Replacement of dietary fish meal with plant sources in rainbow trout (*Oncorhynchus mykiss*); effect on growth performance, immune responses, blood indices and disease resistance

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Abstract

The aim of this study was to examine the effects of replacing fish meal with plant sources on growth performance, immune responses, hematological parameters and disease resistance in rainbow trout. In this study, mean of initial body weight of fish was 15±2 g and the experiment was carried out for a period of 60 days. Four experimental diets were formulated to replace 0, 40, 70 and 100% fish meal with plant protein sources (wheat gluten, corn gluten and soybean meal). According to results, higher plant protein inclusions (70 and 100%) resulted in undesirable effects on growth, nutritional indices, serum total immunoglobulin and alternative complement activity ($P < 0.05$). Otherwise, results suggested that it is possible to replace 40% of fish meal with plant counterparts without any noticeable negative effects on growth and humeral immune parameters (lysozyme activity and total antibody) ($P > 0.05$). Furthermore, replacement of fish meal with plant sources in all treatments had no significant effects on blood parameters (hematocrit, hemoglobin, white blood cells, hetrophil and lymphocytes count). Finally, no significant differences were observed in fish mortality after 15 days of challenges with *Yersinia ruckeri* among treatments ($P > 0.05$).

Keywords: Plant protein, Growth, Immune response, Blood indices, Disease resistance, Rainbow trout

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Introduction

Fish meal and fish oil are the main ingredients used in aqua feed. Due to the expansion of aquaculture, marine fisheries will not be able to meet the demand of aquaculture industry in the near future. Consequently, there has been considerable interest in introducing sustainable alternatives to fish meal and oil which will reduce the dependence on marine raw materials (Tacon, 2005). The new nutritional strategies, such as inclusion of terrestrial vegetable oils (VOs) and meals in fish feeds, are being imposed as a consequence of the limited availability of fish oil (FO) to cope with the increasing demand for these products (Tacon, 2008).

Many plant protein sources can be used to partially or almost totally replace dietary fish meal (Kaushik et al., 1995), provided that the essential amino acid requirements of the fish species are met, the palatability of the diet is improved and the levels of anti-nutritional factors (ANFs) are reduced (Francis et al., 2001). Recent feeding studies with concentrated plant protein on rainbow trout have indicated that these ingredients can replace all the dietary fish meal with either no reduction or just a slight reduction in growth (Kaushik et al., 1995; Gaylord et al., 2006). Whilst fish growth will usually be the main criteria in identifying alternative protein sources with potential for use in aquafeeds, the effect that these proteins have on the immune system and the disease resistance should also be investigated. Early research by Wedemeyer and Ross (1973) demonstrated that feeding rainbow trout with isonitrogenous and isoenergetic diets with different protein sources (maize gluten or cottonseed meal) had no effect on the susceptibility to infection by corynebacterial kidney disease. Later, Rumsey et al. (1995) found that rainbow trout fed with isonitrogenous diets, but differing in their protein source (fish meal or soybean meal) was subject to changes in their immune response. In that case, feeding rainbow trout with soya-bean increased non-specific defense mechanisms. However, growth was depressed in trout given the soya-bean diet. Krogdahl et al. (2000) reported significantly increased levels of both lysozyme and IgM in the mid and distal intestinal mucosa of Atlantic salmon fed soybean molasses. Similarly to Rumsey et al. (1995) immune parameters such as serum lysozyme activity and plasma total immunoglobulin level increased with the inclusion of soya-bean, whilst disease resistance varied depending on the soya-bean product incorporated. Based on the results obtained in earlier studies, we decided to partially replace fish meal with different dietary levels of gluten-based protein in order to examine the effect on growth and immune response in rainbow trout.

Materials and methods

Fish husbandry and diet preparation

Fish were purchased from a local trout farm and acclimated for 2 weeks during which they were fed commercial diet. Forty fish with average weight of 15±0.2 g were stocked in 21 polyethylene tanks (300 L) supplied with freshwater at a flow rate of 7.5 L min^{-1}. Light/dark cycle was

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12 L:12 D. Water quality parameters were monitored daily for each tank and pH, temperature and dissolved oxygen were maintained at 7.3-7.7, 14-15°C and 6.8-7.5 mg L^{-1}, respectively.

Four experimental diets with similar protein, lipid and energy content were formulated to replace 40, 70 and 100% of fish meal with plant protein. Kilka (*Clupeonella* sp.) meal (Kilkapodre-Sahar, Co., Iran) was the primary sources of fish meal in the control and experimental diets. The experimental plant protein source were wheat gluten, corn gluten and soybean meal. Table 1 shows the formulation and proximate composition of the experimental diets. Table 2 shows the fatty acid profile of the experimental diets.

Table 1: Ingredients and proximate composition of experimental diets.

Ingredients (g.kg^{-1})	Dietary treatments			
	control	40%	70%	100%
Kilka fish meal	582.5	350.0	182.5	-
Wheat gluten	-	155	260	420
Corn gluten	-	55	110	100
Soybean meal	-	150	150	150
Kilka fish oil	128.9	140.6	161.3	185.7
Blood meal	40	40	40	40
Wheat meal	145	-	-	-
Wheat starch	52.5	49.4	8.0	-
Filler	-	-	28.2	37.3
Zeolite	5	5	5	5
Vitamin premix[1]	15	15	15	15
Mineral premix[2]	10	10	10	10
L-methionine	12	12	12	12
L-lysine	0	8	8	15
Di-calcium phosphate	5	5	5	5
Calcium carbonate	5	5	5	5
Proximate composition (% dry matter)				
Moisture	8.1	7.6	8.2	8.1
Crude protein	45.3	44.5	45.1	45.5
Crude lipid	19.9	19.8	20.1	19.8
Crude starch	14.9	15.0	14.9	15.4
Gross energy (kcal/g)[3]	5.04	5.03	5.05	5.04

[1]Vitamin mixture: (mg or IU/kg of diet) Vitamin A (as acetate) 1600000 IU; vitamin D3, 400000 IU; choline chloride.12000; niacin, 4000; riboflavin, 8000; pyridoxine, 4000; folic acid, 2000; vitamin B12, 8000; biotin, 1; inositol, 20000; vitamin C, 60000; vitamin H2, 2.4; vitamin B2, 8000; vitamin K3, 2000; vitamin E,40000.

[2]Mineral mixture (g/kg): zinc, 12.5 g; iron, 26 g; manganese, 15.8 g; copper, 4.2 g; cobalt, 0.48 g; selenium, 2 g; iodine, 1 g.

[3]Calculated on the basis of 5.64, 9.43, and 4.11 (kcal/g diet) of protein, fat, and carbohydrate, respectively (NRC 1993).

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Table 2: Fatty acid profile of the experimental diets.

Fatty acid (%)	Dietary treatments [2]			
	control	40%	70%	100%
C14:0	2.07	1.51	2.01	1.74
C16:0	19.68	16.04	17.10	17.96
C18:0	7.25	6.85	10.39	9.13
Total Saturates	30.07	25.22	30.33	29.56
C16:1n7	3.93	3.11	4.36	3.79
C18:1n9	22.09	24.45	26.2	26.40
C18:1n7	3.90	3.89	3.94	3.20
Total Monoenes[3]	33.35	32.41	36.45	35.10
C18:2n6 cis	9.13	10.75	11.89	15.74
C20:4n6	0.73	0.67	0.60	0.51
Total n-6 PUFA	10.35	11.83	12.97	16.68
C18:3n3	2.77	2.51	2.89	3.13
C20:5n3	5.42	5.10	4.62	4.13
C22:6n3	11.18	10.18	9.90	9.17
Total n-3 PUFA	19.58	17.88	17.59	16.56
HUFA N-3	16.81	15.37	14.69	13.43

[1]Values are % of total fatty acid expressed as mean.

[2]See Table 1 for diet abbreviations.

[3]Monoene isomers were pooled.

Briefly, all dry ingredients were thoroughly mixed in a mixer. Oil was added and thoroughly mixed for 5 min and then moistened by adding cold distilled water until stiff dough yielded. The wet dough was grinded and converted to strands (3 mm in diameter) using a meat grinder. The strands were dried at 50°C for 8 h using an oven. Afterwards, they were manually crumbled into appropriate size and sieved. Pellets were stored at 4°C during the experiment. Fish were fed three times per day at 3% body weight for 8 weeks.

Growth parameters

At the start and the end of culture period, 12 fish/tank randomly were sampled for biometry (weight and total length). The same time nine fish per tank were collected to weigh the liver and digestive tract for hepato-somatic index (HSI) and viscera-somatic index (VSI) calculations. The following parameters were calculated:

Specific growth rate (SGR, % d^{-1})=$100\times[(\ln W_f - \ln W_i)\times T^{-1}]$; Daily growth rate (DGR, g d^{-1})=$(W_f - W_i)\times T^{-1}$; Condition factor (CF)=$100\times(W\times TL^{-3})$; days reared; Feed conversion ratio (FCR)=$TFI\times(FB-IB)^{-1}$; Hepato-somatic index (HSI, %)=$100\times(LW\times W^{-1})$; Viscera-somatic index (VSI, %)=$100\times(VW\times W^{-1})$.

Where: W_f and W_i are the final and initial body weights (g), T—time (days), FB and IB are the final and initial absolute weights (g), TFI—total feed intake (g), LW—liver weight (g), VW—viscera weight (g).

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Proximate composition of diets

Chemical analysis of the test diets was determined by drying in oven at 105 °C for 25 h to a constant weight; ash was determined by incineration in a muffle furnace at 600 °C for 6 h; crude protein was determined by the Kjeldahl method (N × 6.25) using an automatic Kjeldahl system (Behrotest WD 40, Germany); Crude lipid content determination was conducted by ether extraction and carbohydrate were calculated by the following formula:

Carbohydrate (g kg^{-1}) =1000− (weight in grams [protein+lipid+moisture+ash]).

All methods are based on those described in the Association of Official Analytical Chemists, (AOAC, 1990) and modified as described in Aksnes et al. (2006).

Gross energy content of the diets and feces was calculated on the basis of 5.64, 9.43, and 4.11 (kcal/g diet) of protein, fat, and carbohydrate, respectively (NRC, 1993). Fatty acid composition was analyzed by gas chromatography (Agilent 7890A GC System, USA) using a BP×70 capillary glass column (0.32mm×50 m, SGE Analytical Science Australia) after esterification in acetyl-chloride/methanol mixture. Fatty acid methyl esters were prepared via a modified procedure of Lepage and Roy (1984).

Immunological analysis

Five fish from each tank were randomly sampled on day 1 of the experiment, prior to the first feeding. A second sampling took place on day 60 of the experimental rearing. Blood was collected by vein puncture using syringes coated with heparin and transferred immediately into sterile tubes and allowed to clot at room temperature for 1 h. Then the blood samples were incubated at 4°C for 5 h. The sera were separated by centrifugation (1500×g for 5 min at 4°C) and stored at -80°C until use.

Serum lysozyme activity

Lysozyme activity in serum was determined according to the method of Demers and Bayne (1997) based on the lysis of the lysozyme sensitive gram positive bacterium, *Micrococcus lysodiekticus* (Sigma). The dilutions of hen egg white lysozyme (Sigma) ranging from 0 to 20 mg ml^{-1} (in 0.1Mphosphateecitrate buffer, pH 5.8) were considered as the standard. This along with the undiluted serum sample (25 ml) was placed into a 96-well plate in triplicate.175 ml of *M. lysodiekticus* suspension (75 mg ml^{-1}) prepared in the same buffer was then added to each well. After rapid mixing, the change in turbidity was measured every 30 s for 5 min at 450 nm and at approximately 20 °C using a microplate reader. The equivalent unit of activity of the samples as compared to the standard were determined and expressed as μg ml^{-1} serum.

Serum alternative complement activity

Alternative complement activity was assessed based on the hemolysis of rabbit red blood cells (RaRBC) as described by Waley and North (1996); Boesen et al. (1999) and Amar et al. (2000). The RaRBC were washed three times in

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ethylene glycol tetra acetic acid-magnesium-gelatin veronal buffer (0.01 M EGTA-Mg-GVB, pH 7) and the cell numbers were adjusted to 2×10^8 cells ml^{-1} in the same buffer. At first, the 100% lysis value was obtained by adding 100 ml of the above RaRBC to 3.4 ml distilled water. The hemolysate was centrifuged and the optical density (O.D.) of the supernatant was determined at 414 nm using a spectrophotometer (Awareness, USA). Following, the test sera were diluted (100 times), and different volumes ranging from 100 to 250 ml (total volume was adjusted to 250 ml with the buffer) was allowed to react with 100 ml of RaRBC in small test tubes. These mixtures were incubated at 20°C for 90 min with intermittent mixing, and then 3.15 ml of 0.85% NaCl solution was added and the tubes were centrifuged at 1600×g for 10 min at 4°C. The O.D. of the supernatant was measured at 414 nm. A lysis curve was obtained by plotting the percentage of haemolysis against the volume of serum added on a log-log graph. The volume yielding 50% haemolysis was used for determining the complement activity of the sample as follow:

ACH50 (Units ml^{-1}) = K × (reciprocal of the serum dilution) × 0.5

Where K is the amount of serum (ml) giving 50% lysis and 0.5 is the correction factor since the assay was performed on half scale of the original method.

Plasma total antibody level

Plasma total immunoglobulin was assayed following the method of Siwicki et al. (1994). The serum samples were diluted with 0.85% NaCl (100 times) and total protein content was determined by Bradford method (Kruger, 1996). One hundred ml of total serum samples were mixed with an equal volume of 12% solution of polyethylene glycol (Sigma) in wells of a 96-well micro titer plate. Following 2 h of incubation at room temperature, plate was centrifuged at 5000×g at 4°C. The supernatant was diluted 50 times with 0.85% of NaCl and the protein content was determined by Bradford method (Kruger, 1996). This value was subtracted from the total protein level and the result was equal to the total immunoglobulin concentration of the serum that was expressed as mg/ml.

Hematological parameters

Nine fish from each group were anaesthetized with clove powder (200 mg l^{-1}) and blood was collected by caudal vein puncture in heparinised syringes. Hematocrit values (Ht) were determined by placing fresh blood in glass capillary tubes and centrifuging for 5 min in a microhematocrit centrifuge. Hemoglobin (Hb) level was determined colorimetrically by measuring the formation of cyanomethemoglobin using a commercial kit. The white blood cells (WBCs) were counted under a light microscope using a Neubauer hemocytomter after dilution with phosphate-buffered-saline. Differential leukocyte counts were determined using blood smears under a light microscope. Cells were identified on the basis of morphology and cell structure as documented in previous fish leucocytes studies (Jalali et al., 2009).

Bacterial challenge

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After a period of 60 days of feeding the fish with the experimental diets, 30 fish from each dietary treatment (10 fish per each tank) were collected and anaesthetized with clove powder (200 mg lit^{-1}). Fish were then challenged by I.P. injection with 0.1 ml of a suspension of *Y. ruckeri* ($BCCM^5$/LMG3279) (1×10^7 cells ml^{-1}). The dead and moribund fishes were removed and examined microbiologically for up to 14 days post infection. Moreover, agglutination test was performed on samples as confirmation test (Tukmechi et al., 2011).

Statistical analysis

All the measurements were made in triplicate. The normalized and homogenized data were subjected to analysis of variance (ANOVA). Correlation coefficients were significant with $P < 0.05$.

Results

The result of growth indices are shown in Table 3. No significant differences were detected in replacement of fish meal with 40% plant protein in compared to control group. However replacement of fish meal with 70% and 100% plant protein resulted in decreased WG, SGR, and DGR and increased FCR. Hepato-somatic index (HSI) and viscera-somatic index (VSI) of control fish did not significantly differ from fish in other experimental treatments. Condition factor (CF) was significantly lower in fish fed diet with complete replacement of fish meal with plant sources.

Table 3: Growth indices of rainbow trout fed with experimental diets for 60 days (n=9 fish/tank)

Performance parameters	Dietary treatments [2]			
	control	40%	70%	100%
Initial body weight (g)	15.6±0.2[a]	15.5±0.3[a]	15.1±0.1[a]	15.5±0.1[a]
Final body weight (g)	71.1±2[a]	69.0±2[a]	56.9±1[b]	47.9±3[c]
Weight Gain (g/fish)	55.4±2[a]	53.5±1[a]	41.7±1[b]	32.4±3[c]
FCR	0.97±0.07[c]	1.04±0.03[c]	1.17±0.03[b]	1.33±0.04[a]
SGR	1.13±0.02[a]	1.11±0.01[a]	0.99±0.01[b]	0.84±0.05[c]
HIS	1.49±0.05[a]	1.42±0.05[a]	1.29±0.11[a]	1.46±0.08[a]
VSI	14.0±0.3[a]	14.2±0.5[a]	14.5±0.6[a]	14.4±0.2[a]
CF	1.14±0.01[a]	1.14±0.05[a]	1.18±0.04[a]	1.06±0.5[b]

[1]Values are means ± SD Values with the same superscripts within the same row are not significantly different.

[2]See Table 1 for the abbreviations of dietary treatments.

The serum lysozyme activity in groups fed on diets with 40, 70 and 100% fish meal replaced by plant sources (42.5±4, 48.66±1 and 47.36±4 μg/ml, respectively) had no significant differences compared to the control group (46.39±1.8 μg/ml) (Fig. 1).

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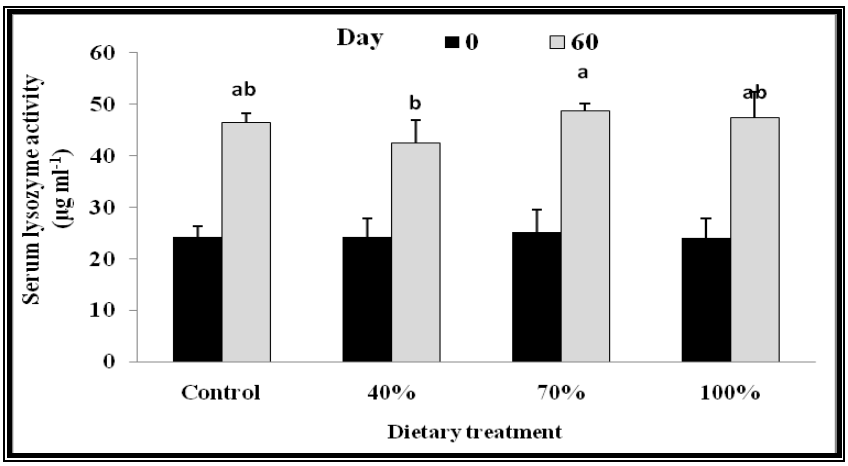

Figure 1: The lysozyme activity of rainbow trout fed with experimental diets for 60 days. Each value (mean ± SD) is the average performance of 15 fish/treatment. Values with the same superscripts within the same column are not significantly different. [1]See Table 1 for the abbreviations of dietary treatments.

Alternative complement activity in %40 (31 ± 4.8 U/ml) and 70% (28.7± 4.1 U/ml) and 100% (27.17 ± 3.8 U/ml) groups were significantly (P < 0.05) lower than in control (34.11 ± 1.3 U/ml) (Fig. 2).

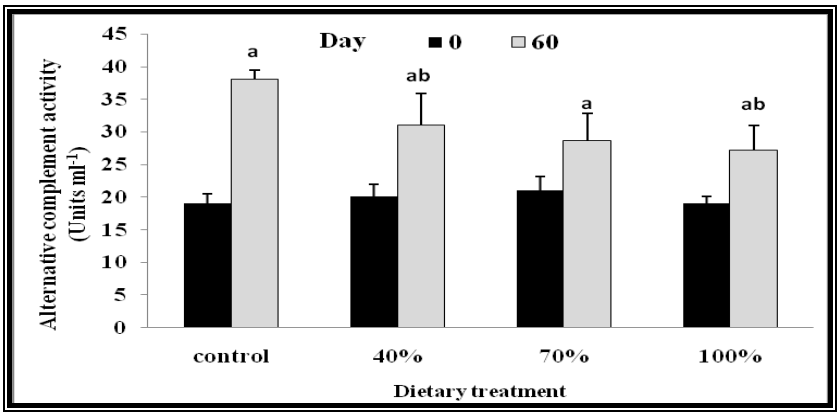

Figure 2: The alternative complement activity of rainbow trout fed with experimental diets for 60 days. Each value (X ± S.D.) is the average performance of 15 fish/treatment. Values with the same superscripts within the same column are not significantly different (P < 0.05).
[1]See Table 1 for the abbreviations of dietary treatments.

The serum total antibody in 70% (5.8±0.8 µg/ml) and 100% (5.7±1.0 µg/ml) groups were significantly (P < 0.05) lower than in control (8.05±1.8 µg/ml). But the serum total antibody in group fed on diet with 40% (6.32±0.3 µg/ml) fish meal replaced by plant sources had no significant differences compared to the control group (Fig. 3).

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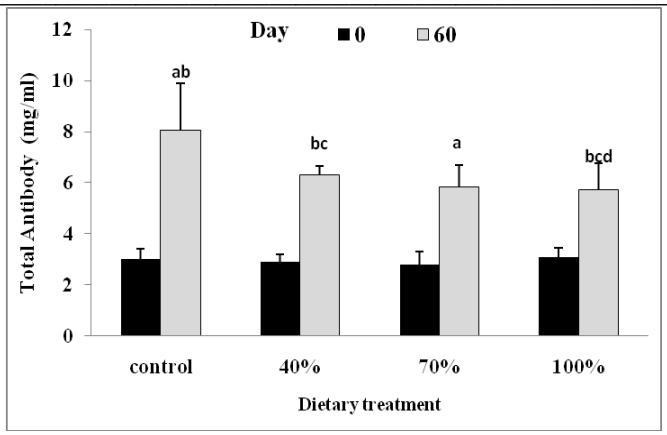

Figure 3: The serum total antibody of rainbow trout fed with experimental diets for 60 days. Each value (X ± SD) is the average performance of 15 fish/treatment. Values with the same superscripts within the same column are not significantly different (P < 0.05). [1]See Table 1 for the abbreviations of dietary treatments.

Hematological parameters in different treatments are shown in Table 4. After the 60 days of feeding with experimental diets, the hematocrit, hemoglobin, white blood cells, heterophils and lymphocytes count of control fish did not significantly differ from fish in other experimental treatments.

Table 4: Hematological parameters and cumulative mortality (%) observed in rainbow trout fed experimental diets for 60 days and then challenged with *Y. ruckeri*. (n=9 fish/tank).[1]

	Dietary treatments [2]			
	Control	40%	70%	100%
Hematocrit (%)	31.33 ± 3.8	32.50 ± 3.9	29.66 ± 2.1	28.50 ± 3.0
Hemoglobin (g/dL)	10.5 ± 1.90	10.3 ± 1.70	9.3 ± 1.96	8.5 ± 1.50
White blood cell count ($\times 10^4$ mm^{-3})	4.92 ± 0.4^{ab}	4.80 ± 0.6^{ab}	4.15 ± 0.5^{b}	4.39 ± 0.3^{b}
Heterophils	70.8 ± 13.7	77.6 ± 6.1	80.8 ± 5.2	75.7 ± 3.4
Lymphocytes (%)	30.8 ± 5.4	21.0 ± 5.1	19.1 ± 5.2	24.3 ± 3.4
Mortality (%)	66.6 ± 5.0^{abc}	63.3 ± 6.5^{bc}	73.9 ± 4.5^{abc}	76.6 ± 5.1^{ab}

[1]Values are means ± SD Values with the same superscripts within the same row are not significantly different (P<0.05). [2]See Table 1 for the abbreviations of dietary treatments.

No significant differences were observed in fish mortality after 15 days of challenges with *Yersinia ruckeri* among treatments (Fig. 4).

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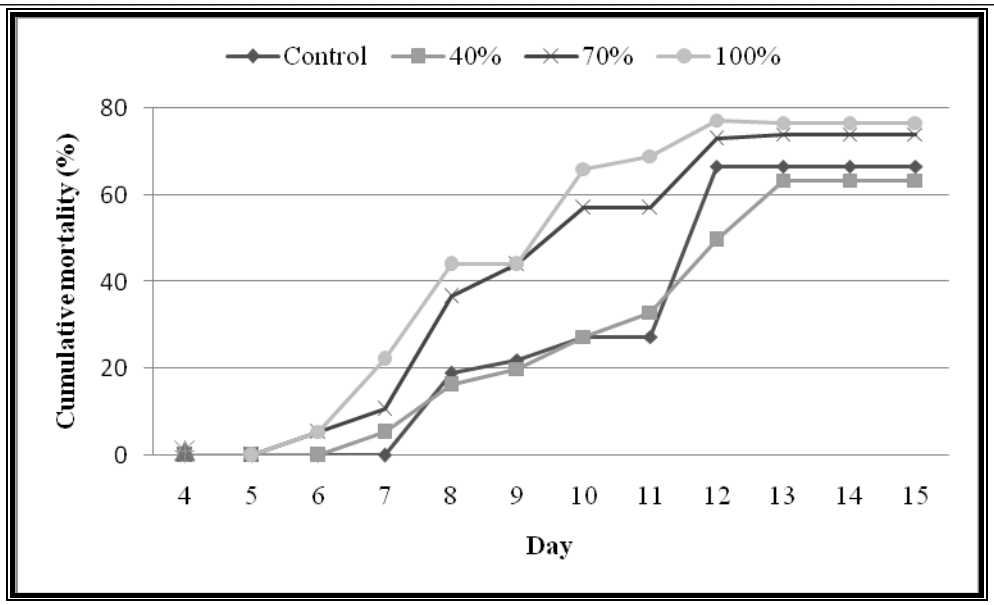

Figure 4: Cumulative mortality (%) observed in rainbow trout fed with experimental diets for 60 days and then challenged with *Y. ruckeri*. No significant differences was seen between the experimental groups (P>0.05).

Discussion

The results obtained in this study indicates that replacement of fish oil and 40% fish meal with plant sources does not have significant effect on fish growth. This result is in agreement with the works done by Bell et al., 2002; Torstensen et al., 2004 in Atlantic salmon (*Salmo salar*). Results showed that substituting 40% of fish meal with wheat gluten based plant ingredients did not adversely affect on fish growth indices, which were in accordance with replacing 30 and 35% of fish meal with wheat gluten in Atlantic salmon and Atlantic halibut, respectively (Storebakken et al., 2000; Helland and Grisdale-helland, 2006) and also were in compliance with substitution of 50% of fish meal with corn gluten in Atlantic salmon (Mente et al., 2003). The growth indices were gradually decreased by concomitant increment of dietary plant protein inclusion from 40% to 70% and 100%. It is proposed that inclusion of higher levels of plant ingredients in salmonids diets has adverse effect on fish performance (Francesco et al., 2004; Gaylord et al., 2006; Palmegiano et al., 2006; Drew et al., 2007).

Plant protein meals contain ANFs; these cause decrease in growth performance and feed efficiency (Olvera-Novoa et al., 2002), and affect digestive enzyme activity and digestion/absorption capacity of animal (Alarcon et al., 1999). Lin et al. (2010) reported the activities of protease in both intestine and hepatopancreas in Juvenile Tilapia were significantly decreased by replacement of fish meal with different plant proteins. Furthermore, the effects of different levels of dietary fish meal replacement with plant protein on digestive enzymes activities of rainbow trout were studied by Santigosa et al. (2008), where they showed that total protease activity of fish fed diet containing fish meal as the only protein source

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reached its highest value three hours after feeding, while the enzymatic activities in fish fed diet containing 50 and 70% plant protein increased very slowly. Group with total fish meal replacement did not reach the highest value of those fed diet with fish meal. They reported decreased digestive enzymes activities can to some extent interpret the lower fish growth rates observed in groups fed diet with 75 and 100% fish meal replacement.

Fish fed different diets did not show any significant differences in hepatosomatic index which is in compliance with some existing literature on rainbow trout (Palmegiano et al., 2006; Drew et al., 2007). Similarly there were no significant differences in VSI amongst fish of different dietary groups, which may be attributed to the shorter experimental period in this study. In contrast to our findings, 24 weeks and 96 days experiments on inclusion of plant protein in rainbow diet (Francesco et al., 2004; Palmegiano et al., 2006, respectively) resulted in significantly higher VSI.

The lysozyme activity, serum total immunoglobulin and alternative complement activity in groups fed with diet with 40, 70 and 100% fish meal replaced by plant sources had no significant difference with control group. This result is in agreement with Bransden et al. (2001) studies which have shown that complete replacement of fish meal with plant sources in Atlantic salmon (*Salmo salar*) diet had no significant effect on lysozyme activity and serum total immunoglobulin. Whilst studies on the use of soybean meal (because of its antigenic properties) in diets for salmonids and its subsequent effects on immune function have been widely undertaken (Rumsey et al., 1995; Krogdahl et al., 2000), few have worked on other proteins. Sitjà-Bobadilla et al. (2005) reported lysozyme activity in high level replaced fish meal by plant sources had no significant difference with FM group. These results are in agreement with our finding. The serum total antibody and alternative complement activity in 70% and 100% groups were significantly lower than in control group. This result is in agreement with Sitjà-Bobadilla et al. (2005) in Gilthead sea bream (*Sparus aurata*).

Replacement of fish meal with plant sources did not alter the number of hematocrit, hemoglobin, white blood cells, heterophils, lymphocytes count and mortality after 15 days of challenge with *Yersinia ruckeri* between experimental treatments. Bransden et al. (2001) demonstrated that Atlantic salmon parr supplied feeds with fish meal as the major protein source and subsequently challenged with *Vibrio anguillarum*, had no significantly mortality rates and some blood chemistry (neutrophil, glucose and total protein) compared to salmon supplied feeds containing plant protein source.

To conclude, the results of the present study showed that it is possible to replace 40% of fish meal with plant counterparts without any noticeable negative effects on growth and humoral immune parameters (lysozyme activity and total antibody) indices. However, higher plant protein inclusion resulted in undesirable effects on growth and serum total immunoglobulin and alternative complement activity. Replacement of fish

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meal with plant sources had no significant effect on blood parameters and fish mortality after 15 days of challenge with *Yersinia ruckeri*.

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