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Growth and hematological changes of Aspikutum, a hybrid of *Leuciscus aspius* female (Linnaeus, 1758) × *Rutilus frisii* male (Kamensky, 1901), fed rations with various protein to lipid ratio

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Abstract

A 60-day trial with six diets (three different protein levels × two different lipid levels) containing 30, 35 and 40% protein with 10 and 15% lipid levels was conducted to determine the optimum dietary protein to lipid ratio and effects on growth performance and hematological indices of Aspikutum, *Leuciscus aspius* female×*Rutilus frisii* male. Two hundred and fifty two fish (31.2±0.4 g; mean±SEM) were distributed in 18 circular concrete tanks (400 L) and were fed 3 times daily according to their apparent satiation. The results showed that the highest growth rate was achieved when fish fed with diet containing 35% protein and 15% lipid. Lipid productive value was significantly decreased with increasing dietary lipid level. Number of red blood cells, white blood cells, hemoglobin concentration, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin, neutrophil and eosinophil were not considerably differing among rearing treatments, while mean corpuscular hemoglobin concentration was significantly higher in fish fed with diet containing 30% protein and 10% lipid. The percentage of lymphocyte increased with increasing dietary lipid level, whereas a decrease of monocyte percentage was observed with increasing of dietary lipid level. Dietary protein and lipid level, as well as the interaction showed no significant differences in total lipid, triglyceride, cholesterol and total protein levels. In general, this study has indicated that diet containing 35% protein and 15% lipid had positive effects on growth performance, while no effects on biochemical and hematological parameters was achieved.

Keywords: Protein, Lipid, Blood parameters, Hybrid, Asp, Caspian Kutum

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Introduction

Successful aquaculture is dependent on the supply of diets containing adequate levels of protein, energy and appropriate balance of nutrients for effective growth and aquatic health under rearing conditions (Cho and Bureau, 1995). Determination of optimal protein content in the diet is the basic factor in ration formulation with high quality and affordable for fish. Therefore, to improve protein utilization for fish growth and reduce the feed conversion ratio, dietary protein could be partially replaced with lipid or carbohydrate (Aliyu-Paiko et al. 2010). On the other hand, inclusion of lipid in diet can improve growth performance, nutritional status and feed palatability (Aliyu-Paiko *et al*., 2010). Appropriate balance between dietary protein and energy is necessary to optimize utilization of protein, increase of growth rate, minimize of excessive accumulation of lipid and glycogen on liver and visceral tissues, reduce undesirable nitrogenous wastes withdrawal and improve the quality of aquaculture effluents (Ai *et al*., 2004; Mohanta *et al*., 2009). Dietary protein and energy level modifies absorption efficiency and it is directly associated with nutrient composition, ingredients quality and formulation process of the diet (Tacon and Forester, 2000). When fish fed with diet containing inappropriate levels of protein and lipid, growth performance is reduced because of undesirable digestible energy or deficiency of essential fatty acids (Arredondo-Figueroa *et al*., 2012). Therefore, it is important to find the proper levels of non-protein energy sources such as carbohydrate and lipid to reduce feed costs and preventing imbalances in either non-protein dietary energy sources (Kim and Lee, 2005).

Kutum (*Rutilus frisii*) is a valuable commercial species in southern parts of the Caspian Sea (Dorafshan and Heyrati, 2006; Fadakar *et al*., 2014) with the average annual catch about 10,000 tons. But, despite its popularity, this fish has not been cultured successfully to a marketable size in Iran (Amini *et al*., 2007). In wild habitats, Kutum is generally fed mollusks and crustaceans and it is known as an omnivore species (Razavi Sayyad, 1995).

Asp (*Leuciscus aspius*) is distributed in the inland waters of Europe, north east of Atlantic Ocean and Caspian Sea (Kottelat and Freyhof, 2007). It is the only carnivorous cyprinid species that feeds primarily other fishes, frogs and duckling (Coad, 2014).

Hybridization is one of the eugenics techniques in aquaculture that it has been expanded for producing hybrid with desirable traits among breeders (Bartley *et al*., 2001). So far, many hybrids have been introduced for use in aquaculture such as hybrids in cyprinids, salmonids, tilapias, catfishes, moronids, sparids, sturgeons and other hybrids in freshwater and marine species (Bartley *et al*., 2001), that indicates importance of hybrids production in different culture systems. In the meantime, hybrid of female Asp ×male Kutum (*Leuciscus aspius* ♀×*Rutilus frisii* ♂) is an example of new hybrid (Falahatkar *et al*., 2013,

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2015; Bagheri *et al*., 2017) which the aim of production and introducing this hybrid to fish farms is increasing share of aquaculture as well as compensate the lack of farming fish of Asp and Caspian Kutum.

Nevertheless, introducing this hybrid as a candidate for aquaculture needs to series of studies to determine the initial requirements for market growing. Therefore, determination of nutritional requirements can be placed in priority of evaluate these necessities. The aim of this study was to estimate the dietary protein to lipid ratio of Aspikutum juveniles. The effects of dietary protein and lipid levels on growth performance, feed utilization and hematological parameters have also investigated.

Materials and methods

Fish production and diet preparation

Hybrid of female Asp×male Kutum (*Leuciscus aspius* ♀×*Rutilus frisii* ♂) was artificially produced at the Dr. Yousefpour Fish Hatchery Center in April 2012. These fish were originally obtained from wild broodstock that were caught from lake behind the Aras Dam and Caspian Sea, respectively. The mean larvae body weight was 2.5 mg when have been transferred to the earthen ponds for rearing (Falahatkar *et al*., 2013). Fish with an average body weight of 19.8±0.2 g have been transferred from the earthen ponds into the 4000 L fiberglass tanks to adapt to the new rearing facilities. During this time, they were fed with an artificial diet until the start of the experiment.

The ingredients, nutrient and energy contents of the experimental diets are presented in Table 1. A 3×2 factorial design was established, and six test diets containing three protein levels (30, 35 and 40%), and two lipid levels (10 and 15%) have been formulated. An equal proportion of fish oil and corn oil was used as the source of lipid. All dry ingredients were finely ground, carefully weighed and mixed manually. The fish oil and corn oil were then added gently while mixing continuously, and then water was added to the mixture for uniform blending. Finally, mixture was passed with a 4-5 mm diameter die. The wet strands were dried in a convection oven at 45°C for 12 h and stored at -20°C until use.

Table 1: Formulation and proximate composition of the experimental diets (P/L: protein to lipid ratio; %).

Ingredients (%)	Diets					
	P30/L10	**P30/L15**	**P35/L10**	**P35/L15**	**P40/L10**	**P40/L15**
Fish meal	23.02	23.7	31	31.46	38.6	38.96
Soybean meal	15	15.98	16.68	18	19	20.5
Wheat flour	25.54	21.67	20.55	16.04	14.76	10.16
Wheat bran	22.5	19.5	18.5	16	15	12.5
Fish oil	0.97	3.58	0.64	3.25	0.32	2.94
Corn oil	0.97	3.57	0.63	3.25	0.32	2.94
Lecithin	3	3	3	3	3	3
Methionine	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	0.5	0.5	0.5	0.5	0.5	0.5
Molasses	2.5	2.5	2.5	2.5	2.5	2.5
Yeast	1.5	1.5	1.5	1.5	1.5	1.5
Vitamin premix[1]	2	2	2	2	2	2
Mineral premix[2]	1.5	1.5	1.5	1.5	1.5	1.5
Dicalcium phosphate[3]	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.4	0.4	0.4	0.4	0.4	0.4
Proximate analysis (%; n=3)						

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Table 1 continued:						
Moisture	9.85±0.48	8.05±0.11	8.91±0.14	9.78±0.22	9.10±0.21	8.52±0.11
Crude protein	30.13±0.30	30.35±0.24	35.78±0.35	35.27±0.20	40.02±0.36	40.37±0.12
Crude lipid	9.54±0.29	14.15±0.48	10.62±0.14	14.47±0.11	10.41±0.12	15.09±0.07
Ash	9.40±0.08	9.40±0.00	10.39±0.17	10.29±0.17	11.49±0.12	11.53±0.06
Gross energy (kJ g^{-1})[4]	17.39	18.66	17.70	18.95	17.98	19.23

[1]Science Laboratories (Qazvin, Iran). Each 1000 g vitamin mixture provides vitamin A, 1 600 000 I.U; vitamin D_3, 400 000 I.U; vitamin E, 40 g; vitamin K_3, 2 g; thiamin, 6 g; riboflavin, 8 g; calcium pantothenate, 12 g; niacin, 40 g; pyridoxine, 4 g; folic acid, 2 g; cyanocobalamin, 8 mg; H_2, 0.24 g; vitamin C, 60 g; inositol, 20 g and BHT, 20 g.
[2]Science Laboratories (Qazvin, Iran). Each 1000 g mineral premix provides ferrous, 26 g; zinc, 12.5 g; selenium, 2 g; cobalt, 480 mg; copper, 4.2 g; manganese,15.8 g; iodine,1 g and choline chloride, 12 g.
[3]Jahan phosphate (Roudsar, Iran).
[4] Based on 23.4 kJ g^{-1} protein, 39.2 kJ g^{-1} lipid, 17.2 kJ g^{-1} carbohydrate.

Experimental design

The study was conducted at the Dr. Yousefpour Fish Hatchery Center in Siahkal, Guilan, Iran. Before the initiation of the experiment, fish were acclimated to laboratory conditions for 10 days by feeding a basal diet containing 28.6±0.1% protein, 14.7±0.3% lipid, 8.4±0.1% ash and 9.6±0.2% moisture. After the acclimation period, 252 fish (average weight of 31.2±0.4 g) were distributed into 18 circular concrete tanks (with the volume of 400 L) with 14 fish per tank. Natural photoperiod was maintained during the feeding trial and water flow rate of 5.3±0.3 L min^{-1} was supplied for each tank. Each diet was given in three replicates. Fish were fed to satiation three times daily (10:00, 15:00 and 20:00 h) for 60 days. Fish were individually weighed at the start and end of feeding trial and weighed every two weeks interval. Water temperature, dissolved oxygen and pH were monitored daily, which the mean water parameters during the experiment were 16.1±0.3°C, 7.5±0.1 mg L^{-1} and 8.3±0.1, respectively.

Samples collection and chemical analysis

At the end of experimental period, all fish were starved for 24 h and anaesthetized using diluted clove powder (300 mg L^{-1}). All fish were weighed and total body length was measured individually. Growth performance and feed utilization were determined in terms of final weight (FW), weight gain (WG), specific growth rate (SGR), feed efficiency (FE), condition factor (CF), survival rate, protein productive value (PPV) and lipid productive value (LPV). A sample of 20 fish at the beginning and 5 fish per tank at the end of the feeding trial were collected and killed with high dose of clove powder (500 mg L^{-1}) and stored at -20 °C for proximate body composition. All chemical composition analyses of diets, also protein and lipid contents of body was measured using the methods of the Association of Official Analytical Chemists (AOAC, 1995). Also, 8 fish from each tank were randomly sampled, and approximately 1.5-2 ml of blood was taken from the caudal vein with a 2 ml heparinized plastic syringe. Because of low amount of blood, samples of 2 fish from each

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tank were pooled to obtain one sample for evaluation of hematological variables. Blood plasma was collected after centrifugation (1500×g for 10 min) and stored at -70 °C until analysis. Plasma parameters including triglyceride, cholesterol, total lipid and total protein levels were measured spectrophotometrically (Unico UV-2100, New Jersey, USA) using standard kits (Zist Chimi Company, Tehran, Iran). Hemoglobin concentration has measured using Drabkin solution (Cyanmethahemoglobin) with a spectrophotometer at 540 nm absorbance (Drabkin, 1945). Hematocrit (Hct) has determined using microhematocrit method of centrifuging the samples at 3500×g for 10 min (Řehulka, 2000). Number of red blood cells (RBCs) and white blood cells (WBCs) were manually counted using ringer solution by hemocytometer (Houston, 1990). Blood indices including mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were determined according to Ranzani-Paiva *et al*. (2004). Differential leukocyte count was performed after preparing the smear and staining with Giemsa solution (Houston, 1990).

Statistical analysis

Normality of data and homogeneity of variances were checked using Kolmogorov-Smirnov and Levene's tests, respectively. To detect statistically significant differences, data were analyzed by Two-Way Analysis of Variance (ANOVA) using protein and lipid concentrations as the two factors. When Two-Way ANOVA showed a significant interaction between these two factors, One-Way ANOVA was used to analyze the data. Multiple comparisons were evaluated using the Tukey's test. All statistical analyses were carried out using the SPSS software (Version 16.0, SPSS, Chicago, IL, USA) with a significance level of $p<0.05$. All data presented in the text are means±standard error (SE).

Results

After 60 days of rearing, most of the growth parameters (FW, WG, SGR and FE) were significantly affected by protein content of the diets ($p<0.05$), so that the highest values of growth and the best FE were observed for fish fed with 35% protein diet. Also, CF was significantly affected by dietary protein and lipid levels ($p<0.05$), with the highest value in fish fed diets 35% protein and 15% lipid. PPV was not significantly affected by both protein and lipid content of the diets as well as the interaction between protein and lipid ($p>0.05$). LPV was influenced by dietary protein and lipid levels ($p<0.05$), so that LPV decreased significantly in groups fed the high protein level. LPV was significantly decreased with increasing dietary lipid levels at the 35% and 40% protein levels. Survival rate was 100% in all of the rearing groups (Table 2).

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Table 2: The effects of different diets with varying protein to lipid ratios (P/L) on growth rate and diet utilization in Aspikutum (*Leuciscus aspius* female×*Rutilus frisii* male) after 60 days rearing period (means±SE).

	Diets						Two-way ANOVA		
	P30/L10	P30/L15	P35/L10	P35/L15	P40/L10	P40/L15	P	L	P×L
Initial body weight(g)	31.19±0.88	31.17±1.08	31.21±0.92	31.26±0.90	31.26±0.98	31.21±0.95	0.989	0.927	0.982
Final body weight (g)	36.38±0.92^{b}	36.82±1.13^{ab}	37.67±1.03^{ab}	39.88±0.91^{a}	36.48±1.03^{ab}	36.81±1.00^{ab}	0.036	0.252	0.512
WG (%)[1]	16.64±1.00^{b}	18.18±3.34^{b}	20.67±1.07^{ab}	27.57±0.87^{a}	16.68±1.39^{b}	17.62±0.88^{b}	0.004	0.055	0.226
SGR (%/day)[2]	0.26±0.01^{b}	0.28±0.05^{b}	0.31±0.01^{ab}	0.40±0.01^{a}	0.26±0.02^{b}	0.27±0.01^{b}	0.005	0.065	0.261
CF[3]	0.73±0.00^{b}	0.72±0.01^{b}	0.72±0.01^{b}	0.77±0.00^{a}	0.72±0.01^{b}	0.73±0.00^{b}	0.003	0.008	0.002
FE (%)[4]	35.84±1.93^{b}	36.94±2.72^{ab}	38.56±0.60^{ab}	44.14±0.80^{a}	35.50±1.52^{b}	35.72± 1.07^{b}	0.015	0.133	0.293
PPV (%)[5]	4.07±0.57	3.25±0.67	2.30±0.23	2.69±0.22	2.89±0.29	2.31±0.36	0.124	0.109	0.118
LPV (%)[6]	9.26±1.43^{a}	7.59±1.10^{ab}	6.46±0.38^{abc}	4.98±0.46^{bc}	8.58±0.20^{ab}	3.27±1.05^{b}	0.011	0.001	0.081
Survival (%)[7]	100	100	100	100	100	100	-	-	-

Values within the same row with different letters are significantly different (p<0.05).
[1]Weight gain=100 × [final body weight (g)-initial body weight (g)]/initial body weight (g)
[2]Specific growth rate=100×[ln final body weight (g)-ln initial body weight (g)]/days of the experiment
[3]Condition factor=100×[fish weight (g) / fish length^{3} (cm)]
[4]Feed efficiency=100×[wet weight gain (g)/feed consumed (g)]
[5]Protein productive value=100×[protein gain (g) / protein intake (g)]
[6]Lipid productive value=100×[lipid gain (g)/ lipid intake (g)]
[7]Survival=100×[final number of surviving fish/initial number of fish]

At the termination of feeding period, hematological indices including RBC, WBC, Hb, Hct, MCV, MCH, neutrophil and eosinophil were not significantly affected by dietary protein and lipid levels as well as the interaction between protein and lipid levels (p>0.05). The highest value of MCHC was obtained in fish fed with diet containing 30% protein and 10% lipid. At the 35% and 40% protein levels, the percentage of lymphocyte was increased with increasing dietary lipid level, whereas a decrease of monocyte percentage was observed with increasing of dietary lipid level (p<0.05; Table 3). Plasma biochemical parameters indicated that dietary protein and lipid levels as well as the interaction had not significant effect on total lipid, triglyceride, cholesterol and total protein concentrations after 60 days of rearing (p>0.05; Table 4).

Table 3: The effects of different diets with varying protein to lipid ratio (P/L) on hematological parameters in Aspikutum (*Leuciscus aspius* female×*Rutilus frisii* male) after 60 days rearing period (n=6; means±SE).

	Diets						Two-way ANOVA		
	P30/L10	P30/L15	P35/L10	P35/L15	P40/L10	P40/L15	P	L	P×L
RBC (×10^{6}/mm^{3})	1.81 ± 0.05	1.82 ± 0.04	1.87 ± 0.04	1.83 ± 0.03	1.89 ± 0.04	1.76 ± 0.04	0.619	0.126	0.166
WBC (×10^{3}/mm^{3})	8.52 ± 0.26	8.68 ± 0.23	8.92 ± 0.19	8.87 ± 0.21	8.95 ± 0.08	8.35 ± 0.21	0.300	0.324	0.152
Hb (g dL^{-1})	13.35 ± 0.34	13.45 ± 0.34	13.67 ± 0.32	13.47 ± 0.26	13.70 ± 0.25	12.85 ± 0.30	0.564	0.163	0.218
Hct (%)	57.00 ± 1.61	58.50 ± 1.63	59.83 ± 1.56	57.67 ± 1.20	59.83 ± 1.01	55.17 ± 1.35	0.623	0.117	0.088
MCV (fL)	315.33 ± 3.61	320.67 ± 2.03	320.33 ± 2.01	313.83 ± 3.81	315.83 ± 2.65	314.17 ± 2.29	0.447	0.632	0.061
MCH (pg cell^{-1})	73.67 ± 0.76	73.33 ± 0.84	73.00 ± 0.26	73.17 ± 0.65	72.17 ± 0.54	72.50 ± 0.72	0.150	0.919	0.842
MCHC (g dL^{-1})	23.34 ± 0.21^{a}	22.50 ± 0.34^{b}	22.50 ± 0.22^{b}	23.00 ± 0.26^{ab}	22.50 ± 0.22^{b}	23.00 ± 0.26^{ab}	0.742	0.786	0.012
Lymphocyte (%)	68.17 ± 1.83^{ab}	67.50 ± 1.52^{ab}	65.00 ± 1.03^{b}	68.83 ± 1.47^{ab}	65.50 ± 0.56^{ab}	69.33 ± 1.43^{a}	0.717	0.016	0.085
Neutrophil (%)	28.33 ± 1.36	28.50 ± 1.18	30.50 ± 0.88	27.83 ± 1.35	30.00 ± 0.73	28.83 ± 1.05	0.566	0.133	0.355
Monocyte (%)	3.00 ± 0.45^{ab}	3.33 ± 0.33^{ab}	3.83 ± 0.31^{a}	2.83 ± 0.17^{b}	3.83 ± 0.31^{a}	2.83 ± 0.31^{b}	0.817	0.032	0.053
Eosinophil (%)	0.50 ± 0.22	0.67 ± 0.42	0.33 ± 0.21	0.67 ± 0.33	0.67 ± 0.33	0.33 ± 0.21	0.266	0.130	0.058

Values within the same row with different letters are significantly different (p<0.05).

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Table 4: The effects of different diets with varying protein to lipid ratio (P/L) on plasma biochemical parameters in Aspikutum (*Lueciscus aspius* female× *Rutilus frisii* male) after 60 days rearing period (n=6; means±SE).

	Diets						Two-way ANOVA		
	P30/L10	P30/L15	P35/L10	P35/L15	P40/L10	P40/L15	P	L	P×L
Triglyceride (mg dL^{-1})	107.83±4.62	109.83±4.45	97.83±5.36	112.50±7.24	116.83±7.08	127.67±13.36	0.674	0.139	0.069
Cholesterol (mg dL^{-1})	188.33±5.74	203±13.16	208.67±5.80	218.17±9.00	211.83±12.01	200±10.54	0.373	0.612	0.211
Total lipid (mg dL^{-1})	366±10.36	380.83±23.49	384±12.36	406.33±17.31	406.50±12.04	412±29.48	0.894	0.337	0.147
Total protein (g dL^{-1})	2.80±0.22	3.00±0.33	2.67±0.17	3.45±0.25	3.30±0.28	2.98±0.28	0.111	0.289	0.627

Discussion

In the present study, FW, WG and SGR have been improved with increasing dietary protein from 30 to 35%, and slightly decreased thereafter with further increases in dietary protein level. These results indicated that the 35% dietary protein could meet protein requirement of this hybrid under the experimental conditions. Mahmoodi *et al*. (2013) reported that Kutum as a parent of this hybrid which fed a 35% protein diet showed better weight gain than fish fed with diets containing 30% or 40% protein.

The decline in growth rate with increasing protein levels has been reported by Countinho *et al*. (2012) in juvenile *Diplodus puntazzo* (weighing 49.3 g), fed diets with protein levels ranging from 15 to 50%. Wang *et al*. (2013) indicated that growth rate of *Pseudobagrus ussuriensis* has been increased with the increases of dietary protein from 35% to 45%, and then decreased with increasing dietary protein. In *Puntius gonionotus*, Mohanta *et al*. (2008b) observed a decline in body weight gain when dietary protein content increased from 30 to 35%. Lee *et al*. (2002) in *Paralichthys olivaceus*, Ozório *et al*. (2009) in *Diplodus vulgaris*, Mohseni *et al*. (2011) in *Acipenser persicus*, and Shapawi *et al*. (2014) in *Epinephelus fuscoguttatus* found significant decrease in weight gain with excessive dietary protein, because at excessively high dietary protein level, the free amino acids accumulated in body fluids may become toxic (Harper *et al*., 1970) or the metabolic cost of nitrogen excretion may reduce the growth (Jauncey, 1982). In this study, the highest value of FE was obtained in fish fed diet containing 35% protein. This result indicated that diet containing 35% protein is sufficient for fish growth and no need more protein. Similar results were also have reported in other studies (Mohanta *et al*., 2008b; Ozório *et al*., 2009; Ebrahimi *et al*., 2013; Wang *et al*., 2013).

In the present study, WG, SGR and FE have not been affected by dietary lipid level, but the results showed that improvement in growth rate and FE was observed in fish fed with 15% lipid than the fish fed 10% lipid. CF was significantly affected by dietary protein and lipid levels in fish fed with 35% protein and 15% lipid diets. According to increase of CF and improvement in growth performance with increasing dietary lipid, the results showed that diets containing 15% lipid can be appropriate for optimal growth of Aspikutum. Thus, improvement in

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growth performance and FE indicates that dietary lipid can provide the energy needed for fish activities and prevent the use of protein for energy supply, thus the protein is used for growth and tissue formation (Dias *et al*., 1998). The protein-sparing action by dietary lipid as energy source has been studied by numerous researchers that dietary lipid have been led to improve feed and protein utilization efficiencies (Dias *et al*., 1998; Torstensen *et al*., 2001; Mohseni *et al*., 2007).

In this study, increase in dietary protein and lipid levels had no significant effect on PPV, while LPV was influenced by dietary protein and lipid levels. In this case, LPV decreased with increasing dietary protein levels. This may be because of the high level of digestible carbohydrate in low protein (30%) diet, which may lead to lipogenesis (Mohanta *et al*., 2008b). These results correlate with the findings of Amin *et al*. (2014) for *Salvelinus fontinalis*. Also, Likmani and Wilson (1982) observed increase in lipogenic activity in channel catfish (*Ictalurus punctatus*) with higher levels of dietary carbohydrate.

Unlike present results, Ozório *et al*. (2009) in two-banded sea bream and Gao *et al*. (2011) in grass carp (*Ctenopharyngodon idella*) observed that LPV have increased with increasing dietary protein levels. Decreasing the LPV with increasing dietary lipid which was observed in the present study may be related to increase share of lipid in energy supply for fish. Similar findings have been reported in other fish species (Peres and Oliva-Teles 1999; Satpathy *et al*., 2003; Du *et al*., 2005; Mohanta *et al*., 2008a; Gao *et al*., 2011).

Our data showed that dietary protein and lipid levels as well as the interaction had no significant effect on hematological parameters (RBC, WBC, Hb, Hct, MCV, MCH, neutrophils and eosinophils) in this hybrid, but MCHC was significantly affected by the interaction between dietary protein and lipid. This result is in agreement with many studies i.g. *Hippoglossus hippoglossus* (Hemre *et al*., 1992), *Gadus morhua* (Rosenlund *et al*., 2004) and *Piaractus mesopotamicus* (De Almeida Bicudo *et al*., 2009). Hematological changes in Atlantic salmon, *Salmo salar*, showed that RBC, Hb, Hct and MCV have not been affected by dietary lipid level, but MCH and MCHC significantly increased with increasing dietary lipid level (Hemre and Sandnes 1999), and no correlations were found between these indices and dietary compositions. However, some authors have reported that dietary protein and lipid levels influence the hematological parameters (Kikuchi *et al*., 2009; Abdel-Tawwab *et al*., 2010).

Docan *et al*. (2012) has reported that inadequate nutrition may be a factor that results in a decreased number of lymphocytes. In the present study, in 35% and 40% protein levels, the highest values of lymphocytes observed in fish fed diets containing 15% lipid. This immune response seems to demonstrate that dietary lipid has the immune role for this hybrid.

At the end of the trial, plasma triglyceride, cholesterol and total lipid

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levels have not been affected by diet composition. In a study on Atlantic cod (*Gadus morhua*) juveniles, Rosenlund *et al*. (2004) observed no difference in these indices. Gao *et al*. (2011) in grass carp and Abbas and Siddiqui (2013) in red snapper (*Lutjanus argentimaculatus*) found no correlations between cholesterol and triglyceride with increasing dietary protein levels. Hemre and Sandnes (1999) in Atlantic salmon and Chatzifotis *et al*. (2010) in Meager (*Argyrosomus regius*) showed that the plasma cholesterol and triglyceride were not affected by different levels of lipid. On the other hand, it is inconsistent with likely observations (Du *et al*., 2005; Cheng *et al*., 2006; Abdel-Tawwab *et al*., 2010; Coldebella *et al*., 2011; Jin *et al*., 2013) where they found at least a significant difference in these parameters with increasing dietary protein and lipid levels.

In our study, there was no significant effect of dietary protein and lipid levels on the plasma total protein concentrations. This is in agreement with the results reported for other fish species such as *Morone saxatilis*×*M. chrysops*, Atlantic cod, grouper, *Epinephelus coioides*, Meager and sharpsnout sea bream (Gummadi and Reigh, 2003; Rosenlund *et al*., 2004; Cheng *et al*., 2006; Chatzifotis *et al*., 2010; Countinho *et al*., 2012).

Unlike these results, Hemre and Sandnes (1999) in Atlantic salmon, Abdel-Tawwab *et al*. (2010) in Nile tilapia (*Oreochromis niloticus*) and Ding *et al*. (2010) in starry flounder (*Platichthys stellatus*) found that plasma total protein was affected by diet composition, and increased with increasing dietary protein and lipid levels. Increase in plasma protein level with increasing dietary protein can be associated with increased absorption of amino acids from protein digestion (Yamamoto *et al*., 2000). Also, in diets containing higher lipid levels, there is a decrease in amino acids oxidation, resulting an increase absorption of proteins which leads to increase plasma total protein (Medale and Guillaume 2001; Ding *et al*., 2010). The discrepancy among the results of various experiments may have depended on digestion efficiency, fish weight, composition of the diet and water temperature (Grove *et al*., 1978; Darcy, 1984).

Overall, the present results indicate that protein requirement for maximum growth and feed utilization of Aspikutum juveniles is around 35%. Also, dietary lipid was effective in sparing protein for growth and tissue formation, and the optimum lipid requirement for this fish was 15%. Nevertheless, given that the Aspikutum is known as a new hybrid, further research is needed to better understand of metabolic utilization of dietary protein, lipid and carbohydrate and the effects of macro and micro-nutrients on hematological parameters, immune responses, digestive enzymes activity and digestive tract histology in various ages and sizes.

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