximus L.*). *Oceanologia Etlimnologia Sinica*, 32 (5):527-533.
McCord J.M. and J. Fridovich, 1969. Superoxide dismutase: an enzymatic function for erythrocuuprein (hemocuprein). *J. Biol. Chem*., 244:6049-6055.
Nijhof N., 1993. Effects of body weight on diet requirements of turbot (*Scophthalmus Maximus*) with special reference to feed intake and body composition. Pages 501-513 in S.J. Kaushik, and P. Luquet, editor. *Fish Nutrition in Practice*, INRA, Paris.
Niu H.X., Lei J.L., Chang J., Jia Y.D. and C.R. Gao, 2013. Effects of Fresh Frozen Trash Fish and Commercial Feeds on Growth, Lipid Metabolism and Antioxidant Function of Turbot (*Scophthalmus maximus* L.). *Chinese J. Anim. Nutr.*, 25(11):2696-2704.
NRC (National Research Council) 2011. Chapter 6: lipid. Pages 181-246 in R. W. Hardy, editor. *Nutrient requirements of fish*. National Academy Press, Washington, USA.
Regost C., Arzel J., Cardinal M., Robin J., Laroche M. and S.J. Kaushik, 2001. Dietary lipid level, hepatic lipogenesis and flesh quality in turbot (*Psetta maxima*). *Aquaculture*, 193: 291-309.
Sæther B.S. and M. Jobling, 2001. Fat content in turbot feed: influence on feed intake, growth and body composition. *Aquacult. Res*., 32:451-458.
Sanz A., Furné M., Trenzado C.E., de Haro C. and M.J. Sánchez-Muros, 2012. Study of the Oxidative State, as a Marker of Welfare, on Gilthead Sea Bream, *Sparus aurata*, Subjected to Handling Stress. *J. World Aquacult. Soc*., 43:707-715.
Sargent J.R., 1995. Origins and functions of egg lipids: Nutritional implications. pp. 353-372. In: N. R. Bromage and R. J. Roberts, editor. *Broodstock Management and Egg and Larval Quality*. Blackwell, Oxford.
Sevgili H., Kurtoğlu A., Oikawa M., Mefut A. and R. Süyek, 2012. The use of farmed salmon oil to replace anchovy oil in diet of turbot, *Psetta maxima*, reared in brackish water. *J. World Aquacult. Soc*., 43**:** 560-570.
Sevgili H., Kurtoğlu A., Oikawa M., Öztürk E., Dedebali N., Emre N. and F. Pak, 2014. High dietary lipids elevate carbon loss without sparing protein in adequate protein-fed juvenile turbot (*Psetta maxima*). *Aquacult. Int*., 22:797-810.
Shi C.Y., Wang Y.G., Yang S.L., Huang J. and Q.Y. Wang QY, 2004. The first report of an iridovirus like agent infection in farmed turbot *Scophthalmus maximus* in China. *Aquaculture*, 236:11-25.

Yigit M., Yardim O. and S. Koshlo, 2002**.** The protein sparing effects of high lipid levels in diets for rainbow trout (*Oncorhynchus mykiss* W. 1792) with special reference to reduction of total nitrogen excretion. *Isr. J. Aquacult. - Bamidgeh,* 54(2):79-88.
Zhang C.N., Wang A.M., Liu W.B., Yang W.P., Yu Y.B., Lu L.L., Huang J.T. and Z.T. Qi, 2013. Effects of dietary lipid levels on fat deposition, lipid metabolizing enzyme and antioxidantic activities of *Chelon haematocheilus*. *J. Fish Sci. of China,* 20(1): 108-115.