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Investigation of growth performance, carcass indices, fillet chemical composition and non-specific immune parameters of Rainbow trout *(Oncorhynchus mykiss)* fed diets containing poultry by-product meal supplemented with canola meal and cottonseed meal

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Abstract
In the present study four isonitrogenous (40% crude protein) and isocaloric (3600 kcal/kg digestible energy) diets were formulate. Control diet was based on fish meal (FM), diet 2 (PFM) contained 25/34% poultry by-product meal and 24/66% fish meal, diet 3 (CPFM) contained 16/14% fish meal, 25/34% poultry by-product meal and 10% cottonseed meal and diet 4 (CCP) was free from fish meal and had 40/24% poultry by-product meal, 10% cottonseed meal and 7/8% canola meal to meet the nutritional requirements of rainbow trout weighing 150±9 grams for a period of three months in order to determine their effects on growth performance, carcass indices, fillet chemical composition and non-specific immune response of rainbow trout. The results of this experiment showed that weight gain, feed intake and body condition score were not affected by the proposed diet formulation, but a significant improvement was observed in feed conversion ratio and specific growth rate ($P<0.05$). Furthermore, it was indicated that lysozyme level and immunoglobulin had no change, but anti-trypsin activity associated with alpha-anti-protease of the fish fed (CCP) was significantly lower ($P<0.05$). Total antiprotease activity of the fish fed (CPFM) and (CCP) was also lower compared with the control diet ($p<0.05$). We can conclude that with increasing the plant protein levels, a significant reduction in serum total protein in all treatments compared to the control group was observed ($P<0.05$). Poultry by-product meal supplemented with cottonseed meal and canola meal used in this study, which has been replaced fish meal up to 100%, resulted in a reduction of non-specific immune parameters, but had no adverse effect on growth performance, carcass indexes and carcass chemical composition.

Keywords: Fish meal, Rainbow trout, Canola meal, Cottonseed meal, Anti-protease, Lysozyme.

1. Introduction
Fish meal is the most efficient and expensive component in formulating food by aquatic feed industries, especially for carnivorous fish. Because of having adequate amount of calcium and phosphorus, as well as high digestibility and good amino acid and fatty acid profiles, high doses of fish meal are consumed by aquatic feed industries [2]. Poultry by-product meal, on the other hand, is one of the supplementary animal protein sources with a good balance of essential amino acids and contains high amounts of calcium and phosphorus, but compared to fish meal, due to the lack of lysine and methionine, addition of synthetic supplements of these amino acids to have optimum growth performance in rainbow trout seems reasonable [23]. Several studies have been conducted regarding the effect of using poultry by-product meal alone or along with plant protein sources as supplements for fish meal on growth performance of rainbow trout. In a study, Sevgili and Erturk (2004) [19] illustrated that using poultry by-product meal up to 20% had no adverse effects on rainbow trout growth performance. The results of the study conducted by Lee *et al*. (2002) [12] were indicative of the fact that poultry by-product meal along with plant protein supplements can only partially replace fish meal in a diet.
Nowadays, due to economical problems of preparing food from animal protein sources, plant protein sources which are usually by-products of oilseed meal are used. Canola meal *(Brassica campestris or Brassica napus)* has a better balance of amino acids than other plant

protein sources. It is also a good source of sulfur amino acids such as methionine, cystine and linolenic acid. Studies on feed conversion ratio, growth rate, feed intake and protein efficiency of experimental fish fed different levels of canola meal replaced fish meal showed no significant difference among groups and with the control group [8].

Thiessen (2004) [24] illustrated that production of canola protein concentrate and purified canola protein free from Erucic acid can lead to an increase in growth rate, feed efficiency and protein consumption in rainbow trout. Magdy *et al*. (2008) [15] in their study on Nile Tilapia investigated that replacing fish meal with plant protein sources and suggested that canola meal along with other plant protein sources can replace fish meal up to 30/5-45% without any adverse effect on feed intake, feed conversion ratio and protein efficiency. According to Yigit *et al*. (2011) [29], the best level for canola meal in rainbow trout fry diets is 8%. According to Lee *et al*. (2010) [11], in case of using dicalcium phosphate as a supplement in rainbow trout diets, animal protein sources supplemented with plant protein sources can be replaced fish meal up to 100%.

Cottonseed meal as another plant protein source used in feeding farmed fish, has less protein and more fiber content. This high level of fiber leads to a reduction in the metabolizable energy level. Cottonseed meal contains 0.4-1.7% gossypol whose role is reducing the efficiency of using dietary protein and dietary iron. Magdy *et al* (2012) [13] observed that replacing cottonseed meal up to 40% in Nile tilapia diet is possible if 580mg/kg iron is supplemented in the diet. As pointed by Bilgüven and Barış (2011) [3], cottonseed meal can be used in older trout feeds since the older the trout becomes, the more it can use cottonseed meal and its gossypol tolerance level increases. Blom *et al*. (2014) [4] indicated that replacing dietary fish meal protein with cottonseed meal protein has no adverse effect on survival and female fish growth. These researchers also showed that cottonseed gossypol in the diet were transferred to the eggs as well.

Several studies have also been conducted regarding the effect of replacing fish meal with animal and plant protein sources on immune response of rainbow trout, whereas Lee *et al*. (2002) [12] illustrated that the hematocrit value of the fish fed diets containing cottonseed meal or animal protein sources reduced significantly compared with the fish fed diets containing fish meal.

As discussed by Bransden *et al*. (2001) [5], replacing fish meal with plant protein sources does not have a severe effect on the production of Neutrophil oxygen radical. In shrimp, *Macrobrachium nipponense,* using animal protein sources like poultry by-product meal as an alternative for fish meal does not result in any significant difference in the production of superoxide anion by hematocytes [28].

Significant reduction of plasma protein level was also found when replacing fish meal with some plant protein sources [21] and [10].

In another study, Bransden *et al*. (2001) [5] observed that Lysozyme, plasma total protein and total immunoglobulin did not change following the replacement of fish meal with some animal protein sources in Atlantic salmon.

The main aim of the present study is to investigate the effect of using poultry by-product meal along with canola meal and cottonseed meal as protein sources replaced fish meal on growth performance, carcass quality and chemical composition and non-specific immune parameters of rainbow trout (*Oncorhynchus mykiss*).

2. Methods and Materials

2.1 Fish and experimental diets

In the present study, a total number of rainbow trout with average weight of 150 ±9 grams were used (60 in each pond). The Fish were kept in 16 octagon concrete ponds (1×1×1m) with inlet water of 1.5-2 lit sec^{-1}. The water temperature was 14.5 ^{0}C with the pH level of 7.3. The dissolved oxygen of water was 7.5 mg/L. Feeding was three times per day according to NRC (1999) [15] based on 2.5% of the body weight.

2.2 Chemical analysis of the experimental diets and carcass composition

The crude protein of the diets and the crude protein of carcass were determined by Kjeldalh (kjeltec Analyzer unit 2300 Foss Model), fat by solvent extraction, ash by placing the samples in a muffle furnace (550 °C) for 12 h, fiber by placing the samples remaining in a muffle furnace (600 °C) for 6 h after acid and alkali hydrolysis and moisture by drying (105 °C) until constant weight has been attained. Nitrogen free extract was calculated by subtracting the values of protein, fat, fiber and ash from the dry matter.

The amino acid profile of the experimental diets was determined using high-performance liquid chromatography (HPLC, Knauer/smatline Model, Germany). All methods are based on those described in the Association of Official Analytical Chemists, (AOAC, 1990) [1] and its modification.

In the present study four isonitrogenous (40% crude protein) and isocaloric (3600 kcal/kg digestible energy) diets were formulated considering the nutrient requirements for cold water fish (NRC,1999) [16] (Tables 1 & 2). Control diet was based on fish meal (FM) containing 50% fish meal as the major source of dietary protein supply, diet 2 (PFM) contained 25/34% poultry by-product meal and 24/66% fish meal, diet 3 (CPFM) contained 16/14% fish meal, 25/34% poultry by-product meal and 10% cottonseed meal and diet 4 (CCP) was free from fish meal and had 40/24% poultry by-product meal, 10% cottonseed meal and 7/8% canola. The pellet sizes were 3-3.5 mm.

2.3 Growth performance and carcass traits

Average weight gain, specific growth rate, body condition factor and feed conversion ratio as the most important factors of growth performance along with Hepatic Somatic Index (HIS), Viscera Somatic Index (VSI) and Carcass Efficiency (CE) were measured in the current study [9].

Weight gain (g) = final weight – initial weight

$$\text{Specific growth rate} = \frac{(\text{Ln final weight} - \text{Ln initial weight}) \times 100}{\text{Experimental period (days)}}$$

$$\text{Condition factor} = \text{weight} / (\text{length})^3 \times 100$$

Feed conversion ratio = food consumption (g) / weight gain (g)

Carcass weight (g) = fish carcass weight – fins, head and viscera's weight

Carcass efficiency = (carcass weight without fins, head and viscera / carcass weight) × 100

Viscera somatic index = (viscera weight / carcass weight) × 100

Hepatic somatic index = (liver weight / carcass weight) × 100

Table 1: Composition of experimental fish diets

Experimental diets				
Ingredients	**FM (Control)**	**PFM**	**CPFM**	**CCP**
Fish Meal	50	24.66	16.14	0
Poultry by product Meal	0	25.34	25.34	40.24
Cottonseed Meal	0	0	10	10
Canola Meal	0	0	0	7.80
Corn	12.48	12	9.62	5.50
Wheat	10	8	7	4.5
Soybean Meal	17.27	19	19.65	19
Soybean Oil	7	7.75	9	9.71
Mineral Premix[1]	0.5	0.5	0.5	0.5
Vitamin Premix[2]	0.5	0.5	0.5	0.5
D-Methionine	0.5	0.5	0.5	0.5
L- Lysine	0.5	0.5	0.5	0.5
Anti Oxidant [3]	0.1	0.1	0.1	0.1
Colin Chloride	0.15	0.15	0.15	0.15
Binder [4]	1	1	1	1
Arginine (%)	2.58	2.51	2.4	2.38
Histidine (%)	1.02	1.02	1	1
Lysine (%)	3.2	3.1	3.02	3.02
Leucine (%)	3.08	3	3	3
Isoleucine (%)	1.87	1.87	1.86	1.86
Cysteine and Methionine (%)	1.95	1.92	1.93	1.9
Phenylalanine (%)	1.74	1.7	1.6	1.61
Tryptophan (%)	0.93	0.91	0.92	0.9
Valine (%)	2.03	2.01	2.02	2

Mineral Premix (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.
[2] Vitamin Premix: (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.
[3] Butil Hydroxi Anisol
[4] Lignosulfate

Table 2: Nutritional composition of experimental diets

		Experimental diets		
Ingredients	**FM (control)**	**PFM**	**CPFM**	**CCP**
Dry Matter (%)	90.96	90.98	90.98	91.52
Crude Protein**(%)**	40.3	40.2	40	40
Crude Fat (%)	16.54	16.98	17.73	20.6
Crude Fiber (%)	2.3	2.98	3.48	3.85
Nitrogen Free extract (%)	24.82	23.52	21.27	17.77
Crude Ash (%)	7	7.3	8.5	9.3
Digestible Energy (Kcal/Kg)	3650	3650	3650	3650

2.4 Blood sampling
At the end of the experiment, 7 fish per tank were euthanized by using MS-222 (methanesulfonate) and bled from the caudal vein. Blood samples were transferred into Eppendorf tubes and allowed to clot at room temperature for 1 h. Then samples were kept at 4 ºC for 5 h. The sera were separated by centrifugation (1500 × g for 5 min at 4 ^{0}C) and stored at -80 ^{C} until required for analysis of non-specific immune parameters.

2.5 Non-specific immune parameters
In order to evaluate non-specific immune parameters, the method described by Cuesta *et al*. (2005) [7] was used. 135 µl of HBSS (Hanks Balanced Salt Solution) without Ca^{+2} or Mg^{+2} were added to 15 µl of serum sample in each well plate. Finally 50 µl of 20 mM 3,30,5,50-tetramethylbenzidine hydrochloride (TMB) (Sigma) and 5 mM H_2O_2 was added. The color-change reaction was stopped after 2 min by adding 50 µl of 2 M sulfuric acid and the optical density values were read at 450 nm by ELISA reader, Hiperion model, MPR^{4+}, made in Germany.

2.5. a. Serum bacterial activity
Serum bactericidal activitywas measured by slight modification of the method described by Villamil *et al*. (2003) [27]. 10^{8} ml^{-1} bacterial suspension of *Yersinia ruckeri* was prepared in Tryptone soy broth (TSB) and 100 µl of suspension was dispersed into each well plate. After adding 33 µl of serum in triplicate in each well, the mixture was incubated at 18ºC for 6 h. For 10 min plate was shaken slightly and then supernatant was discarded. 100 µl of 3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyltetrazolium bromide (MTT) (0.5 mg ml^{-1}) was added to each well. After 15 min in the dark,

the optical density (600 nm) of the viable bacteria was measured.

2.5. b. Serum α_1-antiprotease

The level of serum α_1-antiprotease was measured according to Rao and Chakrabarti (2005) [18] with some modifications. In tubes 10 µl of serum sample was diluted with 20 µg of trypsin dissolved in 100 µl of Tris-HCl (50 mM, pH 8.2). All tubes were made up to 200 µl with Tris-HCl and incubated at room temperature for 1 h. Then, 2 ml of 0.1 mM substrate, BAPNA (Na-benzoyl-DL-arginine-p-nitroanilide HCl, Sigma) dissolved in Tris-HCl (containing 20 mM calcium chloride), was added to all tubes and incubated for a further 15 min. Finally the color-change was stopped by adding 500 µl of 30% acetic acid and the optical density was read at 410 nm in a UV-visible spectrophotometer, Spectronic 2OD.

Finally the color-change was stopped by adding 500 µl of 30% acetic acid and the optical density was read at 410 nm in a UV-visible spectrophotometer, Spectronic 2OD.

2.5. c. Serum total antiprotease

The level of serum total antiprotease was measured according to Rao and Chakrabarti (2005) [18] with some modifications. In tubes 10 µl of serum was diluted with 20 µg of trypsin dissolved in 100 µl of PBS (pH 7.4). All tubes were incubated at room temperature for 30 min. Then, 1 ml of casein dissolved in PBS (2.5 mg ml^{-1}), was added to all tubes and incubated for a further 15 min. Finally the color-change was stopped by adding 500 µl of 10% trichloroacetic acid. All tubes were centrifuged at 3800 rpm for 10 min to remove the precipitate. The optical density was read at 280 nm in a UV-visible spectrophotometer, Spectronic 2OD and trypsin inhibition was checked.

2.5. d. Serum lysozyme activity

Serum lysozyme activity was measured using a turbidimetric microtitre plate technique according to Tukmechi *et al*. (2011) [26] with slight modification. Briefly, a standard suspension of *Micrococcus lysodeikticus* (75 µg ml^{-1}) was prepared with 0.1 M phosphate citrate buffer, pH 5.8. Rainbow trout serum (25 µl) was added to 75 µl of *Micrococcus lysodeikticus* suspension and the decrease in absorbance after 4 and 9 min at 450 nm. One unit of lysozyme activity was defined as reduction in absorbance of 0.001 per min.

2.5. e. Plasma total immunoglobulin content

Plasma total immunoglobulin was determined following the method of Siwicki *et al*. (1994) [22]. The difference in total protein content prior to and after precipitation of the immunoglobulin component with 12 % polyethylene glycol (PEG, Sigma) was determined by Bradford method.

2.6. Statistical Analyses

The effects of experimental diets on growth performance, carcass indexes, fillet chemical composition and non-specific immune parameters of Rainbow trout were studied using a completely randomized design with four replications of four treatments. The obtained data were analyzed using GLM procedure of SAS software (9.1) and mean comparison was performed using Duncan's test.

3. Results

The growth performance factors, weight gain, body condition factor and feed intake of fish fed different experimental diets during the total experimental period showed no significant difference compared with the control diet and in comparison with each other (Table 3). On the other hand, feed conversion ratio and specific growth rate improved increasing the replacement level of fish meal with poultry by-product meal along with plant protein sources ($P<0.05$).

Carcass traits including carcass weight, carcass efficiency and viscera weight and somatic indices of experimental fish showed no significant difference between the groups. Regarding liver weight and hepatic somatic index, a reduction was observed between fish fed experimental diets compared to those fed control diet ($P< 0.05$) (Table 4).

Fillet chemical composition including dry matter, crude protein, crude fat, organic matter and ash of experimental fish fillet showed no significant difference between the groups and were not affected by replacement (Table 5).

The obtained results regarding some immune parameters studied in the present study which are important components of fish immune system are shown in table 6. First, lysozyme, peroxidase and anti-bacterial activity in fish serum was taken into account. The results showed that none of these parameters were affected by experimental diets. Serum peroxidase content and bactericidal activity were not altered by the dietary treatments. In the present study, total antiprotease activity was significantly decreased in diets CPFM and CCP compared with the control diet ($p<0.05$). On the other hand, a reduction in alpha-anti-protease activity was only visible in diet CCP comparing control diet ($P<0.05$). As another result, serum total protein was significantly lower for all treatment groups (the least was in CCP diet) ($p<0.05$), while serum total immunoglobulin was not significantly affected by any diet.

Table 3: Weight gain, feed conversion ratio, specific growth rate and body condition score for rainbow trout fed different diets for 90 days

Growth Performance	Experimental diets			
	FM (control)	PFM	CPFM	CCP
Weight gain (gr)	370.77±15.63	366.88±9.25	361.95±2.35	354.93±9.31
Feed Conversion Ratio	1.20±0.047^{a*}	1.13±0.03^{a}	1.09±0.07^{ab}	1.00±0.032^{b}
Specific Growth Rate (%)	1.34±0.051^{b}	1.43±.086^{ab}	1.47±.086^{ab}	1.67±0.044^{a}
Body Condition Score (%)	1.530±0.130	1.400±0.077	1.385±0.070	1.420±0.196

***** In each row, the means with different letters have significant differences. ($P<0.05$). Values are means± SE for three replications

Table 4: Carcass weight, carcass efficiency, viscera weight, viscera Index, liver weight and hepatic somatic index for rainbow trout fed different diets for 90 days

Growth Performance	Experimental diets			
	FM (control)	PFM	CPFM	CCP
Carcass Weight (gr)	242.11±6.26	234.94±9.85	217.08±7.36	231.94±13.71
Carcass Efficiency (%)	80.13±0.74	81.01±0.51	78.76±1.30	79.69±1.31
Viscera Weight (gr)	62.20±4.70	57.47±3.79	56.20±1.99	58.67±2.93
Viscera Somatic Index (%)	16.99±0.57	16.71±0.45	17.82±0.74	17.65±0.69
Liver Weight (gr)	5.78±0.24a*	5.03±0.47ab	4.67±.21b	4.41±0.11b
Hepatic Somatic Index (%)	1.595±0.027a	1.425±0.065b	1.387±0.043b	1.385±0.023b

* In each row, the means with different letters have significant differences (P<0.05). Values are means± SE for three replications.

Table 5: Dry matter, crude protein, ether extract, organic matter and ash in the fillet of rainbow trout fed different diets for 90 days

	Experimental diets			
Chemical Composition	FM (control)	PFM	CPFM	CCP
Dry Matter (%)	26.32±0.36	26.05±1.13	26.12±0.55	26.23±0.77
Crude Protein (%)	19.72±0.53	19.65±0.56	19.80±0.48	19.82±0.52
Ether Extract (%)	5.55±0.28	5.80±0.18	5.70±0.11	6.10±0.12
Organic Matter (%)	25.03±0.35	24.81±1.06	24.85±0.59	24.95±0.93
Ash (%)	1.295±0.10	1.237±0.094	1.272±0.102	1.282±0.169

Values are means± SE for three replications**.**

Table 6: Non-specific immune parameters for rainbow trout fed different diets for 90 days

	Experimental diets			
Immune parameters	FM (control)	PFM	CPFM	CCP
Peroxidase content (450 nm)	$0.268 \pm 0.004^{a*}$	0.268 ± 0.005^{a}	0.275 ± 0.005^{a}	0.282 ± 0.005^{a}
Bactericidal activity (600 nm)	0.727 ± 0.039^{a}	0.746 ± 0.032^{a}	0.662 ± 0.025^{a}	0.797 ± 0.042^{a}
α_1-antiprotease (410 nm)	0.047 ± 0.005^{a}	0.049 ± 0.002^{a}	0.045 ± 0.003^{ab}	0.037 ± 0.002^{b}
Total antiprotease (280 nm)	2.484 ± 0.015^{a}	2.431 ± 0.017^{ab}	2.387 ± 0.015^{b}	2.370 ± 0.013^{b}
Total protein (mg ml^{-1})	48.3 ± 1.5^{a}	41.5 ± 0.8^{b}	41.8 ± 1^{b}	37.6 ± 1^{c}
Total Ig (mg ml^{-1})	4.94 ± 0.8^{a}	4.27 ± 0.8^{a}	4.68 ± 1.15^{a}	4.8 ± 0.33^{a}
Lysozyme (U ml^{-1})	2.944 ± 0.526^{a}	3 ± 0.370^{a}	3.111 ± 0.504^{a}	3 ± 0.363^{a}

* In each row, the means with different letters have significant differences (P<0.05). Values are means± SE for three replications.

4. Discussion

The lack of significant difference in weight gain between experimental groups and control group indicates that cottonseed meal can easily replace fish meal in rainbow trout diets. On the other hand, up to 10% of cottonseed meal and up to 7.8% of canola meal along with 40.24% poultry by-product meal can replace fish meal, even without processing and isolation of anti-nutritional substances. The lack of significant difference in weight gain and body condition score was indicative of the biological value of the experimental diets of the present study. The results of the current study regarding weight gain and body condition factor was in line with the findings of Lee *et al*. (2010) [11], Magdy *et al*. (2012) [13] and Magdy *et al*. (2008) [15].

In CCP diet, as fish meal replacing level increased and canola meal was added to the chemical composition of the diet, improvements in specific growth rate and feed conversion ratio became visible and significant differences were observed in specific growth rate and feed conversion ratio in CCP diet (the only diet containing canola meal) compared with control diet (P<0.05). Canola meal is a rich source of linolenic fatty acid and methionine and cystine, all of which can increase metabolism and growth. That is why canola meal was added to CCP diet. The results of the current study regarding canola meal were in accordance with the findings of Thiessen *et al*. (2004) [24] and Plaipetch & Yakupitiyage (2012) [17].

In the present study, when using a balanced diet in terms of plant protein sources, liver weight and HSI did not change in rainbow trout, but in diets having high levels of fish meal, a significant increase in liver weight and Hepatic Somatic Index (HSI) was seen (P<0.05). This increase in HSI and liver weight was probably due to the presence of decarboxylated free amino acids as a result of bacterial enzymes and production of biogenic amines in fish meal used in FM and PFM diets. Biogenic amines are excreted by the liver and this will probably lead to an increase in liver activity through an increase in the number of hepatocytes which in turn results in an increase in liver weight and hepatic index [20].

The lack of significant difference in fillet chemical composition of experimental fish showed that poultry by-product meal supplemented with plant protein sources could replace fish meal up to 100% and resulted in a similar fillet chemical composition with that of the control diet. This was similar to the results obtained by Tidwell *et al*. (2005) [25] whose study illustrated that fillet chemical composition of Largemouth Bass was not affected by animal and plant protein replacement, but was in conflict with the findings of Bilgüven & Barış (2011) [3]. The conflict was due to the use of high percentage of cottonseed meal containing gossypol by researchers which led to the reduction of tissue protein.

The obtained results regarding the amount of ash in the current study was less than that obtained by Steffens (1994) [23]. This difference can be justified by considering the fact that in the present study pure fish fillet was used to measure the amount of ash, but in Steffens's study (1994) [23] the whole body of the fish (fillet with bones and scales) had been used for this purpose.

It is worth noting that when fish encounters pathogens, myeloperoxidase and eosinophil peroxidase in granules of phagocytic cells are released. Released granules use H_2O and halide ions to form chlorides and chloramines to help them in their battles with the disease [6].

Infected nutrients preferably move towards the immune system and have no effect on growth. In this case, amino acids are distributed to the liver in order to synthesize proteins such as antiprotease and compensate for the low level of nutrient absorption before the infection leads to the deficiency of the immune response [5]. Replacing fish meal with higher levels of poultry by-product meal supplemented with plant protein sources had no effect on anti-bacterial activity, immunoglobulin, lysozyme and peroxidase. These results were in line with the findings of Bransden *et al*. (2001) [5] and Yang *et al*. (2004) [28].

Fish serum contains substances with antioxidant enzyme activity and thus defends the body against pathogens by inhibiting the extracellular enzymes. The inhibition of some proteases by fish serum is mainly due to α_1-proteinase inhibitor, α_2-anti-plasmin and α_2 macroglobulin [18].

In the current study, the amount of alpha-anti-protease decreased significantly in CCP diet in which the total replacement had occurred. Total anti-protease in CPFM diet and CCP diet had a significant reduction ($P<0.05$). Serum total protein was also decreased significantly in PFM, CPFM and CCP diets, respectively. This decrease is mainly related to inhibitors of plant protein sources that are considered as anti-nutritional. For example, free gossypol of cottonseed meal interacts with free epsilon amine of lysine and other amino acids and causes protein denaturation which in turn reduces the nutritional value of the protein and purity of protein adsorption. In other words, the binding property of gossypol molecules with minerals such as iron causes their low availability in the body and results in hematocrit deficiency [14]. In the current study, due to the use of low levels of plant protein sources as supplements of poultry by-product meal, no adverse effect was observed on growth performance, carcass quality and carcass chemical composition. This replacement resulted in a decrease in immune system, but this reduction in non-specific immune system did not lead to experimental fish mortality. As a general conclusion, it can be said that fish meal was totally replaced by 40.24% poultry by-product meal along with 7.8% canola meal and 10% cottonseed.

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