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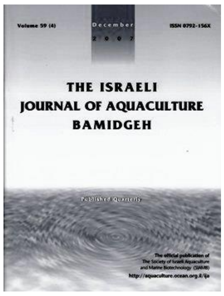

Effect of the Substitution of Fish Oil with a Mixture of Plant-Based Oils in Diets of Rainbow Trout (*Oncorhynchus mykiss* Walbaum) Fingerlings on Growth, Phosphorus and Nitrogen Excretion

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Key words: growth, lipids, plant oils, phosphorus, rainbow trout

Abstract

A feeding trial was performed to determine the effect of substituting fish oil with a 1:1 mixture of soybean and linseed oil on growth, phosphorus and nitrogen excretion, and lipid deposition of rainbow trout fingerlings. Diets with soy protein concentrate and soybean meal were prepared with 25, 50, and 75% substitution of fish oil with plant oils. Soy protein concentrate and soybean meal were used with fish oil at 100% in the control diet. Triplicate groups of 15 juveniles with an initial weight of 0.74 ± 0.05 g (mean ± standard error) were fed the experimental diets for 70 days. Growth performance, protein, and lipid digestibility, oxygen consumption, nitrogen, and phosphorus excretion, and lipid deposition (liver and muscle) were determined. The mixture of plant oils did not affect the growth performance of fingerlings. Apparent digestibility of lipids decreased as the fish oil in the diet decreased. A significant decrease of phosphorus excretion was observed as plant oils in the diets were increased. Lipid deposition was higher in liver unrelated to plant oil concentration in the diet. The results show that it is possible to use high quantities of plant ingredients in the diets without affecting the growth performance and lipid deposition, as well as significantly decreasing phosphorus excretion.

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Introduction

The growth and intensification of salmonid culture has increased demand for feeds (Olsen et al., 2003). Fish meal (FM) and fish oil (FO) are important ingredients in salmonid feeds however the supply of both these ingredients is becoming increasingly unstable and unreliable. (Gatlin et al., 2007; Kaushik and Seiliez, 2010). The aquaculture industry, thus, must find alternative ingredients at competitive prices. Plant based products have been suggested as a substitute for both FM and FO. Studies have shown that substitutions of FO with soy, linseed, palm, or canola oils (Dernekbaşi et al., 2011), do not affect the growth of salmonids (Greene and Selivonchick, 1990; Richard et al., 2006; Pettersson et al., 2009) when FM is used as the main protein source.

However, when plant based protein is used in combination with such oils, growth has been affected. Substitution of 80% of FM with wheat and corn gluten, and soybean meal and 70% of fish oil with a mixture of linseed, palm, and rapeseed oils, has been found to reduce growth of Atlantic salmon, *Salmo salar* (Torstensen et al., 2008). Total substitution of FM and FO with plant proteins (soymeal, corn gluten meal and wheat gluten) and oils (linseed, palm and rapeseed) affected the growth of rainbow trout due to lower feed intake and protein utilization (Panserat et al., 2009). Growth of Coho salmon (*Oncorhynchus kisutch*) was retarded when fed a diet with proteins from poultry byproduct meal, blood meal, canola meal and wheat gluten, and plant oils (canola and linseed) to substitute FM and FO, respectively (Twibell et al., 2012). This data indicates that total substitution of FM and FO negatively affects growth. The aim of this study was to determine the effect of diets with 75% substitution of fish meal with plant protein from soybean meal (SBM) and soy protein concentrate (SPC), and 25, 50, and 75% of fish oil with a mixture 1:1 soy and linseed oils on the growth performance, oxygen consumption, phosphorus, and nitrogen excretion, and lipid deposition of rainbow trout fingerlings (*Oncorhynchus mykiss*).

Materials and Methods

Experimental diets. Eight diets were formulated with a minimum content of 40% crude protein, and 10% lipids, to supply the requirements previously determined for rainbow trout (NRC, 2011). Four diets were formulated with 400 g/kg SPC (Profine-E, crude protein, 64 ± 1%; crude lipids, 0.5 ± 0.1%; Vimifos S.A de C.V., México) and 200 g/kg of Peruvian FM (crude protein, 65 ± 3%; crude lipid, 11 ± 2%; Vimifos S.A. de C.V., México) and SPC as protein sources. Four other diets (Table 1) were formulated with 400 g/kg SBM (crude protein, 50 ± 5%; crude lipid, 1 ± 0.8%; Nutricasa, S.A. de C.V., México) and 200 g/kg of FM (diets SBM).

Table 1. Formulation and proximate composition (mean ± SE) of the diets for fingerlings of rainbow trout.

Ingredients	*Diets (g/kg)*							
	SPC-25	*SPC-50*	*SPC-75*	*SPC-C*	*SMB-25*	*SBM-50*	*SBM-75*	*SBM-C*
Fish meal[1]	200	200	200	200	200	200	200	200
Soy protein concentrate[1]	400	400	400	400	0	0	0	0
Soybean meal[2]	0	0	0	0	400	400	400	400
Cod liver oil[3]	37.5	25	12.5	50	37.5	25	37.5	50
Mixture of plant oil[4]	12.5	25	37.5	0	12.5	25	12.5	0
Protease[5]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Phytase[5]	0	0	0	0	0.8	0.8	0.8	0.8
α-cellulose[6]	109	109	109	109	108.2	108.2	108.2	108.2
Other[7]	240.2	240.2	240.2	240.2	240.2	240.2	240.2	240.2
Proximate composition								
Protein[8]	42±1	40 ± 1	42 ± 3	43 ± 2	42 ± 1	38 ± 0.5	39 ± 0.2	39 ± 1
Lipid[8]	9±0.5	10 ± 0.6	10 ± 0.3	10 ± 0.4	11 ± 1	10 ± 1	12 ± 1	10 ± 0.1
Ash[8]	9±0.1	9 ± 0.4	9 ± 0.2	9 ± 0.2	9 ± 0.2	9 ± 0.3	9 ± 0.3	9 ± 0.2
Moisture	5±0.5	4 ± 0.1	5 ± 0.3	5 ± 0.1	4 ± 0.6	5 ± 0.6	5 ± 0.2	4 ± 0.5

[1] Vimifos S.A de C.V., Mexico
[2] Nutricasa, S.A. de C.V., Mexico
[3] Drotasa S.A. de C.V., Mexico
[4] Soy and linseed oils, 1:1. soy oil (Ragasa, S.A. de C.V., Mexico) and linseed (Drotasa S.A. de C.V., Mexico)
[5] DSM Nutritional Products of Mexico, Mexico
[6] Sigma Aldrich Co., USA
[7] Other ingredients (g/kg): soybean lecithin (Abastecedora de Productos Naturales, S.A. de C.V., Mexico), 50; dextrin (Sigma Aldrich Co., USA), 100; mixture of vitamins and minerals (DSM Nutritional Products of Mexico, Mexico), 40; DL-methionine, 0.2 (Evonik Mexico, S.A de C.V.); wheat gluten (Sigma Aldrich Co., USA), 50.
[8] % dry weight basis

For each of the diets with SPC and SBM, the FO (cod liver oil, Drotasa S.A. de C.V., Mexico) was substituted at 25, 50, and 75% with a mixture 1:1 of soy (Ragasa, S.A. de C.V., Mexico) and linseed (Drotasa S.A. de C.V., Mexico) oils (MSL). Both SPC and SBM diets with a 100% of FO were used as control groups. Besides the meals and oils, soybean lecithin (Abastecedora de Productos Naturales, S.A. de C.V., Mérida, México), dextrin (Sigma Aldrich Co., St. Louis, MO, USA), a mixture of vitamins and minerals (Micro Rovimix for carnivorous fish, DSM Nutritional Products of Mexico, Guadalajara, Mexico) and wheat gluten (Sigma Aldrich Co., St. Louis, MO, USA) were used. To improve the digestibility of the protein fraction, protease (Ronozyme ProAct, DSM Nutritional Products of Mexico, Guadalajara, Mexico) was added to the diets. To reduce the effect of phytic acid in the soybean meal (Cheng et al. 2004) diets, SBM was also added with phytase (Ronozyme P-(CT), 10,000 FYT/g, DSM Nutritional Products of Mexico, Guadalajara, Mexico). α-cellulose (Sigma Aldrich Co., St. Louis, MO, USA) was used as filler up to 100%. Diets were prepared by mixing all the powdered ingredients for a period of 20 min. Then the oils and purified water (40%) were added and mixed again for other 20 min (Cruz et al., 2011). The resulting wet dough was passed through a meat chopper to obtain 5-mm diameter pellets, which were dried at a constant temperature of 60 °C for 60 min and then stored at -24 °C. Before use, the pellets were crumbled and sifted to 0.5 mm particles.

Test fish. Rainbow trout (*Oncorhynchus mykiss*) 60 days post-hatching were obtained from the "Centro de Producción Acuícola "El Zarco", in the municipality of Ocoyoacac, State of México, México. They were transported to the Laboratorio de Producción Acuícola de la Facultad de Estudios Profesionales Iztacala, UNAM. Fish were kept in 1,000 L tanks for a period of 2 weeks and fed with a commercial diet (Api-trucha 1, 50% crude protein, Malta-Cleyton de México) until the start of the feeding trial.

Feeding trial. For the feeding experiment, 24 polypropylene tanks (100 L capacity each, filled to 80 L) were used. Fifteen fish, initial weight 0.74 ± 0.05 g (mean ± SEM) were randomly stocked in each tank. The test diets were fed to triplicate groups of fingerlings. Fish were fed equal amounts of their respective diets at 7% of their body weight, twice daily at 0800 and 1600 h. The fingerlings were weighed every 10 days and the ration size was adjusted accordingly. Feces were siphoned from the bottom of each tank 30 min after feeding. Uneaten feed was removed and quantified for measurement of diet intake. During the feeding trial, water parameters were (mean ± SD): dissolved oxygen, 5.5 ± 1.0 mg/l; ammonium and ammonia, 0.00 mg/l; pH 7.9 ± 0.5; water temperature, 13 ± 1 °C. Water flow in each tank was of 2 L/min. The feeding trial continued for 70 days, after which the organisms were starved for 24 h, and weighed to determine their growth performance. 10 fish from each tank were collected randomly and kept for an additional 10 days to determine apparent digestibility. The remainder of the fish were used to evaluate oxygen consumption, P and N excretion, and were then sacrificed with an overdose of MS-222 (ethyl 3-aminobenzoate, methanosulfonic acid, Sigma Aldrich Comp., St. Louis, MO, USA) and dissected to obtain liver and muscle samples.

Digestibility determination. The apparent digestibility coefficients (ADC) of the lipid and protein fractions were determined by using chromium oxide (Cr_2O_3, J.T. Baker, Phillipsburg, N.J., USA) as an inert marker at a concentration of 1% in each diet. The diets with the marker were well received by the fingerlings. Fish were fed for a period of 10 days, during which feces were collected on daily basis. Fecal matter was collected 30 min after feeding (Windell et al., 1978) to reduce the leaching of nutrients. It was then dried in an oven and 500 mg was digested with perchloric acid and nitric acid at 250 C. The content of chrome was quantified according with the formula reported by Furukawa & Tsukahara (1966):

$$[Cr_2O_3] = ((Y-0.032) / 0.2089) / 4$$

Where, Y= absorbance at 350 nm

The ADCs for the protein and lipid were calculated according to the formula of Austreng (1978):

$$ADC (\%) = 100 - ((\%MD / \%MF) \times (\%NF / \%ND) \times 100)$$

Where, MD= marker on diet; MF= marker on feces; NF= nutrient on feces and ND= nutrient on diet.

Oxygen consumption, nitrogen, and phosphorus excretion. The fish were fasted for a period of 24 h prior to analysis, and then weighed. A closed recirculation system of 1 L flasks connected in series was used. The flasks were slowly filled with water and one fish was placed in each flask. Water samples were taken and the dissolved oxygen was measured; then the flasks were hermetically sealed. After 30 min, the flasks were opened, dissolved oxygen was measured, and a water sample was taken to determine the P and N. The dissolved oxygen was measured with an oxygen meter (model 85, YSI Incorporated, OH, USA) and oxygen consumption was calculated as the difference between the initial and the final concentration. P excretion (as PO_4^{3-}) was determined by the molybdovanadate method, and nitrogen (as NH_3-N) by the Nessler method (Clescerl et al., 1995) respectively.

Chemical Analysis. The protein content in diets and feces was determined with the technique reported by AOAC (1990), while lipid content in feeds, diets, muscle, and liver was determined by chloroform and methanol extraction (Blight and Dyer, 1959).

Statistical Analysis. Data on final weight (FW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed efficiency rate (FER), protein efficiency rate (PER), survival, ADC, oxygen consumption, P and N excretion, contents of lipid and protein in muscle and liver were tested for normality with the Shapiro and Wilk W test and the Bartlett's test, respectively (Zar, 1989). Since all data was shown to be normal and homoscedastic, a one-way ANOVA test was used (StatPlus for Mac 2009, AnalystSoft Inc., USA). Significant differences among the treatments were determined by a Fisher LSD test (Zar, 1999), with a significance level of 5% ($p<0.05$) for each set of comparisons.

Results

Means of the final weight (FW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed efficiency rate (FER) and protein efficiency rate (PER) are shown in Table 2.

Table 2. Growth performance of rainbow trout fingerlings fed diets with soy products and fish oil substitution with a mixture of plant oils (soy and linseed, 1:1). Data are the means of triplicate groups ± SEM. Means with different superscripts in the same column differ significantly ($P<0.05$).

Diets	*Final weight (g)*	*WG (%)*[1]	*SGR (%/day)*[2]	*FI (g/day/fish)*	*FER*	*PER*
SPC-25	6.4 ± 0.03	821 ± 63	3.0 ± 0.1	0.14 ± 0.006[a]	0.63 ± 0.006[a]	1.49 ± 0.01
SPC-50	6.6 ± 0.23	907 ± 23	3.1 ± 0.03	0.15 ± 0.004[a]	0.61 ± 0.003[a]	1.53 ± 0.008
SPC-75	6.6 ± 0.17	932 ± 32	3.2 ± 0.04	0.15 ± 0.004[a]	0.63 ± 0.01[a]	1.50 ± 0.02
SPC-C	6.6 ± 0.26	897 ± 45	3.1 ± 0.07	0.14 ± 0.001[a]	0.63 ± 0.01[a]	1.48 ± 0.04
SBM-25	6.6 ± 0.22	781 ± 38	3.2 ± 0.06	0.18 ± 0.004[b]	0.48 ± 0.006[b]	1.33 ± 0.10
SBM-50	6.9 ± 0.12	872 ± 26	3.2 ± 0.03	0.19 ± 0.002[b]	0.50 ± 0.003[b]	1.20 ± 0.01
SBM-75	6.7 ± 0.17	813 ± 34	3.2 ± 0.05	0.17 ± 0.003[b]	0.50 ± 0.003[b]	1.19 ± 0.01
SBM-C	6.5 ± 0.07	734 ± 64	3.0 ± 0.1	0.19 ± 0.002[b]	0.49 ± 0.001[b]	1.34 ± 0.09

[1]Weight gain = ((Final weight–initial weight) /initial weight) x 100
[2]Specific growth rate = ((ln final weight – ln initial weight)/70) x 100
[3]Feed efficiency ratio = Weight gain (g) / total feed ingested (g on dry weight basis)
[4]Protein efficiency ratio = Weight gain (g) / total protein ingested (g on dry weight basis)

After 70 days of being fed the diets, no significant differences were observed in growth performance of the rainbow trout fingerlings. Higher values of WG and SGR were observed in fish fed the diets with SPC, regardless of the plant oil mixtures. Values of FI were significantly higher in the fingerlings fed the SBM diets than those fed the SPC diets, but higher values of FER and PER were observed in the latter group.

No significant differences were observed in survival among the groups, fish fed the SPC-50 diet showed a 96 ± 3 % and the SPC-75 group, 97 ± 2%. The survival for the other treatments was 100%.

The ADCs of the lipid fraction are show in Fig. 1a and significantly lower values were observed as the plant oil increased in the diets; however, all values were higher than 95%. Regarding the ADCs of the protein fraction (Fig. 1b), a significantly higher value was observed in the fish diet SPC-C than in the other groups. No significant differences were observed among the groups fed the diets with SBM.

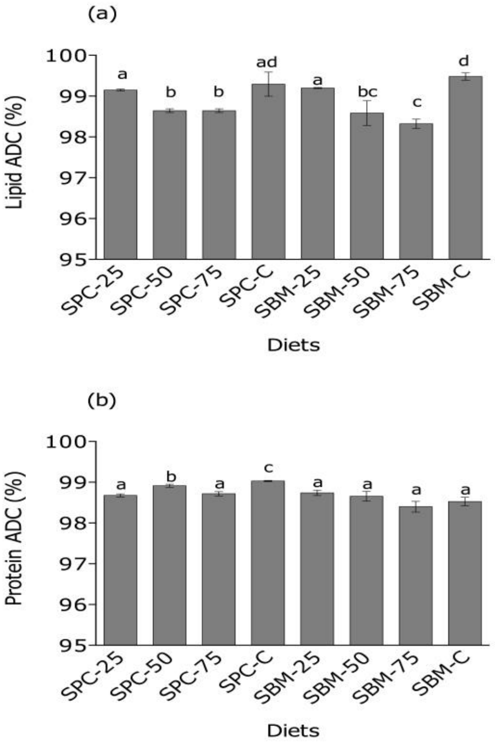

Fig. 1. Apparent digestibility coefficients (ADC) of lipid (a) and protein (b) of rainbow trout fingerlings fed diets with soy products and fish oil substitution with a mixture of plant oils (soy and linseed, 1:1). Bars represent the means of three replications ± SEM. Bars with different letters are significantly different ($P<0.05$).

Lower values were observed in oxygen consumption as the ratio of plant derivatives increased in the diets (Fig. 2a). The oxygen consumption of the fish fed diet SBM-75 was significantly lower than the rest of the groups. Fig. 2b shows the N excretion (expressed as mg of NH_3-N/l/g of fish) was significantly lower in the fish fed diets with SBM, than those fed on SPC diets. Among the latter, lower values were observed as the mixture of plant oils increased in the diets.

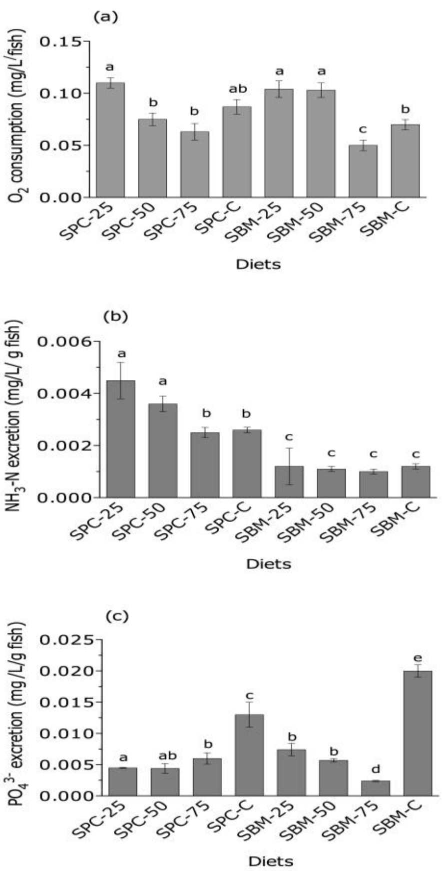

Fig. 2. Oxygen consumption (a), nitrogen (b) and phosphorus (c) excretion of rainbow trout fingerlings fed diets with soy products and fish oil substitution with a mixture of plant oils (soy and linseed, 1:1). Bars represent the means of three replications ± SEM. Bars with different letters are significantly different ($P<0.05$).

In general, lipid deposition in the liver was higher than in muscle (Fig. 3). The lipid content in muscle was not significantly different between the groups, while in the group fed diet SPC-75 the lipid content in the liver was significantly lower. Higher deposition was observed in the fish fed the diets with SBM.

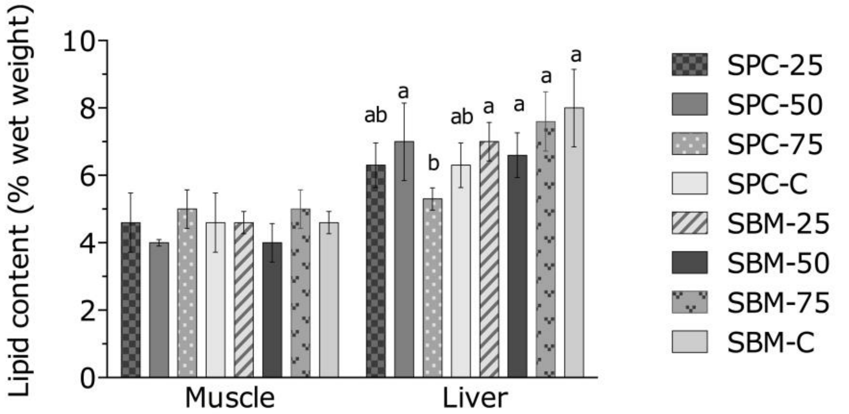

Fig. 3. Lipid contents in muscle and liver of rainbow trout fingerlings fed diets with soy products and fish oil substitution with a mixture of plant oils (soy and linseed, 1:1). Bars represent the means of three replicates ± SEM. Bars with different letters are significantly different ($P<0.05$).

Discussion

The supply of FM and FO is expected to become increasingly limited in the coming decades (Hardy 2010), and the rapidly growing aquaculture industry must find alternative ingredients for the future (Gatlin et al., 2007; Kaushik and Seiliez, 2010). Our study has examined the use of soybean products (meal and protein concentrate) as main sources of protein and the substitution of fish oil with a mixture of soy and linseed oil in rainbow trout fingerlings, showing the possibility of substituting up to 75% of FO without affecting the growth, lipid deposition in liver and muscle, and decreasing phosphorus excretion as well.

Several studies have shown that partial or total substitution of fish oil with plant-based oils did not affect the growth of salmonids, when FM was used as protein source (Dernekbaşi et al., 2011; Greene and Selivonchick, 1990; Richard et al., 2006; Pettersson et al., 2009). However, a decrease in the growth and feed intake has been observed when plant substitutions are used as the sole protein source in combination with plant oils (Panserat et al., 2009; Twibell et al., 2012). Use of small portions of FM and/or FO might aid diet consumption (Torstensen et al., 2008) and maintain palatability of diets (Pettersson et al., 2009). Inclusion of FM might maintain an adequate level of essential amino acids, such as methionine, lysine, or threonine, to supply the requirements of rainbow trout (Gatlin et al., 2007; NRC, 2011). After 70 days of feeding the fingerlings with SBM and SPC, and with the different substitutions of FO, no negative effects were observed in growth performance. The observed WG and SGR was higher than in trout fed with partial substitutions of the FM with soybean meal and total substitutions with soybean meal and *Spirulina* powder, respectively. (Cruz et al., 2011; Hernández et al., 2012) Growth performance was also higher than in rainbow trout fed a diet with total substitution of FM and FO with ingredients of plant origin (Panserat et al., 2009) and in juvenile Coho salmon fed a mixture of animal and plant proteins with plant oils (Twibell et al., 2012).

The SGRs in this experiment are higher that in rainbow trout fed diets with partial substitution of FM and FO with soybean meal and canola oil, respectively (Güroy et al. 2012). The oxygen consumption was lower than that reported by Cruz et al. (2011) and Hernández et al. (2012), and the tendency toward lower values of consumption as the plant oils increased in the diets, indicates that less energy is used in oxidation thereby resulting in better digestion of nutrients (Bureau et al., 2002). The ADCs of the lipid portion were over 98% for all diets confirming that soy and linseed oils are incorporated by rainbow trout fingerlings. The ADCs values were slightly higher than in rainbow trout fed diets with canola oil, (Güroy et al., 2012) suggesting that a mixture of oils might be more effective than a single source of oil.

No relationship was observed between fish oil substitution and N excretion which is generally associated with protein intake and digestibility (Bureau et al., 2002). The ADCs of protein were higher than 98%, and similar to those reported for rainbow trout fed soybean meal and soy protein concentrate (Cruz et al., 2011; Médale et al., 1998), while the N excretion values are lower than those previously reported with soy meal (Cruz et al., 2011; Hernández et al., 2012).

Phosphorus, particularly the dissolved form, released from farms, is one of the main concerns of the aquaculture industry (Dalsgaard et al., 2009), as it increases the nutrient levels in the surrounding water bodies and contributes to eutrophication (Bureau and Cho, 1999). P excretion from fish fed diets with substitution of FO showed lower values than those reported by Cruz et al. (2011) and Hernández et al. (2012). The use of the mixture with soy and linseed oils significantly decreased the P excretion which might indicate that P content in the diets with FO as the sole lipid source, is higher than the rainbow trout fingerlings requirements. The excreted P may come from the phospholipids contained in the FO (Richard et al., 2006).

Previous studies reported that the utilization of plant oils does not affect the lipid deposition on organs of rainbow trout (Richard et al., 2006). During this research it was observed that lipid deposits are higher in the liver, than in the muscle. Concentrations in both organs are similar to those previously reported in rainbow trout fed with soy or linseed oil (Greene and Selivonchick, 1990), rapeseed oil (Pettersson et al., 2009) or a mixture of rapeseed, palm and linseed oils (Richard et al., 2006). In our study it was not possible to establish the fatty acid profile of the liver and muscle, but it has been found that the use of plant oils might have an effect on the long chain poly-unsaturated fatty acids (Twibell et al., 2012; Overturf et al., 2013), as such oils are rich in mono-unsaturated fatty acids and might decrease the level of eicosapentaenoic acid (EPA) and docohexaenoic acid (DHA) (Bell et al., 2003).

The present research shows the possibility of using soy protein concentrate or soybean meal as the main protein sources in diets for rainbow trout fingerlings and the substitution level of up to 75% of the FO. We used a 1:1 mixture of soy and linseed oils as partial substitutes for the fish oil. It was observed that maintaining small portions of FM and FO have a positive effect on the growth of rainbow trout fingerlings. One of the most important aspects of the present research is the significant decrease of P excretion when a mixture of plant oils is used, and this may be very important for the future of aquaculture.

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