

Effects of fish oil and partial fish meal substitution with oilseed oils and meals on growth performance, nutrient utilization and health of the rainbow trout *Oncorhynchus mykiss*

Derya Güroy · Betül Güroy · Daniel Lee Merrifield · Ahmet Adem Tekinay · Simon John Davies · İzzet Şahin

Received: 15 April 2011 / Accepted: 9 September 2011 / Published online: 15 October 2011
© Springer Science+Business Media B.V. 2011

Abstract A feeding trial was conducted to investigate the effects of the replacement of dietary fish meal and fish oil with oilseed meals (soybean or canola) and canola oil on growth, nutrient utilization, body composition, diet digestibility and hematological parameters of rainbow trout *Oncorhynchus mykiss*. Seven diets were used; the control diet (designated FM) contained fish meal and fish oil as the main protein and lipid sources. For the experimental diets, 40% of fish meal protein was substituted with soybean meal, canola meal or a soybean/canola meal mixture, and these diets (designated SM, CM and SCM, respectively) contained fish oil as the lipid source. Three additional diets (SM + CO, CM + CO and SCM + CO) were formulated with the same vegetable protein meals but with fish oil replaced by canola oil. Fish were fed twice daily to apparent satiation for 11 weeks. The growth of fish fed the CM + CO diet was significantly lower than that of fish fed the FM, SCM, SM + CO and SCM + CO diets. The feed conversion ratio (FCR) was significantly better in fish fed the FM and SCM + CO diets than in fish fed the CM and CM + CO diets. Furthermore, feed intake was significantly lower for fish fed the CM + CO diet than in fish fed the SCM diet, and lipid digestibility of the CM + CO diet was significantly lower than that of all other diets. No significant differences of body composition were observed. Circulating leukocyte levels, leukocyte ratios and serum lysozyme activity remained unaffected by dietary treatment. However, it was observed that fish fed the CM + CO diet displayed hematocrit levels significantly lower ($P < 0.05$) than that of fish fed the other diets. The results indicate that when diets contain either fish oil or canola oil, canola meal and soybean meal can be incorporated into rainbow trout feeds at a combined 32% inclusion level (replacing 40% of fish meal protein) without inducing significant negative effects on growth, nutrient utilization or health.

D. Güroy (✉) · B. Güroy · İ. Şahin
Department of Aquaculture, Armutlu Vocational College, University of Yalova, 77500 Yalova, Turkey
e-mail: dguroy@yalova.edu.tr

D. L. Merrifield · S. J. Davies
Aquaculture and Fish Nutrition Research Group, School of Biomedical and Biological Sciences,
The University of Plymouth, Plymouth, Devon PL4 8AA, UK

A. A. Tekinay
Department of Aquaculture, Faculty of Fisheries, University of İzmir Katip Çelebi,
35580 Izmir, Turkey

Keywords Rainbow trout · Canola meal · Soybean meal · Canola oil · Growth · Hematology

Introduction

Rainbow trout are one of the most widely cultured freshwater fish species with over 570,000 mt produced in 2008 (FAO 2010). Compound feeds constitute the most significant operating cost with protein and lipid being the most costly constituents in aquafeeds (Higgs and Dong 2000; Li et al. 2000).

Fish meal supports good fish growth and flesh quality because of its high digestibility, complimentary amino acid profile and high palatability. Cultured rainbow trout have traditionally been fed diets with high inclusions of fish oil and fish meal, but these raw materials are unsustainable, expensive and add pressure to wild fish stocks. Therefore, replacing fish meal and fish oil with alternative plant ingredients has become a major drive within the aquaculture sector (Gatlin et al. 2007).

Oilseed meal protein sources, such as soybeans, canola and cottonseed, are considered as potential alternatives for fish meal, due to their competitive prices and relative availability (Gatlin et al. 2007; Hardy 2010). Soybean and canola meals are used as primary plant protein sources in fish feeds owing to their relatively high crude protein level, high protein availability and complimentary amino acid composition compared with other oilseed meals (Hertrampf and Piedad-Pascual 2000; Lee et al. 2002, 2006). Many researchers have examined the efficacy of fish meal substitution in aquafeeds with a variety of oilseed meals at different levels of inclusion for a range of fish including rainbow trout (soybean meal: Refstie et al. 1997, 2000; Barrows et al. 2007; cottonseed meal: Luo et al. 2006; canola products: Drew et al. 2007; Shafaeipour et al. 2008). It has been demonstrated that soybean products (Rumsey et al. 1994) and canola meal (Shafaeipour et al. 2008) can be used to replace up to 30% of fish meal in rainbow trout diets without reducing growth performance.

For the most part, the prior discussed studies have used fish oil as the major lipid source in the experimental diets tested. But vegetable oils should be utilized in aquafeeds as potential alternative lipid sources because increasing global fish oil production is not sustainable (Bell 2008). A number of studies have reported that the dietary use of vegetable oils for partial, or in some cases total, replacement of fish oil is possible for several species without detrimental impacts on growth performance (Brandsen et al. 2003; Torstensen et al. 2004; Izquierdo et al. 2005; Pratoomyot et al. 2008). Despite this however, there is limited information available regarding the effects of the simultaneous use of both oilseed meal and oilseed oil inclusion in diets for rainbow trout. Therefore, the aim of the present study was to assess the effects of the replacement of dietary fish meal and fish oil with various levels of oilseed meals (namely soybean or canola) and oilseed oil (canola oil) on rainbow trout growth, nutrient utilization, body composition, diet digestibility and hematological parameters.

Materials and methods

Experimental system and fish

A nutrition trial was conducted in 21 cylindrical fiberglass tanks (150 l) within a recirculation freshwater system located at Canakkale Onsekiz Mart University, Faculty

of Fisheries and Fish Nutrition Laboratory. The temperature was maintained at $16 \pm 1^\circ\text{C}$, dissolved oxygen at $8.3 \pm 0.02 \text{ mg l}^{-1}$ and pH at 7.4 ± 0.02 throughout the trial. Water flow rate was approximately 12.0 l min^{-1} for each tank. A natural photoperiod was maintained throughout the experimental period, and all tanks had similar light conditions.

Rainbow trout, weighing 60 g on average, were obtained from a private trout farm and acclimated to laboratory conditions for 3 weeks, during which, they were fed a commercial diet (Bagci Feed Company; crude protein: 45%, crude lipid: 16%). Fish (initial weight $\sim 70 \text{ g}$) were randomly distributed into the experimental tanks (12 fish per tank) and designated to one of the seven dietary treatments. Each treatment was conducted in triplicate. Fish were batch weighed every 2 weeks to observe growth and feed utilization parameters. Fish were fed by hand three times a day to apparent satiation, and feed intake was recorded daily for 11 weeks.

Experimental diet formulation

The formulation and chemical analysis of the experimental diets are shown in Table 1. The amino acid composition of the feeds and protein sources are displayed in Table 2. A total of seven diets were formulated; the control diet (FM) contained only fish meal and fish oil as the main protein and lipid sources, respectively. For the experimental diets, 40% of the fish meal protein was substituted with either soybean meal (SM), canola meal (CM) or a soybean meal/canola meal mixture (1:1 ratio on protein basis; SCM). These diets (FM, SM, CM and SCM) contained fish oil as the lipid source, while three further experimental diets (SM + CO, CM + CO and SCM + CO) were formulated with the same vegetable protein meals but with the 100% replacement of fish oil with canola oil.

Feed ingredients were thoroughly mixed and moistened with deionized water prior to pelleting (3 mm pellet size) in a meat grinder. The diets were then dried in a forced air oven (40°C) until the moisture content was reduced to less than 10%. The diets were stored in sealed plastic bags at -20°C prior to use.

Fish growth performance, nutrient utilization and somatic indices

Fish growth performance and nutrient utilization were calculated according to the following formulae:

$$\text{Feed conversion ratio (FCR)} = \text{FI}/\text{WG}.$$

$$\text{Specific growth rate (SGR)} (\% \text{ day}^{-1}) = 100 (\ln \text{FBW} - \ln \text{IBW}/T)$$

$$\text{Protein efficiency ratio (PER)} = 100 (\text{WG}/\text{PI})$$

$$\text{Thermal - unit growth coefficient (TGC)} = 100 ((\text{FBW}^{1/3} - \text{IBW}^{1/3})/(\text{Temp} \times T))$$

$$\text{Net protein utilization (NPU)} (\%) = 100 (\text{PG}/\text{PI}).$$

Six fish were randomly taken from the initial pool at the beginning of the experiment, and three fish from each tank (nine fish per group) were randomly sampled at the end of the trial to determine somatic indices.

The somatic indices were calculated according to the following formulae:

Table 1 Formulation and proximate composition of experimental diets

| | FM | SM | CM | SCM | SM + CO | CM + CO | SCM + CO |
|----------------------------------|------|------|------|------|---------|---------|----------|
| Ingredients (%) | | | | | | | |
| Fish meal ^a | 50.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 | 35.0 |
| Soybean meal ^b | 0.0 | 29.0 | 0.0 | 14.5 | 29.0 | 0.0 | 14.5 |
| Canola meal ^c | 0.0 | 0.0 | 35.0 | 17.5 | 0.0 | 35.0 | 17.5 |
| Corn gluten ^d | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Wheat meal ^e | 27.3 | 11.9 | 6.5 | 9.3 | 11.9 | 6.5 | 9.3 |
| Fish oil ^f | 12.0 | 13.4 | 12.8 | 13.0 | 0.0 | 0.0 | 0.0 |
| Canola oil ^g | 0.0 | 0.0 | 0.0 | 0.0 | 13.4 | 12.8 | 13.0 |
| Vitamin premix ^h | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Mineral premix ⁱ | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Celite ^j | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Proximate composition (%) | | | | | | | |
| Moisture | 9.9 | 9.8 | 9.8 | 9.2 | 9.0 | 9.1 | 9.3 |
| Crude protein | 46.2 | 47.4 | 46.5 | 46.7 | 47.0 | 48.0 | 45.8 |
| Crude lipid | 18.1 | 18.8 | 17.8 | 18.1 | 18.4 | 18.0 | 18.2 |
| Crude ash | 9.1 | 8.4 | 6.2 | 7.3 | 7.5 | 6.9 | 7.4 |
| Nitrogen-free extract | 26.6 | 25.4 | 29.5 | 27.9 | 27.1 | 27.1 | 28.6 |

^a Anchovy fish meal. Can Kardesler Fish Meal Corporation, Samsun, Turkey

^b Abalioglu Ltd, Denizli Turkey

^c Agromey Feed Mill Company, İzmir, Turkey

^d Agromey Feed Mill Company, İzmir, Turkey

^e Kepez Un, Çanakkale, Turkey

^f Anchovy fish oil. Can Kardesler Fish Meal Corporation, Samsun, Turkey

^g Agromey Feed Mill Company, İzmir, Turkey

^h Per g mixture: vitamin A: 342 IU; vitamin D₃: 329 IU; vitamin E: 0.0274 IU; vitamin K₃: 5.48 mg; vitamin B₁: 2.05 mg; vitamin B₂: 3.42 mg; vitamin B₃: 20.5 mg; vitamin B₅: 5.48 mg; vitamin B₆: 2.05 mg; vitamin B₁₂: 2.74 mg; vitamin C: 24.0 mg, Kartal Chemical Incorporated, Kocaeli, Turkey

ⁱ Per g mixture: biotin: 0.411 mg; folic acid: 0.685 mg; Zn: 12.3 mg; Mn: 4.80 mg; Cu: 1.64 mg; I: 0.274 mg; Se: 0.0274 mg; Ca: 125 mg; K: 189 mg, Kartal Chemical Incorporated, Kocaeli, Turkey

^j Celite Celite Corporation, World Minerals Co., Lompoc, CA, USA

$$\text{Condition factor (K) (\%)} = 100 (\text{BW}/(\text{FL}^3))$$

$$\text{Dress-out (DO) (\%)} = 100 ((\text{BW} - \text{VW})/\text{FBW})$$

$$\text{Hepatosomatic (HSI)} = 100 (\text{LW}/\text{BW});$$

$$\text{Viscerosomatic (VSI)} = 100 (\text{VW}/\text{BW})$$

where FBW is the final body weight (g), IBW is initial body weight (g), BW is body weight (g), FI = feed intake (g), WG = weight gain (g), T = time (days), PI = dietary protein intake (g), Temp = temperature (°C), PG = protein gain (g), FL = fish length (cm), LW = liver weight (g) and VW = viscera weight (g).

Table 2 Amino acid composition of experimental diets (g/100 g N)

| | Fish meal | Soybean meal | Canola meal | FM | SM | CM | SCM | SM + CO | CM + CO | SCM + CO | Requirement of rainbow trout ^a |
|---------------|-----------|--------------|-------------|------|------|------|------|---------|---------|----------|---|
| Methionine | 1.82 | 0.54 | 0.7 | 1.17 | 1.01 | 1.09 | 1.05 | 1.03 | 1.09 | 1.00 | 1.45 ^b |
| Cystine | 0.67 | 0.55 | 0.47 | 0.50 | 0.53 | 0.52 | 0.53 | 0.55 | 0.51 | 0.55 | |
| Lysine | 5.7 | 2.4 | 2.3 | 3.03 | 2.83 | 2.92 | 2.87 | 2.81 | 2.90 | 2.89 | 1.80 |
| Tryptophan | 0.67 | 0.52 | 0.44 | 0.40 | 0.43 | 0.43 | 0.43 | 0.45 | 0.44 | 0.43 | 0.20 |
| Threonine | 2.49 | 1.69 | 1.71 | 1.52 | 1.59 | 1.69 | 1.64 | 1.60 | 1.71 | 1.67 | 0.80 |
| Isoleucine | 3.64 | 2.18 | 1.51 | 2.17 | 2.19 | 2.06 | 2.12 | 2.17 | 2.03 | 2.11 | 0.80 |
| Histidine | 1.53 | 1.01 | 1.1 | 0.94 | 0.97 | 1.05 | 1.01 | 0.99 | 1.04 | 1.00 | 0.70 |
| Valine | 3.26 | 2.02 | 1.94 | 2.03 | 2.05 | 2.12 | 2.09 | 2.07 | 2.11 | 2.10 | 1.30 |
| Leucine | 4.69 | 2.8 | 2.6 | 3.45 | 3.46 | 3.53 | 3.50 | 3.40 | 3.57 | 3.58 | 1.40 |
| Arginine | 3.73 | 2.8 | 2.3 | 2.16 | 2.35 | 2.33 | 2.34 | 2.33 | 2.30 | 2.38 | 2.00 |
| Phenylalanine | 2.68 | 2.1 | 1.5 | 1.85 | 1.99 | 1.87 | 1.93 | 2.00 | 1.91 | 1.94 | 1.86 |

^a Hardy (2002)

^b Methionine + Cystine

Digestibility trial

For the determination of apparent digestibility coefficients (ADCs) of dry matter, protein and lipid, the remaining fish from the growth trial were anesthetized with MS-222 and manually stripped of feces 6 h after feeding. All diets contained Celite (10 g kg⁻¹) as the indigestible marker. Wet fecal samples were collected and dried at 50°C (48 h) before analysis. Analysis of acid insoluble ash (Celite) in the diets and feces was performed according to standard AOAC (2000) methodology.

Digestibility coefficients of nutrients were determined by diet and feces analysis using the following formula:

$$\text{ADC}\% = 100 (1 - (\text{marker in diet}/\text{marker in feces}) \times (\text{nutrient in feces}/\text{nutrient in diet}))$$

Proximate composition

Twelve fish were randomly taken from the beginning of the experiment from the initial batch of fish, and three fish from each tank (nine fish per group) were randomly sampled at the end of the trial to determine whole-body proximate analysis. Analysis of crude protein, moisture, and ash in diets and the whole-body of fish was performed according to standard AOAC (2000) procedures. Dry matter was determined by drying at 105°C until a constant weight was obtained. Ash content was measured by burning in a muffle furnace at 525°C for 12 h. Crude protein was analyzed by the Kjeldahl method after acid digestion using the Gerhardt system. Dietary and whole-body lipids were extracted according to the procedure of Folch et al. (1957) with chloroform/methanol (2:1 v/v; AOAC 2000). Nitrogen-free extract (NFE) was calculated by taking the sum values for crude protein, lipid and ash and then subtracting this from 100.

Dietary amino acid analysis

Sixty milligrams of sample (protein source or diet) was hydrolyzed in 10 ml of 6 M HCl in screw-capped tubes. The tubes were flushed with nitrogen and then heated at 110°C for 24 h. The hydrolysates were rotary-evaporated to dryness under vacuum at 40°C and then re-dissolved in a sodium citrate buffer at pH 2.2. The amino acids (AA) were separated by ion-exchange chromatography on a Shimadzu RF-10AXL sodium column and detected following post-column derivatization with ninhydrin, by measuring absorbance at 350–450 nm. Identification and quantification of the detected AA were performed using external standards after adjustments by linear regression. The AA standard was purchased from Sigma-Aldrich Co, USA as a synthetic AA mixture.

Hematology

Blood was sampled from three fish per tank at the end of the trial. Samples were taken from the caudal arch using a 25-gauge needle and 2-ml syringe. Hematocrit levels were determined by drawing fresh blood into microhematocrit tubes and centrifugation at 3,600g for 6 min in a microhematocrit centrifuge. Hematocrit values were measured and read as % packed cell volume (%PCV). Blood smears were prepared, stained with Giemsa (BDH) and mounted in DPX (BDH). Total leukocyte levels were quantified as total number of leukocytes per 1,000 blood cells. Thrombocytes, lymphocytes, monocytes and granulocytes were identified following the descriptions of Rowley (1990). A minimum of

100 cells per sample were counted from nine fish per dietary group, and values were expressed as a percentage of the total leukocytes (Merrifield et al. 2010a). Additional blood was left to clot for 3 h before serum was removed. Serum samples were stored at -80°C until analysis of lysozyme activity. Lysozyme activity was assessed using the turbidometric assay of Ellis (1990). Briefly, 50 μl of serum was added to 950 μl of the *Micrococcus lysodeikticus* (Sigma, St Louis, MO, USA) solution (200 mg ml^{-1} in 0.05 M Na_2HPO_4 at pH 6.2). Turbidity at 530 nm was measured after 0.5 and 4.5 min at 23°C . One unit of lysozyme activity was defined as a decrease in absorbance of 0.001 units per minute.

Statistical analysis

All data were subjected to an analysis of variance (ANOVA) and post hoc LSD using the statistical software package Statgraphics 7.0 (Manugistics Incorporated, Rockville, MD, USA; Zar 2001). The mean values for three replicate tanks were used for statistical analyses with the data, which were analyzed with nested design model using individual measurements within a tank. All percentage data and ratios were arcsine transformed before being subjected to the analysis. The results were treated as statistically significant at the $P < 0.05$ level.

Results

The overall growth performance and nutrient utilization data are displayed in Table 3. The final mean weights of fish at the end of the 11-week feeding trial ranged from 158.8 g (CM + CO diet) to 197.1 g (FM diet). Some statistical differences were observed for the mean final weight, SGR and thermal growth coefficient (TGC) with fish fed CM + CO displaying significantly lower final weight (158.8 g), SGR ($1.07\% \text{ day}^{-1}$) and TGC (0.106) than fish fed the FM, SCM, SM + CO and SCM + CO diets.

The lowest feed intake ($\text{g fish}^{-1} \text{ day}^{-1}$) was recorded in fish fed the CM + CO diet, which was significantly lower than the FI of fish fed the SCM diet (Table 3). Feed conversion ratio (FCR) values for all groups ranged from 1.14 (FM and SCM + CO) to 1.33 (CM and CM + CO). The lowest FCR of 1.14 for fish fed the FM and SCM + CO diets was significantly ($P < 0.05$) better than trout fed the CM and CM + CO diets (1.33).

Rainbow trout fed the FM and SCM + CO diets displayed a PER of 1.90, which was significantly higher ($P < 0.05$) than the group fed the CM + CO diet (1.34). However, fish fed SM, CM, SCM and SM + CO diets did not produce significant ($P > 0.05$) differences of PER despite general trends toward lower PER values (1.65, 1.63, 1.68 and 1.66, respectively). Net protein utilization (NPU) of the CM + CO group (34.27%) was significantly lower ($P < 0.05$) than that of the FM (42.90%) and the SCM + CO (41.70%) fed rainbow trout, respectively.

Data on the apparent digestibility coefficients (ADCs) of the experimental diets are presented in Table 4. Dry matter digestibility values for all groups varied from 67.8 (CM + CO Diet) to 70.2% (SCM + CO Diet). No statistical difference was found among dietary treatments in terms of the apparent digestibility coefficients of dry matter. On the other hand, apparent digestibility of protein (ranging from 88.7 to 93.1%) was significantly affected ($P < 0.05$) by the various dietary treatments. The lowest protein ADC value was found for the CM + CO diet, but this was only significantly lower than that of the

Table 3 Growth performance and nutrient utilization of rainbow trout after 11 weeks of feeding on experimental diets ($n = 3$)

| | FM | SM | CM | SCM | SM + CO | CM + CO | SCM + CO | <i>P</i> value |
|---|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------|
| Initial mean weight (g) | 70.10 ± 0.08 | 69.90 ± 0.35 | 69.90 ± 0.16 | 69.90 ± 0.29 | 69.80 ± 0.32 | 69.90 ± 0.15 | 70.00 ± 0.12 | |
| Final mean weight (g) | 197.10 ± 4.41 ^b | 172.8 ± 6.37 ^{ab} | 170.2 ± 7.90 ^{ab} | 185.7 ± 3.05 ^b | 178.6 ± 8.72 ^b | 158.80 ± 7.80 ^a | 194.20 ± 2.80 ^b | 0.017 |
| Specific growth rate (SGR; % day ⁻¹) | 1.34 ± 0.03 ^b | 1.18 ± 0.08 ^{ab} | 1.15 ± 0.05 ^{ab} | 1.27 ± 0.02 ^b | 1.21 ± 0.04 ^b | 1.07 ± 0.06 ^a | 1.33 ± 0.07 ^b | 0.015 |
| Thermal growth coefficient (TGC) | 0.138 ± 0.01 ^b | 0.118 ± 0.02 ^{ab} | 0.115 ± 0.02 ^{ab} | 0.129 ± 0.02 ^b | 0.122 ± 0.01 ^b | 0.106 ± 0.02 ^a | 0.136 ± 0.01 ^b | 0.039 |
| Feed intake (FI; g fish ⁻¹ day ⁻¹) | 1.88 ± 0.03 ^{ab} | 1.71 ± 0.01 ^{ab} | 1.72 ± 0.01 ^{ab} | 1.92 ± 0.02 ^b | 1.80 ± 0.03 ^{ab} | 1.49 ± 0.05 ^a | 1.85 ± 0.06 ^{ab} | 0.032 |
| Feed conversion ratio (FCR) | 1.14 ± 0.02 ^a | 1.28 ± 0.13 ^{ab} | 1.33 ± 0.14 ^b | 1.28 ± 0.08 ^{ab} | 1.29 ± 0.18 ^{ab} | 1.33 ± 0.09 ^b | 1.14 ± 0.06 ^a | 0.024 |
| Protein efficiency ratio (PER) | 1.90 ± 0.04 ^b | 1.65 ± 0.05 ^{ab} | 1.63 ± 0.05 ^{ab} | 1.68 ± 0.06 ^{ab} | 1.66 ± 0.14 ^{ab} | 1.34 ± 0.07 ^a | 1.90 ± 0.06 ^b | 0.025 |
| Net protein utilization (NPU%) | 42.90 ± 2.32 ^b | 37.46 ± 2.45 ^{ab} | 36.35 ± 3.25 ^{ab} | 37.21 ± 1.69 ^{ab} | 37.33 ± 6.42 ^{ab} | 34.27 ± 5.89 ^a | 41.70 ± 2.17 ^b | 0.031 |

In the same line, values with different superscript letters are significantly different ($P < 0.05$). Data are expressed as mean ± SD

Table 4 Apparent digestibility coefficients (ADC) of rainbow trout after 11 weeks of feeding on experimental diets ($n = 3$)

| Parameters (%) | FM | SM | CM | SCM | SM + CO | CM + CO | SCM + CO | <i>P</i> value |
|----------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------------|--------------------------|----------------|
| Dry matter | 70.1 ± 0.11 | 69.3 ± 0.22 | 68.6 ± 0.36 | 69.7 ± 0.45 | 69.9 ± 0.69 | 67.8 ± 0.58 | 70.2 ± 0.34 | 0.078 |
| Protein | 91.2 ± 0.25 ^{ab} | 91.5 ± 0.28 ^{ab} | 90.9 ± 0.45 ^{ab} | 92.3 ± 0.52 ^{ab} | 91.8 ± 0.64 ^{ab} | 88.7 ± 0.60 ^a | 93.1 ± 0.27 ^b | 0.024 |
| Lipid | 93.4 ± 0.18 ^b | 94.7 ± 0.22 ^b | 93.6 ± 0.41 ^b | 95.3 ± 0.46 ^b | 94.2 ± 0.59 ^b | 88.2 ± .51 ^a | 95.8 ± 0.24 ^b | 0.034 |

In the same line, values with different superscript letters are significantly different ($P < 0.05$). Data are expressed as mean ± SD

SCM + CO diet. Apparent digestibility of lipid varied from 88.2 to 95.8%, with the CM + CO group (88.2%) significantly lower than all other groups.

Data on biometric parameters of fish fed the different diets are displayed in Table 5. With the exception of condition factor (CF), which was significantly higher ($P < 0.05$) in fish fed the SCM + CO diet than those fed the CM + CO diet, no statistical differences of the biometric parameters were observed.

The whole-body composition of fish fed the experimental diets is presented in Table 6. No significant differences were observed in body protein (17.9–19.2%), lipid (14.0–16.3%) moisture (64.2–65.8%) or ash (2.4–2.9%) among the dietary treatments ($P > 0.05$).

Hematological and immunological data are presented in Table 7. Rainbow trout fed the CM + CO diet had hematocrit levels significantly lower ($P < 0.05$) than those fed the other treatments. Circulating leukocytes levels, differential leukocyte proportions and serum lysozyme activity were not affected by dietary treatment.

Discussion

The current investigations were performed to test the feasibility of including dietary soybean or canola meals with canola oil in practical diets for rainbow trout. The data indicate that the soybean meal can be included at a level of 29.0% in diets with fish oil or canola oil without significantly reducing growth performance, although it should be noted that trends toward reduced performance was observed. Several studies have partially, or fully, replaced fish meal with soybean meal in aquafeeds, and results with rainbow trout indicate that inclusion rates in the diet of 15 and 20% does not adversely affect growth performance (Refstie et al. 2000; Hardy 2002; Cheng et al. 2003). Levels above this can be detrimental due to the presence of antinutritional factors (ANFs), which can retard growth and induce enteritis in salmonids (Krogdahl et al. 2003, 2010; Merrifield et al. 2009). It is possible that the presence of ANFs and subsequent enteritis could be at least a partial cause for the trends toward reduced growth of SM fed fish observed in the present study.

Hilton and Slinger (1986) incorporated canola meal into practical diets (from 13.5 to 40.4% inclusion) for juvenile rainbow trout and concluded that canola meal should not be included in rainbow trout diets due to observed reductions in growth and poor dietary digestibility. It must be noted however that during the last 25 years, production technologies have advanced and present-day canola meals display somewhat different properties to that used by Hilton and Slinger (1986), generally reflected by higher protein contents, lower fiber contents and higher digestibility. Indeed, in a more recent study, Thiessen et al. (2004) replaced 50 (30–33% dietary inclusion) and 75% (45–49% dietary inclusion) of dietary fish meal in rainbow trout diets with a canola protein concentrate and reported no significant reductions in growth performance. The present study indicates that although diets containing 35% canola meal did not significantly reduce growth performance when using fish oil (CM group), trends toward lower growth performance, PER and NPU were observed compared with the FM and SCM + CO groups, and elevated FCR was observed compared with the FM group. Therefore, the findings of the present study lie somewhere between those of Hilton and Slinger (1986) and Thiessen et al. (2004), indicating that low-level canola meal inclusion could be used, but levels of 35% (i.e. 40% fish meal replacement) begin to cause problems pertaining to higher FCR and trends toward depressed growth and protein utilization. Furthermore, the use of 35% canola meal in conjunction with canola oil (CM + CO group) significantly impaired final weight, SGR, TGC, PER and NPU. These parameters were all significantly lower in the CM + CO fed

Table 5 Biological indices of rainbow trout after 11 weeks of feeding on experimental diets (*n* = 9)

| Parameters (%) | Initial | FM | SM | CM | SCM | SM + CO | CM + CO | SCM + CO | <i>P</i> value |
|----------------------------|--------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|--------------------------|--------------------------|----------------|
| Condition factor (CF) | 1.40 ± 0.26 | 1.63 ± 0.13 ^{ab} | 1.67 ± 0.21 ^{ab} | 1.65 ± 0.13 ^{ab} | 1.66 ± 0.11 ^{ab} | 1.67 ± 0.14 ^{ab} | 1.58 ± 0.09 ^a | 1.79 ± 0.17 ^b | 0.019 |
| Hepatosomatic index (HSI) | 0.81 ± 0.26 | 1.11 ± 0.27 | 1.00 ± .22 | 0.96 ± 0.26 | 1.03 ± 0.19 | 0.93 ± 0.21 | 1.03 ± 0.09 | 0.79 ± 0.27 | 0.112 |
| Viscerosomatic index (VSI) | 15.75 ± 1.30 | 14.34 ± 2.43 | 13.82 ± 1.34 | 15.86 ± 0.68 | 15.53 ± 1.20 | 14.7 ± 1.10 | 14.53 ± 2.00 | 13.09 ± 2.40 | 0.248 |
| Dress-out (DO) | 84.25 ± 2.12 | 85.66 ± 3.56 | 86.18 ± 2.46 | 84.14 ± 1.79 | 84.47 ± 2.40 | 85.3 ± 2.20 | 85.47 ± 3.05 | 86.91 ± 3.52 | 0.261 |

In the same line, values with different superscript letters are significantly different (*P* < 0.05). Data are expressed as mean ± SD

Table 6 Whole-body chemical composition of rainbow trout after 11 weeks of feeding on experimental diets ($n = 9$)

| Parameters (%) | Initial | FM | SM | CM | SCM | SM + CO | CM + CO | SCM + CO | <i>P</i> value |
|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|----------------|
| Moisture | 71.8 ± 2.26 | 65.8 ± 1.60 | 65.7 ± 2.18 | 64.2 ± 0.99 | 64.8 ± 1.83 | 65.8 ± 0.82 | 65.5 ± 2.94 | 65.5 ± 1.67 | 0.125 |
| Protein | 13.0 ± 1.25 | 19.2 ± 1.41 | 18.7 ± 1.44 | 18.5 ± 1.23 | 18.7 ± 1.32 | 18.8 ± 1.54 | 17.9 ± 1.42 | 18.7 ± 1.05 | 0.235 |
| Lipid | 8.4 ± 0.85 | 16.3 ± 0.91 | 15.1 ± 1.69 | 15.2 ± 2.04 | 14.7 ± 1.80 | 14.5 ± 1.47 | 14.9 ± 1.11 | 14.0 ± 0.93 | 0.157 |
| Ash | 3.3 ± 0.26 | 2.4 ± 0.25 | 2.9 ± 0.32 | 2.4 ± 0.23 | 2.5 ± 0.31 | 2.7 ± 0.22 | 2.6 ± 0.23 | 2.5 ± 0.34 | 0.162 |

In the same line, values with different superscript letters are significantly different ($P < 0.05$). Data are expressed as mean ± SD

Table 7 Hematological and immunological parameters of rainbow trout after 11 weeks of feeding on experimental diets ($n = 9$)

| | FM | SM | CM | SCM | SM + CO | CM + CO | SCM + CO | P value |
|--------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------|--------------------------|--------------------------|---------|
| PCV (%) | 33.4 ± 3.05 ^b | 34.8 ± 2.64 ^b | 31.4 ± 2.70 ^b | 32.3 ± 2.48 ^b | 33.9 ± 1.28 | 28.7 ± 2.53 ^a | 35.8 ± 1.89 ^b | 0.026 |
| Lysozyme (U ml ⁻¹) | 402.5 ± 26.25 | 406.1 ± 28.34 | 427.7 ± 29.61 | 379.1 ± 35.93 | 373.6 ± 30.39 | 369.8 ± 36.82 | 467.3 ± 32.58 | 0.082 |
| Leukocyte count ¹ | 22.6 ± 1.8 | 21.4 ± 1.6 | 25.3 ± 1.8 | 24.8 ± 1.7 | 25.0 ± 1.8 | 20.5 ± 1.1 | 25.6 ± 1.4 | 0.128 |
| Lymphocytes (%) | 87.0 ± 5.4 | 86.0 ± 4.6 | 87.3 ± 4.2 | 88.7 ± 3.4 | 86.3 ± 3.7 | 87.9 ± 3.8 | 88.6 ± 3.5 | 0.602 |
| Neutrophil (%) | 5.8 ± 1.8 | 6.3 ± 1.8 | 6.5 ± 1.5 | 4.2 ± 1.6 | 5.3 ± 1.1 | 5.7 ± 1.0 | 4.9 ± 1.1 | 0.534 |
| Thrombocytes (%) | 4.2 ± 0.7 | 4.3 ± 0.8 | 3.4 ± 0.8 | 4.0 ± 0.7 | 4.5 ± 0.9 | 3.7 ± 0.8 | 3.6 ± 0.7 | 0.528 |
| Granulocytes (%) | 3.0 ± 0.6 | 3.4 ± 0.6 | 2.8 ± 0.5 | 3.1 ± 0.7 | 3.9 ± 0.5 | 2.7 ± 0.6 | 2.9 ± 0.4 | 0.429 |

In the same line, values with different superscript letters are significantly different ($P < 0.05$). Data are expressed as mean ± SD

¹ Expressed as leukocytes per 1,000 blood cells

fish than the FM and SCM + CO fed fish. The reason for this does not appear to be due to limiting essential amino acid (EAA) provision, as all diets in the present study exceed previously determined rainbow trout EAA requirements (Hardy 2002; Table 2), although that is not to say that there may not be differences in bioavailability. These reductions could be attributed to EAA availability and/or the presence of ANFs, reduced feed intake (daily feed intake of the CM + CO group was ca. 20% lower than that of FM and SCM + CO groups) and/or reduced nutrient digestibility. Indeed, it was observed in the present study that the ADC values of the CM + CO diets were the lowest of all the experimental diets, being significantly lower in the case of lipid (compared with FM and SCM + CO) and protein (compared with SCM + CO). Similar results of low digestibility have previously been reported in rainbow trout (Hilton and Slinger 1986) and Japanese sea bass (Cheng et al. 2010) fed canola meal. The lower apparent digestibility could be due to the higher levels of fiber, phytic acid, sinapine and tannins in canola meal and erucic acid in canola oil (Mc Curdy and March 1992; Forster et al. 1999; Mwachireya et al. 1999).

The results of Shafaiepour et al. (2008) suggest that the combined use of soybean meal and canola meal might help to overcome the limitations imposed by the singular use of the respective ingredients; Shafaiepour et al. (2008) demonstrated that a diet containing 30% solvent-extracted canola meal and 24.4% soybean meal could be used in rainbow trout diets without affecting growth rate and nutrient utilization. The present study confirms that high-level mixtures of these ingredients can be utilized in rainbow trout diets. More importantly, the present study shows for the first time that canola meal and soybean meal can be incorporated in the diets of rainbow trout at a combined level of 32.0% (replacing 40% of dietary fish meal) with the full replacement of fish oil with a vegetable oil (namely canola oil; SCM + CO group) without effecting growth performance. Little is known about the effect of a simultaneous replacement of both fish meal and fish oil in rainbow trout diets, but some studies have shown that vegetable oils can replace 100% of fish oil in fish meal-based rainbow trout diets, without adverse effects on growth, nutrient utilization and survival (Caballero et al. 2002). In the present study, the use of canola oil as a substitute for fish oil in the SM + CO and SCM + CO diets did not adversely affect the growth of rainbow trout over the 11-week experimental period. However, as discussed previously, the growth performance of rainbow trout fed the canola meal diet with canola oil (CM + CO group) was inferior compared with trout fed the canola meal diet with fish oil (CM group). A number of studies on several fish species have reported no significant effects of canola oil substitution on growth while using fishmeal as a protein source (Guillou et al. 1995; Dosanjh et al. 1998; Caballero et al. 2002; Bell et al. 2003; Turchini et al. 2003; Torstensen et al. 2004; Francis et al. 2006). However, using canola oil to substitute fish oil in semi-purified diets has been reported to have negative effects on Murray cod *Maccullochella peelii peelii* (Francis et al. 2007) and barramundi *Lates calcarifer* (Raso and Anderson 2003).

Recently, it has been reported that the partial replacement (33 and 66%) of fish oil by a combination of vegetable oils (rapeseed, linseed and palm oil) in a plant protein-rich formulation had no detrimental effects on growth performance of juvenile gilthead sea bream *Sparus aurata* (Fountoulaki et al. 2009). A previous study demonstrated that total replacement of fish oil with a rapeseed, linseed and palm oil blend in a plant meal-based diet reduced growth indices of gilthead sea bream (Benedito-Palos et al. 2007). In discussing the limitations of vegetable oil inclusion in aquafeeds, the fatty acid profiles of the lipid sources and the formulated test diets as well as the fatty acid requirements of the intended fish species should be considered.

In the present study, leukocyte levels and the proportions of lymphocytes, neutrophils, thrombocytes and granulocytes were not significantly affected by the different dietary treatments. The values reported for the hematological parameters are within the range reported for normal, healthy juvenile rainbow trout (Merrifield et al. 2010b, c, d). However, lower hematocrit values were found in the group fed CM + CO. Similarly, Dabrowski et al. (2000) and Lee et al. (2002, 2006) have reported lower hematocrit levels in rainbow trout fed diets containing a high level of oilseed meal (cottonseed meal). The reason for the observed lower hematocrit level could be the cumulative effect of gossypol and/or decreased availability of iron in canola meal leading to lower erythrocyte levels or increased fragility (Colin-Negrete et al. 1996). It is recommended that future studies assessing the combination of canola meal and canola oil should investigate blood chemistry and hematological indices in order to ascertain if it is necessary to elevate dietary iron levels in canola-based diets. It appears from the present study however that any such deficiency and subsequent impact on hematology is avoided with the combined use (of levels of up to 32%) of canola with soybean meal.

The present study suggests that the combination of canola meal and soybean meal can be incorporated in the diets of rainbow trout at 32.0% when using either fish oil or canola oil, without detrimentally effecting growth performance, nutrient utilization and the basic health parameters investigated. Further investigation of the interactions between plant protein and oil sources on lipid metabolism and deposition is required in order to formulate effective sustainable production diets for rainbow trout and other carnivorous species.

Acknowledgments The authors kindly thank Zeki Sönmez, Volkan Tepe, Seda Karakoca, Sinem Kucukoguzsoyulu, Mustafa Karga and Özden Oruçoğlu for their technical assistance. We would also like to thank Agromey Feed Mill Company and Kartal Chemical Incorporated for providing feed ingredients.

References

- AOAC (Association of Official Analytical Chemists) (2000) Official methods of analysis, 19th edn. AOAC, Arlington, VA
- Barrows FT, Stone DAJ, Hardy RW (2007) The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout *Oncorhynchus mykiss*. *Aquaculture* 265:244–252
- Bell JG (2008) Alternative lipid sources for fish feed and farmed fish quality. In: Lie Ø (ed) Improving farmed fish quality and safety. Woodhead Publishing Limited, UK, pp 275–299
- Bell JG, Tocher DR, Henderson RJ, Dick JR, Crampton VO (2003) Altered fatty acid compositions in Atlantic salmon *Salmo salar* fed diets containing linseed and rapeseed oils can be partially restored by a subsequent fish oil finishing diet. *J Nutr* 133:2793–2801
- Benedito-Palos BL, Saera-Vila SA, Caldach-Giner AJ, Kaushik S, Pérez-Sánchez JP (2007) Combined replacement of fish meal and oil in practical diets for fast growing juveniles of gilthead sea bream *Sparus aurata* networking of systemic and local components of GH/IGF axis. *Aquaculture* 267: 199–212
- Brandsen MP, Carter CG, Nichols PD (2003) Replacement of fish oil with sunflower oil in feeds for Atlantic salmon *Salmo salar* effect on growth performance, tissue fatty acid composition and disease resistance. *Comp Biochem Phys B* 135:611–625
- Caballero MJ, Obach A, Rosenlund G, Montero D, Gisvold M, Izquierdo MS (2002) Impact of different dietary lipid sources on growth, lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 214:253–271
- Cheng ZJ, Hardy RW, Blair M (2003) Effects of supplementing methionine hydroxy analogue in soybean meal diets and distiller's dried grain-based diets on the performance and nutrient retention of rainbow trout *Oncorhynchus mykiss*. *Aquacult Res* 34:1303–1310
- Cheng Z, Ai Q, Mai K, Xu W, Ma H, Li Y, Zhang J (2010) Effects of dietary canola meal on growth performance, digestion and metabolism of Japanese seabass, *Lateolabrax japonicus*. *Aquaculture* 305:102–108

- Colin-Negrete CJ, Kiesling HE, Ross TT, Smith JF (1996) Effect of whole cottonseed on serum constituents, fragility of erythrocyte cells, and reproduction of growing Holstein heifers. *J Dairy Sci* 79:2016–2023
- Dabrowski K, Rinchar J, Lee KJ, Blom JH, Ciereszko A, Ottobre J (2000) Effects of diets containing gossypol on reproductive capacity of rainbow trout *Oncorhynchus mykiss*. *Biol Reprod* 62:227–234
- Dosanjh BS, Higgs DA, McKenzie DJ, Randall DJ, Eales JG, Rowshandeli N, Rowshandeli M, Deacon G (1998) Influence of dietary blends of menhaden oil and canola oil on growth, muscle lipid composition, and thyroidal status of Atlantic salmon *Salmo salar* in seawater. *Fish Physiol Biochem* 19:123–134
- Drew MD, Ogunkoya AE, Janz DM, Van Kesel AG (2007) Dietary influence of replacing fish meal and oil with canola protein concentrate and vegetable oils on growth performance, fatty acid composition and organochlorine residues in rainbow trout *Oncorhynchus mykiss*. *Aquaculture* 267:260–268
- Ellis AE (1990) Lysozyme assays. In: Stolen JS, Fletcher TC, Anderson DP, Roberson BS, Van Muiswinkel WB (eds) *Techniques in fish immunology*. SOS Publications, Fair Haven, pp 101–103
- FAO (2010) Fisheries and aquaculture department. <http://www.fao.org/fishery>. Cited 15 Oct 2010
- Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509
- Forster I, Higgs DA, Dosanjh BS, Rowshandeli M, Parr J (1999) Potential for dietary phytase to improve the nutritive value of canola protein concentrate and decrease phosphorus output in rainbow trout *Oncorhynchus mykiss* held in 11 C freshwater. *Aquaculture* 179:109–125
- Fountoulaki E, Vasilaki A, Hurtado R, Grigorakis K, Karacostas I, Nengas I, Ringos G, Kotzamanis Y, Venon B, Alexis MN (2009) Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream *Sparus aurata* effect on growth performance, flesh quality and fillet fatty acid profile. Recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. *Aquaculture* 289:317–326
- Francis DS, Turchini GM, Jones PL, De Silva SS (2006) Effects of dietary oil source on growth and fillet fatty acid composition of Murray cod, *Maccullochella peelii peelii*. *Aquaculture* 253:547–556
- Francis DS, Turchini GM, Jones PL, De Silva SS (2007) Growth performance, feed efficiency and fatty acid composition of juvenile Murray cod, *Maccullochella peelii peelii*, fed graded levels of canola and linseed oil. *Aquacult Nutr* 13:335–350
- Gatlin DM III, Barrows FT, Bellis D, Brown P, Campen J, Dabrowski K, Gaylord TG, Hardy RW, Herman E, Hu G, Krogdahl A, Nelson R, Overturf K, Rust M, Sealey W, Skonberg D, Souza E, Stone D, Wilson R, Wurtele E (2007) Expanding the utilization of sustainable plant products in aquafeeds—a review. *Aquacult Res* 38:551–579
- Guillou A, Soucy P, Khalil M, Adambounou L (1995) Effects of dietary vegetable and marine lipid on growth, muscle fatty acid composition and organoleptic quality of flesh of brook charr *Salvelinus fontinalis*. *Aquaculture* 136:351–362
- Hardy RW (2002) Rainbow trout, *Oncorhynchus mykiss*. In: Webster CD, Lim CE (eds) *Nutrient requirements and feeding of finfish for aquaculture*. CABI Publishing, Wallingford, pp 184–202
- Hardy RW (2010) Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquacult Res* 41:770–776
- Hertrampf JW, Piedad-Pascual F (2000) *Handbook on ingredients for aquaculture feeds*. Kluwer, The Netherlands
- Higgs DA, Dong FM (2000) Lipids and fatty acids. In: Stickney RR (ed) *Encyclopedia of aquaculture*. Wiley, New York, pp 476–496
- Hilton JW, Slinger SJ (1986) Digestibility and utilization of canola meal in practical-type diets for rainbow trout *Salmo gairdneri*. *Can J Fish Aquat Sci* 43:1149–1155
- Izquierdo MS, Montero D, Robaina L, Caballero MJ, Rosenlund G, Gines R (2005) Alterations in fillet fatty acid profile and flesh quality in gilthead seabream (*Sparus aurata*) fed vegetable oils for a long term period. Recovery of fatty acid profiles by fish oil feeding. *Aquaculture* 250:431–444
- Krogdahl A, Bakke AM, Baevefjord G (2003) Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar* L.). *Aquacult Nutr* 9:361–371
- Krogdahl A, Penn M, Thorsen J, Refstie S, Bakke AM (2010) Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. *Aquacult Res* 41:333–344
- Lee KJ, Dabrowski K, Blom JH, Bai SC, Stromberg PC (2002) A mixture of cottonseed meal, soybean meal and animal byproduct mixture as a fish meal substitute: growth and tissue gossypol enantiomers in juvenile rainbow trout *Oncorhynchus mykiss*. *J Anim Physiol Anim Nutr* 86:201–213
- Lee KJ, Rinchar J, Dabrowski K, Babiak I, Ottobre JS, Christensen JE (2006) Long-term effects of dietary cottonseed meal on growth and reproductive performance of rainbow trout: three-year study. *Anim Feed Sci Technol* 126:93–106

- Li MH, Robinson EH, Hardy RW (2000) Dietary protein requirements. In: Stickney RR (ed) Encyclopedia of aquaculture. Wiley, New York, pp 202–209
- Luo L, Xue M, Wu X, Cai X, Cao H, Liang Y (2006) Partial or total replacement of fishmeal by solvent-extracted cottonseed meal in diets for juvenile rainbow trout *Oncorhynchus mykiss*. Aquacult Nutr 12:418–424
- Mc Curdy SM, March BE (1992) Processing of canola meal for incorporation in trout and salmon diets. J Am Oil Chem Soc 69:213–220
- Merrifield DL, Bradley G, Baker RTM, Dimitroglou A, Davies SJ (2009) Soybean meal alters autochthonous microbial populations, microvilli morphology and compromises intestinal enterocyte integrity of rainbow trout (*Oncorhynchus mykiss* Walbaum). J Fish Dis 32:755–766
- Merrifield DL, Güroy D, Güroy B, Emery M, Llewellyn C, Skill S, Davies SJ (2010a) Preliminary assessment of Chlorogloeopsis as a dietary supplement for red tilapia *Oreochromis niloticus*. Aquaculture 299:128–133
- Merrifield DL, Bradley G, Baker RTM, Dimitroglou A, Davies SJ (2010b) Probiotic applications for rainbow trout *Oncorhynchus mykiss* I. Effects on growth performance, feed utilisation, intestinal microbiota and related health criteria. Aquacult Nutr 16:504–510
- Merrifield DL, Bradley G, Baker RTM, Davies SJ (2010c) Probiotic applications for rainbow trout *Oncorhynchus mykiss* II. Effects on growth performance, feed utilisation, intestinal microbiota and related health criteria post antibiotic treatment. Aquacult Nutr 19:496–503
- Merrifield DL, Bradley G, Harper G, Baker RTM, Munn CB, Davies SJ (2010d) Assessment of the effects of vegetative and lyophilised *Pediococcus acidilactici* on growth, feed utilisation, intestinal colonisation and health parameters of rainbow trout *Oncorhynchus mykiss*. Aquacult Nutr 17:73–79
- Mwachireya SA, Beames RM, Higgs DA, Dosanjh BS (1999) Digestibility of canola protein products derived from the physical, enzymatic and chemical processing of commercial canola meal in rainbow trout *Oncorhynchus mykiss* held in fresh water. Aquacult Nutr 5:73–82
- Pratoomyot J, Bendiksen EA, Bell JG, Tocher DR (2008) Comparison of effects of vegetable oils blended with southern hemisphere fish oil and decontaminated northern hemisphere fish oil on growth performance, composition and gene expression in Atlantic salmon *Salmo salar*. Aquaculture 280:170–178
- Raso S, Anderson TA (2003) Effect of dietary fish oil replacement on growth and carcass proximate composition of juvenile barramundi *Lates calcarifer*. Aquacult Res 34:813–819
- Refsstie S, Helland SJ, Storebakken T, Baeverfjord G, Lein I, Roem AJ (1997) Adaptation to soybean meal in diets for rainbow trout, *Oncorhynchus mykiss*. Aquaculture 153:263–272
- Refsstie S, Korsoen OJ, Storebakken T (2000) Differing nutritional responses to dietary soybean meal in rainbow trout *Oncorhynchus mykiss* and Atlantic salmon *Salmo salar*. Aquaculture 190:49–63
- Rowley AF (1990) Collection, separation and identification of fish leucocytes. In: Stolen JS, Fletcher TC, Anderson DP, Roberson BS, Van Muiswinkel WB (eds) Techniques in fish immunology. SOS Publications, Fair Haven, pp 113–136
- Rumsey GL, Siwicki AK, Anderson DP, Bowser PR (1994) Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. Vet Immunol Immunol 41:323–339
- Shafaeipour A, Yavari V, Falahatkar B, Maremmazi JG, Gorjipour E (2008) Effects of canola meal on physiological and biochemical parameters in rainbow trout *Oncorhynchus mykiss*. Aquacult Nutr 14: 110–119
- Thiessen DL, Maenz DD, Newkirk RW, Classen HL, Drew MD (2004) Replacement of fishmeal by canola protein concentrate in diets fed to rainbow trout *Oncorhynchus mykiss*. Aquacult Nutr 10:379–388
- Torstensen BE, Froyland L, Lie Ø (2004) Replacing dietary fish oil with increasing levels of rapeseed oil and olive oil—effects on Atlantic salmon *Salmo salar* tissue and lipoprotein composition and lipogenic enzyme activities. Aquacult Nutr 10:175–192
- Turchini GM, Mentasti T, Froyland L, Orban E, Caprino F, Moretti VM, Valfre F (2003) Effects of alternative dietary lipid sources on performance, tissue chemical composition, mitochondrial fatty acid oxidation capabilities and sensory characteristics in brown trout *Salmo trutta*. Aquaculture 225: 251–267
- Zar JH (2001) Biostatistical analysis, 4th edn. Prentice-Hall Inc., Upper Saddle River, NJ