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.

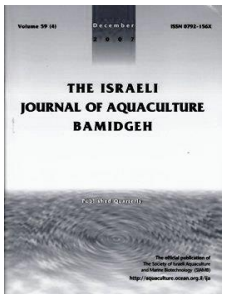
This transformation from a subscription printed version to an on-line OA journal, aims at supporting the concept that scientific peer-reviewed publications should be made available to all, including those with limited resources. The OA IJA does not enforce author or subscription fees and will endeavor 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



Effects of Soya Saponins on Feed Intake, Growth Performance, and Cholesterol Metabolism in Juvenile Turbot (*Scophthalmus maximus* L)

Biao Yun[1,2], Qinghui Ai[1]*, Xueqiao Qian[2] , Kangsen Mai[1]

[1]*The Key Laboratory of Mariculture (Education Ministry of China), Ocean University of China, Qingdao, 266003, P.R. China*

[2] *Research Center of Husbandry & Aquaculture Guang Dong Haid Group Co., LTD No.5, 8th Street, Fuping Road, Xiaoping Industrial Zone, Panyu District, Guang Zhou, P.R. China, 511400*

(Received 9.2.2014, Accepted 3.7.2014)

Key words: Turbot; soya saponins; feed intake; growth performance; cholesterol metabolism; fishmeal

Abstract

An 8-week feeding trial was conducted to investigate the effects of soya saponins on feed intake, growth performance, and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus* L).

A control diet and two experimental diets were prepared with the supplementation of soya saponins, concentrations being as follows: 0.0% (Diet 1, Control), 0.25% (Diet 2) and 0.5% (Diet 3), respectively. The results showed that 0.25% of dietary soya saponins produced comparable growth performance with the control diet. However, the growth performance of fish fed the diet with 0.5% soya saponins was significantly lower. The selected parameters measured in plasma, liver, and feces of each group were not significantly different. These results suggested that 0.25% dietary soya saponins did not produce negative effects, but 0.5% of dietary soya saponins significantly reduced fish growth. Dietary soya saponin supplementation to FM-based diet did not significantly affect cholesterol metabolism.

* Corresponding author: Qinghui Ai, Tel.: +86 532 82032038; e-mail: qhai@ouc.edu.cn

Introduction

Soybean meal (SBM) has been widely used as the most cost-effective alternative protein source for fish meal (FM) in fish feeds (Refstie et al., 2000; Chou et al., 2004; Wang et al., 2006). Growth performance was not affected in Black Sea Turbot *(Psetta maeotica)* when fishmeal was partially replaced with defatted soybean (Ergun et al., 2008) but was lowered in many carnivorous fish species fed SBM-based diets, e.g. rainbow trout (*Oncorhynchus mykiss*) (Refstie et al., 2000), cobia (*Rachycentron canadum*) (Chou et al., 2004), cuneate drum (*Nibea miichthioides*) (Wang et al., 2006) and sharpsnout seabream (*Diplodus puntazzo*) (Hernández et al., 2007). The detrimental effect may be due to antinutritional factors (ANFs) in SBM which contains trypsin inhibitors, lectins, antivitamins, soy antigens, phytic acid, and saponins (Francis et al., 2001a; Gatlin III et al., 2007). The trypsin inhibitors, lectins, and antivitamins are heat sensitive and may be destroyed by thermal treatment; antigenic compounds and phytate levels in fish diets are usually unlikely to affect fish growth performance (Francis et al., 2001a).

Soya saponins are triterpenoid glycosides and can be divided into two major groups: A and B (Knudsen et al., 2008). They derive from a number of plant species in leaves, stem, seeds, bark, blossoms, fruit, and roots (Francis et al., 2001c). SBM usually contains approximately 0.43–0.67% soya saponins (Ireland et al., 1986; Bureau et al., 1998). The effects of dietary soya saponins on growth response of fish are inconsistent. A significant reduction in growth and intestinal damage of chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout was observed when fish were fed a diet containing 0.3% soya saponins (Bureau et al., 1998). However, there was no significant difference in feed intake and growth of channel catfish (*Ictalurus punctatus*) when fed the diet with 0.26% purified soya saponins (97% of soya saponin B) (Twibell and Wilson, 2004).Dietary *Quillaja* saponins at a rate of 0.015% showed potential for growth promotion in common carp (*Cyprinus carpio* L.) (Francis et al., 2001b) and tilapia (*Oreochromis niloticus*) (Francis et al., 2001c).

The hypocholesterolemic effect was observed in plasma of many fish species fed plant-based diets compared with fish fed FM-based diet. The species studied include turbot (*Psetta maxima*) (Regost et al., 1999), gilthead sea bream (*Sparus aurata*) (Gomez-Requeni et al., 2004; Venou et al., 2006), Atlantic cod (*Gadus morhua* L) (Hansen et al., 2007), rainbow trout (Kaushik et al., 1995; Yamamoto et al., 2007), and parrot (*Oplegnathus fasciatus*) (Lim and Lee, 2009). The hypocholesterolemic effect has also been observed in plasma of turbot (*Scophthalmus maximus* L) fed plant-based diets compared with fish fed FM-based diet (Yun et al., 2011).

Turbot is a carnivorous species widely cultured in Europe and East Asia because of its high quality flesh and rapid growth. Dietary effects of soya saponins have not been reported on FI and growth performance of juvenile turbot. It is also unknown if and how dietary soya saponins affect cholesterol metabolism in fish fed FM-based diet. Therefore, the aim of this study was to investigate the effects of soya saponins on feed intake, growth performance, and cholesterol metabolism in juvenile turbot fed FM-based diet.

Materials and Methods

Feed Ingredients and Diet Formulation. Soya saponins (84.96% purity) were obtained from North China Pharmaceutical (Shijiazhuang, China) Co., Ltd. The remaining soya saponins were mainly soybean protein (8.26%) and soybean oligosaccharide (4.77%) according to the supplier. Using FM as the primary protein source, fish oil and soybean oil as the lipid sources, and wheat flour as the carbohydrate source, a control diet (Diet 1) with 61% FM was formulated. The other two isonitrogenous and isolipidic experimental diets were prepared with supplementation of 0.3% and 0.6% soya saponins (84.96% purity), respectively. Composition of the diets is given in Table 1. The planned soya saponin concentrations in the diets were 0.0%, 0.25% and 0.5% respectively. L-threonine, L-arginine, L-valine, L-isoleucine, L-leucme and L-phenylalanine (Crystalline amino acids, CAAs) were supplemented to meet the essential amino acids (EAA) requirements based on whole body amino acid profile of juvenile turbot (Kaushik, 1998).

Table 1. Formulation (% diet), proximate composition (% diet) and energy content (MJ /kg) of the experimental diets

Ingredients	*Diet 1*	*Diet 2*	*Diet 3*
Fish meal[a]	61	61	61
Wheat flour	28.95	28.65	28.35
Fish oil	2	2	2
Soybean oil	4	4	4
Soybean lecithin	1	1	1
Vitamin premix[b]	0.8	0.85	0.85
Mineral premix[b]	0.5	0.5	0.5
Amino acid premix[c]	1.65	1.65	1.65
Ethoxyquin	0.0	0.05	0.05
Soya saponins product[d]	0.0	0.3	0.6
Analyzed nutrients compositions (on dry matter basis %)			
Soya saponins[e]	0.0	0.25	0.5
Dry matter	90.47	91.29	93.70
Crude protein	52.52	52.31	52.32
Crude lipid	12.51	11.94	12.40
Gross energy	20.9	20.99	21.00
Ash	9.19	9.57	9.52

[a] Fish meal: steam-dried fishmeal (COPENCA Group. Lima, Peru)
[b] Yun et al. (2011)
[c] Amino acid premix (g/kg diet): L-arginine 5.0, L-valine 3.0, L-threonine 1.0, L-isoleucine, 2.0, L-leucme, 3.0, L-phenylalanine, 2.5.
[d] Soya saponins product (84.96% pure) was obtained from North China Pharmaceutical (Shijiazhuang, China) Co., Ltd. The remaining of soya saponins product was mainly soybean protein (8.26%) and soybean oligosaccharide (4.77 %) according to the supplier.
[e] The value was calculated according to soya saponins product (84.96% purity).

Ingredients were ground into fine powder and sieved through a 246-μm mesh. The soya saponins were first dissolved in deionized water, mixed with the ingredients thoroughly, and then mixed with fish oil, and then water to produce stiff dough. The dough was then pelleted with an experimental feed mill (F-26 (II), South China University of Technology, China), and dried for about 12 h in a ventilated oven at 45^{0}C, and then stored at -20^{0}C until use.

Fish, Experimental Conditions and Samples Collection. Juvenile turbot were obtained from Yellow sea fisheries Co., Ltd (Haiyang, Shandong, China). Fish were acclimated to the system and fed the control diet for 2 weeks before the trials. Juvenile turbot (initial body weight: 5.91±0.02 g) were randomly distributed into 9 flat-bottomed, constantly aerated, tanks filled with 300 l sea water which was continuously pumped from the coast adjacent to the experiment station, passed through sand filters into each tank at approximately 1.5 l/min. Three replicate tanks were assigned to each diet group and 60 fish of similar size were bulk weighed and stocked in each tank. During the 8-week feeding period, fish were fed the experimental diets to apparent satiation twice daily at 07:00 and 18:00. Any uneaten feed was collected 1 h after each meal, dried to constant weight at 70°C and reweighed. Leaching loss in the uneaten diet was estimated by leaving five samples of each diet in tanks without fish for 1 h, recovering, drying and reweighing. At the end of the experiment, 5 fish from each tank were sampled for morphometric parameters. Individual body weight, body length, liver weight and visceral weight were recorded to calculate condition factor, hepatosomatic index, and viscerosomatic index. All experimental fish were anesthetized with Eugenol (1:10000) (Shanghai Reagent Corporation, Shanghai, China) before sampling. Blood samples were taken from the caudal vein using heparinized syringes. Plasma samples were prepared after centrifugation (4000 g for 10 min) at 4^{0}C and immediately stored at -80^{0}C until analysis. Liver samples were frozen in liquid nitrogen and stored at -80^{0}C for subsequent determination of lipid content. The feces collection method was the same as described in Deng et al. (2010).

During the 8-week feeding period, water temperature was controlled at 19-22^0 C, pH 7.5-8.0, salinity 30-33‰, ammonia nitrogen was lower than 0.1 mg/l, nitrite was lower than 0.1 mg/l, and dissolved oxygen was higher than 6.0 mg/l.

Chemical analysis Dry matter, crude protein, crude lipid, ash, and gross energy were analyzed for content and influence of experimental diets (AOAC, 1995). Dry matter was analyzed by drying the samples to constant weight at 105^{0}C. Crude protein was determined through digestion using the Kjeldahl method and estimated by multiplying nitrogen by 6.25 (N x 6.25). Crude lipid was measured by ether extraction using Soxhlet method. Ash was examined by combustion in a muffle furnace at 550^{0}C for 16 h. Gross

energy was determined by Parr 1281 Automatic Bomb Calorimeter (Parr, Moline, IL, USA). Duplicate analyses were conducted for each sample.

The concentration of total cholesterol (TC), free cholesterol (FC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in plasma were determined by colorimetric enzymatic methods using commercial kits (FC kit supplied by Shanghai Mingdian Bioengineering Co., Shanghai, China; other kits supplied by Changchun Huili bio-Tech Co., Changchun, China) (Yun et al., 2011). After extraction of the lipids from 500 mg liver, and feces with chloroform: methanol (2:1, v/v) (Folch et al., 1957), the TC content in liver and feces, and FC content in liver were determined using the same kits as for the plasma. The volume of lipid solution was made to 10 ml with chloroform: methanol (2:1, v/v). One milliliter of this extract was sampled, dried under a pure nitrogen stream, and the obtained residue was mixed with 1 ml isopropyl alcohol containing 100 g Triton X-100/l (Reagent Grade) (Yun et al., 2011). The amount of cholesterol esters in plasma and liver was calculated by subtracting the FC value from the TC value. Duplicate analyses were conducted for each sample.

Calculations and statistical methods. Growth parameters were calculated as follows:
Weight gain rate, WGR (%) = 100 × [(final body weight − initial body weight) / initial body weight].
Feed intake, FI (%/day) = 100 × total amount of the feed consumed (g) / [(initial body weight + final body weight) / 2] / days.
Feed efficiency ratio, FER = weight gained (g) / total amount of the feed consumed (g).
Survival rate, SR (%) = 100 × (final fish number / initial fish number).
Condition factor, CF = 100 × fish weight / (body length)^3.
Hepatosomatic index, HSI (%) = 100 × (liver weight / body weight).
Viserosomatic index, VSI (%) = 100 × (visceral weight / body weight).

Software SPSS, 11.5 was used for all statistical evaluations. All data were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test. Differences were regarded as significant when $P<0.05$. All data are expressed as means ± standard error (S.E).

Results

All experimental fish showed high survival rate (>99%) (Table 2), and no significant difference was observed among dietary treatments ($P>0.05$). Growth performance in fish fed FM-based diet with 0.25% soya saponins (Diet 2) was not inhibited, and FI and FER were not significantly affected compared with fish fed Diet 1 (Control). However, fish fed the diet with 0.5% soya saponins (Diet 3) showed significantly lower final body weight (FBW), WGR and FER than fish fed the control diet ($P<0.05$, Table 2), but FI was not significantly affected.

Table 2. Growth performance and survival rate of turbot fed the experimental diets (Means ± SE, n = 3)*

	Diet 1	*Diet 2*	*Diet 3*
IBW[1]	5.89±0.0	5.92±0.01	5.92±0.01
FBW[2]	32.7±0.2^b	32.3±0.6^b	30.4±1.3^a
WGR[3]	455.0±3.8^b	445.3±9.5^b	412.9±21.0^a
FER[4]	1.30±0.0	1.32±0.03	1.24±0.03^a
FI[5]	1.88±0.0	1.91±0.05	1.88±0.01
SR[6]	100±0.0	99.4±0.6	100±0.0

[1] IBW: initial body weight.
[2] FBW: final body weight.
[3] Weight gain rate (WGR) (%) = 100 × [(FBW-IBW)/IBW].
[4]Feed efficiency ratio (FER) = (FBW-IBW)/ total amount of the feed consumed.
[5] Feed intake (FI) (%/day) = 100 × total amount of the feed consumed / [(IBW + FBW) / 2] /days.
[6]Survival rate (SR) (%) = 100 × (final fish number / initial fish number).
*****Values in the same row with different superscripts are significantly different ($P<0.05$).

Table 3. Condition factor, hepatosomatic index and viserosomatic index of turbot fed the experimental diets (Means ± SE, n = 3)*

	Diet 1	*Diet 2*	*Diet 3*
CF[1]	3.30±0.03	3.28±0.12	3.37±0.08
HSI[2]	1.41±0.06	1.39±0.13	1.32±0.08
VSI[3]	5.40±0.15	5.20±0.05	5.15±0.06

[1] Condition factor, CF = 100 × fish weight/(body length)^3.
[2] Hepatosomatic index, HSI (%) = 100 × (liver weight / body weight).
[3] Viserosomatic index, VSI (%) = 100 × (viseral weight / body weight).
*****Values in the same row with different superscripts are significantly different ($P<0.05$).

CF, HSI and VSI of turbot at the end of growth trial are presented in Table 3. HSI (from 1.32 to 1.41) and VSI (from 5.15 to 5.40) of fish given increasingly greater dietary soya saponins, showed a decreasing trend but no statistical differences were observed among dietary treatments with respect to CF, HSI and VSI (P>0.05).

Plasma TC (4.13–4.66 mmol/L), FC (2.27–2.62 mmol/L), cholesterol esters (1.51–2.11 mmol/L), HDL-C (2.99–3.13 mmol/L), LDL-C (1.87–1.93 mmol/L) and HDL-C/LDL-C (1.52–1.65) after 24 h starvation in each group were not significantly different (P>0.05, Table 4). No significant differences were detected among dietary treatments with respect to TC (3.30–3.92 g /kg wet liver), FC (2.47–3.24 g/kg wet liver) and cholesterol esters (0.68–0.86 g/kg wet liver) in liver (P>0.05, Table 5). No statistical difference was observed between the dietary treatments (P>0.05) in TC (5.83–6.37 g/kg dry matter) in feces, (Table 5).

Table 4. Cholesterol concentrates in plasma of turbot fed the experimental diet (mmol/L, Means ± SE, n = 3)*

	Diet 1	*Diet 2*	*Diet 3*
Total cholesterol	4.66±0.13	4.13±0.79	4.36±0.50
Free cholesterol	2.55±0.36	2.62±0.31	2.27±0.12
Cholesterol esters	2.11±0.28	1.51±0.71	2.09±0.52
HDL-C[1]	3.13±0.35	2.99±1.18	3.13±0.97
LDL-C[2]	1.92±0.20	1.93±0.54	1.87±0.39
HDL-C/LDL-C	1.64±0.15	1.52±0.09	1.65±0.09

[1]HDL-C: high-density lipoprotein cholesterol;
[2]LDL-C: low-density lipoprotein cholesterol.
*****Values in the same row with different superscripts are significantly different (P<0.05).

Table 5. Lipid profiles in liver (g/kg wet liver) and feces (g/kg dry matter) of turbot fed the experimental diet (Means ± SE, n = 3)*

	Diet 1	*Diet 2*	*Diet 3*
Liver total cholesterol	3.30±0.90	3.92±0.61	3.70±0.51
Liver free cholesterol	2.47±0.66	3.24±0.70	2.84±0.62
Liver cholesterol esters	0.83±0.27	0.68±0.54	0.86±0.17
Feces total cholesterol	6.11±0.41	5.83±1.04	6.37±0.13

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

Discussion

Usually, SBM contains approximately 0.43–0.67% soya saponins (Ireland et al., 1986; Bureau et al., 1998). This data was used as a reference for experimental diets designed for many fish species (Bureau et al., 1998; Twibell and Wilson, 2004; Knudsen et al., 2008; Chen et al., 2011). However, the reported effects of supplemental dietary soya saponins on growth responses of fish differed. In the present study, growth performance in turbot fed FM-based diet with 0.25% soya saponins (Diet 2) was not depressed compared with fish fed Diet 1 (Control). This result was similar with that of Chen et al. (2011) where growth of Japanese flounder (*Paralichthys olivaceus*) fed 0.32% soya saponins added to their diet containing 68% FM was not depressed. Similar results have also been obtained in channel catfish fed casein-based diets (Twibell and Wilson, 2004). However, Bureau et al. (1998) concluded that 0.3% *Quillaja* saponins added to salmonid feed containing 32% of SPC caused significant intestinal damage, and depressed growth of chinook salmon and rainbow trout. These differences could be due to different contents of soya saponins, and different protein sources used in diets. It has been reported that 0.16% soya saponins added to feed containing 62% of FM neither depress growth nor induce intestinal damage of Atlantic salmon, but fish fed the diet with 0.17% soya saponins in combination with lupin kernel meal displayed significant enteritis (Knudsen et al. 2008).

A high level of soya saponins (0.64%) greatly depressed the growth performance of Japanese flounder fed FM-based diet (Chen et al., 2011). Similarly, in this study, turbot fed the diet with 0.5% soya saponins (Diet 3) significantly depressed growth compared with the control diet. This suggests that saponin levels (0.5%) have a deleterious effect as they exceed the endurance limitation of turbot even if fish are fed a FM-based diet. Saponins have been implicated in reduced feed intake and resultant reduced growth by imparting an undesirable taste to the feed (Dersjant-Li, 2002). In the present study, FI of fish fed diet with 0.5% soya saponins (Diet 3) was not affected. This result suggests

that the deleterious effects of soya saponin supplementation in diet, on fish may not be attributed to FI. In this study, fish fed the diet with 0.5% soya saponins (Diet 3) showed significantly lower FER than that of fish fed the control diet ($P<0.05$). Moreover, the mechanism of these deleterious effects of soya saponins in diet on fish is possibly related to impairment of distal intestinal histology, apparent digestibility coefficients of nutrients, and subsequently reduction of feed efficiency ratio (Chen et al., 2011).

In the present study, hypocholesterolemic effect was not observed in the plasma of fish fed diets with 0.25% or 0.5% soya saponins as compared with that of fish fed the control diet. Furthermore, no significant differences were detected among dietary treatments with respect to FC and cholesterol esters in plasma.

Cholesterol metabolism is related to uptake, biosynthesis, transport, and export (Maita et al., 2006; Yun et al., 2011). The main cholesterol synthesis (endogenous cholesterol) was conducted in the liver of fish (Maita et al., 2006). In this study, no significant differences were detected between dietary treatments with respect to TC, FC and cholesterol esters in liver. It is well known that LDL carries cholesterol from the liver to peripheral tissues, while HDL carries cholesterol from peripheral tissues to the liver (Chen et al., 2003; Deng et al., 2010). Therefore, HDL-C/LDL-C ratio could be used as a marker of cholesterol transport. In the present study, there were no statistical differences in plasma HDL-C, LDL-C and HDL-C/LDL-C levels in turbot fed diets with 0.25% or 0.5% soya saponins compared with fish fed the diet without soya saponin supplementation (control diet), which indicated that cholesterol transport was not significantly affected by the supplemented dietary soya saponins. Feces cholesterol (0.583–0.637% dry matter) was not significantly affected by dietary soya saponins. The present study suggests that dietary soya saponin supplementation did not show any effect on cholesterol metabolism of juvenile turbot fed a FM-based diet. This result is the first reported in fish.

In conclusion, results of the present study showed that a diet with 0.25% soya saponins did not significantly affect fish growth performance. However, fish fed the diet with 0.5% soya saponins suffered significantly depressed growth and feed utilization, but had no significant effect on FI. Moreover, dietary soya saponin supplementation to FM-based diet had no significant effect on cholesterol metabolism in fish.

Acknowledgements

This research was supported by the Earmarked Fund for Modern Agro-industry Technology Research System No. nycytx-50-G07.

References

Association of Official Analytical Chemist (AOAC), 1995. Official Methods of Analysis of AOAC International, 16th edn. AOAC Inc., Arlington, VA, USA.

Bureau D.P., Harris A.M. and C.Y. Cho, 1998. The effects of purified alcohol extracts from soy products on feed intake and growth of Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 161: 27–43.

Chen J.Y., Chen J.C. and J.L. Wu, 2003. Molecular cloning and functional analysis of zebrafish high-density lipoprotein-binding protein. *Comp. Biochem. Phys. B*, 136: 117–130.

Chen W., Ai Q.H., Mai K.S., Xu W., Liufu Z.G., Zhang W.B. and Y.H. Cai, 2011. Effects of dietary soybean saponins on feed intake, growth performance, digestibility and intestinal structure in juvenile Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, 318: 95–100.

Chou R.L., Her B.Y., Su M.S., Hwang G., Wu Y.H. and H.Y. Chen, 2004. Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. *Aquaculture*, 229: 325–333.

Deng J.M., Mai K.S., Ai Q.H., Zhang W.B., Wang X.J., Tan B.P., Xu W., Liufu Z.G. and H.M. Ma, 2010. Interactive effects of dietary cholesterol and protein sources on growth performance and cholesterol metabolism of Japanese flounder (*Paralichthys olivaceus*). *Aquacult. Nutr.*, 16: 419–429.

Dersjant-Li Y., 2002. The use of soy protein in aquafeeds. In: Cruz-Suarez L.E., Ricque-Marie D., Tapia-Salazar M., Gaxiola-Cortes M.G. & Simoes N. (Eds.), Advances in Aquaculture Nutrition: Sixth International Symposium of Aquaculture Nutrition, Cancun, Quintana Roo, Mexico.

Ergun S., Yigit M., Turker A. and Harmantepe B., 2008. Partial Replacement of Fishmeal by Defatted Soybean Meal in Diets for Black Sea Turbot *(Psetta maeotica)*: Growth and Nutrient Utilization in Winter. *Isr. J. Aquacult. - Bamidgeh,* 60(3), 2008, 177-184

Folch J., Lees M. and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol.l and Chem.*, 226: 497–509.

Francis G., Makkar H.P.S. and K. Becker, 2001a. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, 199:197–227.

Francis G., Makkar H.P.S. and K. Becker, 2001b. Effects of cyclic and regular feeding of a Quillaja saponin supplemented diet on growth and metabolism of common carp (*Cyprinus carpio* L.). *Fish Physiol. Biochem.*, 24:343–350.

Francis G., Makkar H.P.S. and K. Becker, 2001c. Effects of Quillaja saponins on growth, metabolism, egg production and muscle cholesterol in individually reared Nile tilapia (*Oreochromis niloticus*). *Comparative Biochemistry and Physiology C*, 129: 105–114.

Gatlin III D.M., Barrows F.T. and P. Brown, 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquacult. Res.*, 38:551–579.

Gomez-Requeni P., Mingarro M., Calduch-Giner J.A., Medale F., Martin S.A.M., Houlihan D.F., Kaushik S. and J. Perez-Sanchez, 2004. Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). *Aquaculture*, 232: 493–510.

Hansen A.C., Rosenlund G., Karlsen Ø., Koppe W. and G.I. Hemre, 2007. Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L) I: Effects on growth and protein retention. *Aquaculture*, 272: 599–611.

Hernández M.D., Martínez F.J., Jover M. and B. García García, 2007. Effects of partial replacement of fish meal by soybean meal in Sharpsnout seabream (*Diplodus puntazzo*) diet. *Aquaculture*, 263:159–167.

Ireland P.A., Dziedzic S.Z. and M.W. Kearsley, 1986. Saponin content of soya and some commercial soya products by means of high performance liquid chromatography of the sapogenins. ., 34: 694–698.

Kaushik S.J., 1998. Whole body amino acid composition of European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and turbot (*Psetta maxima*) with an estimation of their IAA requirement profiles. *Aquatic Living Resour.*, 11: 355–358.

Kaushik S.J., Cravedi J.P., Lalles J.P., Sumpter J., Fauconneau B. and M. Laroche, 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 133: 257–274.

Knudsen D., Jutfelt F., Sundh H., Sundetll T.K., Koppe W. and H. FrØkiær, 2008. Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean induced enteritis in Atlantic salmon (*Salmo salar* L.). *Brit. J. Nutr.*, 100:120–129.

Lim S.J. and K.J. Lee, 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. *Aquaculture*, 290:283–289.

Maita M., Maekawa J., Satoh K.I., Futami K. and S. Satoh, 2006. Disease resistance and hypocholesterolemia in yellowtail *Seriola quinqueradiata* fed a non-fishmeal diet. *Fish. Sci.*, 72: 513–519.

Refstie S., Korsøen Ø., Storebakken T., Baeverfjord G., Lein I. and A.J. Roem, 2000. Differing nutritional responses to dietary soy-bean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). *Aquaculture*, 190: 9–63.

Regost C., Arzel J. and S.J. Kaushik, 1999. Partial or total replacement of fish meal by corn gluten meal in diet for turbot (*Psetta maxima*). Aquaculture, 180: 99–117.
Twibell R.G. and R.P. Wilson, 2004. Preliminary evidence that cholesterol improves growth and feed intake of soybean meal-based diets in aquaria studies with juvenile channel catfish, *Ictalurus punctatus*. *Aquaculture*, 236: 539–546.
Venou B., Alexis M.N., Fountoulaki E. and J. Haralabous, 2006. Effect of extrusion and inclusion level of soybean meal on diet digestibility, performance and nutrient utilization of gilthead sea bream (*Sparus aurata*). *Aquaculture*, 261: 343–356.
Wang Y., Kong L.J., Cui Z.H., Li K. and D.P. Bureau, 2006. Effect of replacing fish meal with soybean meal on growth, feed utilization and carcass composition of cuneate drum (*Nibea miichthioides*). *Aquaculture*, 261: 1307–1313.
Yamamoto T., Suzuki N., Furuita H., Sugita T., Tanaka N. and T. Goto, 2007. Supplemental effect of bile salts to soybean meal-based diet on growth and feed utilization of rainbow trout *Oncorhynchus mykiss*. *Fish. Sci.*, 73: 123–131.
Yun B., Mai K.S., Zhang W.B. and W. Xu, 2011. Effects of dietary cholesterol on growth performance, feed intake and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus* L.) fed high plant protein diets. *Aquaculture*, 319: 105–110.