Fishery Technology 56 (2019) : 184 - 189

Effects of Dietary Protein Levels on Haemato-biochemical Response and DNA: RNA ratio of Pabda, *Ompok bimaculatus* (Bloch, 1794)

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Abstract

Five iso-energetic semi-purified diets with varying levels of crude protein *viz.,* P25 (25.10%), P30 (29.93%), P35 (35.28%), P40 (39.73%) and P45 (44.49%) were fed to Pabda, *Ompok bimaculatus* fingerlings (average weight 3.29±0.01 g) in triplicate groups (11 healthy fish per replicate) for a period of 60 days to study the haemato-biochemical parameters and nucleotide concentration of the fish. Fish were stocked in indoor plastic tanks, provided with 500 l of aerated freshwater in each tank. Each group was fed to satiation twice a day for 60 days. Significant differences were observed in haemato-biochemical parameter, RNA concentration and RNA:DNA ratios with different dietary protein levels. These parameters showed an increasing trend with an increase in the dietary protein levels up to P35 diets ($p<0.05$), decreased thereafter. Fish fed with low and high level protein diets showed lesser values of red blood cells, haemoglobin content, haematocrit and total plasma protein when compared with optimum protein level (35.28%). Moreover, the highest glucose level in plasma was observed in fish fed with the lowest protein diet whereas, no significant difference was observed in their WBC counts. Significantly higher ($p<0.05$) muscle RNA and RNA: DNA ratio was found in P35 fed group diets. All these parameters were negatively affected by the further increase in protein level in the diet. It may be concluded that the dietary protein level influences the haemato-biochemical response and nucleotide concentration of *O. bimaculatus,* which showed an increasing trend with increase in dietary protein to optimum level (P35) and decreased after that.

Keywords: Feed formulation, Haemato-biochemical parameters, *Ompok bimaculatus,* Protein diet, RNA: DNA ratio

Introduction

The Indian butter cat fish or Pabda, *Ompok bimaculatus* (Bloch, 1794) is an indigenous silurid (Day, 1981) which is known for its excellent taste, nutritional profile, soft bony structure, rich lipo-protein content and high market value (Biswas et al., 2018). It is considered to be a species which can be diversified towards both species and culture system diversification (Rawat et al., 2018). The healthy culture of pabda depends on the nutritional status of the supplementary feeds. Protein makes up a larger proportion of the fish feed and at the same time is also the one of the most important dietary nutrients affecting growth, reproduction, survival and yield of fish as well as economics of the farming system by influencing the feed cost (Lovell, 1998; Siddiqui & Khan, 2009). An ideal dietary protein level should ensure maximum growth and health condition of fish. Haematological indices are important tools for the evaluation of physiological response, health condition, feed quality and nutritional status of the fish under different conditions of life and environment (Svobodova et al., 2005). The changes in levels of fish blood parameters such as red blood cell, white blood cell, haemoglobin and hematocrit will provide an insight of health condition of fish (Harikrishnan et al., 2011). Hemoglobin is a protein responsible for transport of O_2 and CO_2 in fish body, and its attentiveness is closely related to red blood cell counts (Clark et al., 1979). White blood cells in fish have immune functional role and can be used to detect certain diseases and

Received 04 May 2018; Revised 24 March 2019 ; Accepted 24 June 2019

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injury in fish body (Qiang et al., 2013). Changes in blood parameters are governed in part by the nutritional status of the fish (Kumar et al., 2005; Zhou et al., 2012). RNA: DNA ratio act as an indicator of protein synthesis and thus growth in fish (Khan & Jafri, 1991; Steinhart & Eckman, 1992; Gangadhara et al., 1997). Consequently, RNA: DNA ratio increased rapidly with age of fish (Steinhart & Eckman, 1992). RNA is directly involved in protein synthesis and so increase in RNA content are observed during periods of rapid growth, while DNA content is generally constant (Bulow, 1970; Mohanta et al., 2008).

Some researchers have reported that an increased dietary protein level could raise haemoglobin concentration and red blood cell counts in fish species (Sakthivel, 1988; Abdel-Tawwab et al., 2010; Ahmed & Maqbool, 2017). Information regarding the effect of dietary protein levels on biochemical parameters of *O. bimaculatus* is very limited. Therefore by establishing the relationship between dietary protein level and haematological parameters, monitoring of fish stocks and prediction on their haematological needs will be possible. Hence, the present study attempts to relate the protein requirement of fish to the health condition with special reference to the haematological parameters and RNA: DNA ratio.

Material and Methods

Five iso-energetic experimental diets *viz.,* P25 (25.01%), P30 (29.93%), P35 (35.28%), P40 (39.73%) and P45 (44.49%) with crude protein shown in parenthesis, with energy ranging from 15.6 MJ kg^{-1} (dry matter basis) were formulated. Formulation and proximate composition of the experimental diets are presented in Table 1. These diets were formulated as per the procedure described by Rawat et al. (2018). The experimental diets were produced using a hand pelletizer with 2 mm die to prepare the pellets. The pellets were dried overnight at 40-50°C in dryer and stored in plastic bags at 4°C until used.

The study was carried out for 60 days at the wet-laboratory of College of Fisheries, Central Agricultural University (Imphal), Tripura, India. *O. bimaculatus* fingerlings (average weight: 3.29±0.01 g) were procured from the nursery tank of College of Fisheries, Lembucherra farm and stocked in 1000 l FRP tanks at the laboratory. Fish were acclimatized to laboratory conditions for two week with a commercial diet (CP=30%; COF-CAU pellet feed, India), before commencement of the study. Fifteen indoor plastic containers of 1000 l capacity each were assigned for the five dietary treatment groups namely P25, P30, P35, P40 and P45 in triplicates each. The tanks were maintained with 500 l of static water with continuous aeration and a total of eleven fish were stocked in each of the tank, following a completely randomized design. All groups of fishes were hand-fed with experimental diet twice a day to apparent satiation between 8.00 a.m. to 10.00 a.m. and 3.00–4.00 p.m. After 2 h of feeding period, unfed and faecal matters were removed, dried and weighed. An equal volume of water was added to the tank to replace the siphoned water. The water quality parameters were monitored periodically during the experimental period and important water quality parameters such as temperature (°C), pH, dissolved oxygen (mg L^{-1}), total alkalinity (mg L^{-1}), total hardness (mg L^{-1}), total ammonia (mg L^{-1}), and nitrite (mg L^{-1}) were estimated following standard methods (APHA, 2005).

Feeding was stopped 24 h before the end of experiment, three fishes per treatment were randomly sacrificed with an over dose of clove oil (50 μL L^{-1}) and were taken for haematological studies. Blood samples were drawn from the caudal vein of fish by using 1.0 ml hypo-dermal syringe and 24-gauge needle. Pooled blood samples were immediately transferred into vials coated with thin layer of EDTA. The collected blood was centrifuged at 3000 rpm for 5 min at 4°C. Plasma was collected and stored at -20°C until use. Haematological parameters *viz.,* RBC or EC (Red blood corpuscles or erythrocytes counts), WBC or LC (White blood corpuscles or leucocyte counts), packed cell volume or Haematocrit (PCV; %) and haemoglobin (Hb; g dl^{-1}) were done following the method of Schaperclaus et al. (1991).

$$EC\ mm^{-3} = \text{Erythrocytes in 80 small squares} / (80 \times 1/400 \times 1/10 \times 1/200)$$

$$LC\ mm^{-3} = \text{No. of leucocytes in 4 squares of } 1\ mm^2 / (4 \times 1/10 \times 1/200)$$

Glucose (GLU) and total plasma protein level in plasma was determined by using standard procedure as described by Khan et al. (2018) and calculated based of the following formula:

Table 1. Formulation and composition of experimental diets

Ingredients	Dietary protein levels (%) P25	P30	P35	P40	P45
Ingredients composition of the diet (%)					
Fishmeal[1]	17	17	17	17	17
Corn flour[1]	08	08	08	08	08
Broken wheat flour[1]	20	20	20	20	20
Casein[2]	14	19	24	29	34
Gelatine[2]	03	04	05	06	07
White dextrin[2]	30	24	18	12	06
Carboxymethyl cellulose[2]	01	01	01	01	01
Cod liver oil[1]	02	02	02	02	02
Soybean oil[3]	03	03	03	03	03
Vit-Min Premix[4]	02	02	02	02	02
Proximate composition of the diet (% dry matter basis)					
Crude protein	25.10	29.93	35.28	39.73	44.49
Crude lipid	5.29	5.30	5.20	5.26	5.22
Ash	4.85	4.86	4.88	5.04	4.93
Nitrogen free extract (NFE)	56.85	52.13	46.84	42.06	37.42
Energy (MJ kg^{-1})[5]	15.67	15.70	15.67	15.63	15.64
P/E ratio (g protein MJ^{-1})	16.01	19.06	22.51	25.40	28.44

[1]Obtained from College of Fisheries, CAU, Lembucherra, Agartala, India.

[2]Hi-media Laboratories, Mumbai, India.

[3]Ruchi Soya Pvt. Ltd., Raighad, India.

[4]Vit-Min premix manufactured by Anand International Biopharma, Delhi: Composition-Vit A-1,000,000 IU, Vit D-200,000 IU, Vit E-1,000 mg, Vit C-10,000 mg, Vit D1-800 mg, Vit B2-500 mg, Vit B6-500 mg, Vit B12-40 mg, L-Lysine-1,000 mg, DL Methionine-1,000 mg, Choline Chloride-2,000 mg, Niacinamide-5,000 mg, Calcium D Panthenate-1,000 mg, Biotin-150 mg, Ferrous Sulphate-28,000 mg, CuSO4-12,000 mg, ZnSO4-12,000 mg, MnSO4-6,800 mg, MgSO4-24,000 mg, CoSO4-40 mg, Sodium Selinate-20 mg, KI-240 mg, Inositol-1,000 mg

[5]Calculated by using Bomb Calorimeter

Total Glucose (mg dl^{-1}) =
Absorbance of test x 10 / Absorbance of standard

Total Protein (g dl^{-1}) =
Absorbance of test x 6 / Absorbance of standard

Similarly, three fish per treatment were randomly pooled for the nucleic acid concentration analysis. For DNA and RNA study, muscle sample from each treatment was collected 12 h after last feeding and stored at –20°C (Mitra & Mukhapadhyay, 2002). The muscle DNA analysis was conducted according to Ceriotti (1955) and the RNA by Burton (1956).

One-way ANOVA followed by Duncan's multiple range tests were performed to determine the significant differences between the means, if any. Comparisons were made at the 5% probability level. Results are presented as mean±SE (standard error).

Results and Discussion

The water quality parameters were analysed periodically during the study period and were within the optimum level for fish growth (Biswas et al., 2018). Important water quality parameters including temperature ranged between 24.7 to 28.9°C; pH, 7.2-8.6; dissolved oxygen was above 6 mg L^{-1}; ammonia-nitrogen (NH_3-N), 0.05-0.60 mg L^{-1} and nitrite-nitrogen (NO_2-N), 0.02-0.20 mg L^{-1}. The haematological parameters of *O. bimaculatus* fingerlings fed different dietary protein levels are pre-

Table 2. Haematological parameters in *Ompok bimaculatus* fingerlings fed diets containing different protein levels

Parameters	Dietary protein levels %					P-value
	P25	P30	P35	P40	P45	
Total RBC ($\times10^6$ cells mm^{-3})[1]	2.40±0.01[a]	2.69±0.01[b]	3.31±0.02[e]	3.10±0.01[d]	2.79±0.03[c]	0.008
Total WBC ($\times10^4$ cells mm^{-3})[2]	46.92±1.20	47.99±2.03	48.78±1.16	48.20±2.87	48.11±1.48	0.815
Hb (g%)[3]	5.54±0.29[a]	8.53±0.14[b]	10.57±0.38[d]	9.50±0.28[c]	8.22±0.20[b]	0.000
PCV (%)[4]	25.33±0.88[a]	29.00±0.58[b]	36.00±0.58[d]	32.00±0.58[c]	24.67±0.88[a]	0.001

*Mean values ± SE; Mean values sharing the same superscript are not significantly different ($p>0.05$). [1]Red blood cell count; [2]White blood cell count; [3]Haemoglobin concentration; [4]Haematocrit

sented in Table 2. In the present study, significant difference was observed in RBC, haematocrit and haemoglobin concentration among dietary protein treatments, showing a general trend of linear increase with the increase of dietary protein level up to P35 and then decreases beyond that. Highest RBC (3.31±0.02 mm^{-3}), haematocrit (36.00±0.58%) and haemoglobin (10.57±0.38 g dl^{-1}) values were found for fish fed with P35 dietary proteins, whereas significantly lowest value was noted ($p<0.05$) in both higher (P45) and lower (P25) protein diets. The increase in RBC count at the optimal protein (P35) level might be due to its early release from the storage pool in the spleen, therefore, haemoglobin synthesis occurs in the erythrocytes following RBC release into circulation (Tibbetts et al., 2005; Qiang et al., 2013; Ahmed & Maqbool, 2017). However, a significant reduction of red cells at lower and higher protein diets was also observed. Because of a lack of sufficient data on haematology of this species, it is impossible to decide whether the reduction in RBC counts caused from the dietary treatment or an experimental error. Further, the study also predicted that the reduction of red cells at lower (P25) and higher protein diets (P45) is possibly due to the inhibition of erythropoiesis or by the destruction of red cells (Sakthivel, 1988; Qiang et al., 2013). In concurrent to this finding, Sakthivel (1988) reported that the haemoglobin and RBC count of common carp (*Cyprinus carpio*) at 38% dietary protein level were significantly higher than those at 14 and 58% dietary protein level. Similar observations were reported in species such as Nile tilapia (Abedel-Tawwab et al., 2010) and common carp (Ahmed & Maqbool, 2017). However, the fish fed at varied levels of dietary protein didn't show any difference ($p>0.05$) in their WBC counts. A similar observation was also reported by Ahmed & Maqbool (2017) in common carp. The biochemical parameters (Total protein and Glucose) of pabda fingerlings are presented in Fig. 1. In the present study, the plasma glucose level was found to be significantly higher ($p<0.05$) in the P25 group than in the other groups. A similar finding was also reported in other fish species such as red spotted grouper (Wang et al., 2016) and bluegill sunfish (Yang et al., 2016). Besides, a significantly higher total plasma protein was observed in P35 treatment group followed by P40 group compared to the other groups. Wang et al. (2016) reported that the plasma protein could reflect the protein metabolism in vivo and the growth of fish. Hence, the fish that were fed diets with moderate dietary protein levels had comparatively higher plasma protein content than those fed with diets containing 25.10 and 44.49% protein.

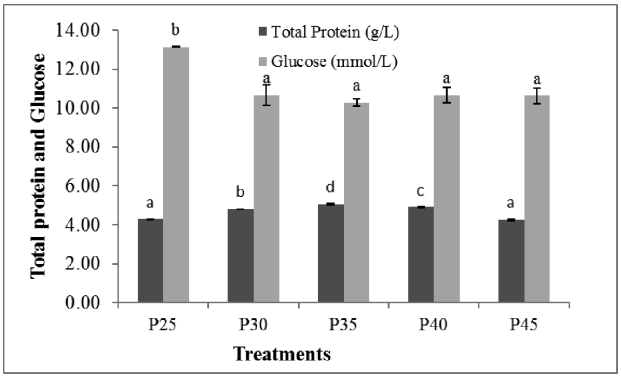

Fig. 1. Total protein (g L^{-1}) and Glucose (mmol L^{-1}) of *Ompok bimaculatus* fingerlings fed with diets containing different protein levels. Data (mean±SE) with different letters (a, b, c & d) significantly differ ($p<0.05$) among different experimental groups.

Nucleic acids play a vital role in growth and development. DNA is a prerequisite for RNA

Table 3. RNA and DNA concentrations in *Ompok bimaculatus* fingerlings fed diets containing different protein levels

Parameters	Dietary protein levels %					P-value
	P25	P30	P35	P40	P45	
Nucleic acid concentration						
Muscle RNA[1]	1.20±0.01[a]	1.26±0.01[b]	1.39±0.01[d]	1.31±0.01[c]	1.26±0.01[b]	0.000
Muscle DNA[1]	0.35±0.00	0.35±0.00	0.36±0.00	0.35±0.00	0.35±0.00	>0.05
RNA : DNA ratio	3.46±0.02[a]	3.64±0.06[b]	4.05±0.07[d]	3.81±0.04[c]	3.64±.0.02[b]	0.000

*Mean values with the same superscripts in each row are not significant (p>0.05). Values are means ± S.E. [1]μg mg^{-1} muscle tissue

synthesis, which is required for protein synthesis (Mitra & Mukhopadhyay, 2002; Labh, 2015). The RNA and DNA concentration and RNA: DNA ratios of the fingerling *O. bimaculatus* were shown in Table 2. The DNA content of a cell remains stable under changing environmental conditions and hence acts as an indicator of biomass (Mohanta et al., 2008; Labh, 2015). In the present study, the muscle DNA content was almost constant (i.e. 0.35–0.36 μg mg^{-1} muscle tissue) in all the dietary groups. The muscle RNA content and RNA: DNA ratio was significantly higher at its optimum protein fed group (P35) than that of the fish fed with low and high protein diets. This elevated RNA: DNA ratios were related with higher levels of RNA and increase in RNA: DNA ratios in *O. bimaculatus* at its optimum protein level corresponding to growth increment which could be attributed to higher protein synthesis. Mohanta et al. (2008) also found that the RNA: DNA ratio increased as the dietary protein increased from 203 to 301 g kg^{-1} in the diet fed to silver barb but it was reduced by a further increase of dietary protein to 400 g kg^{-1}. Similar observations are reported in other warm water fish species like Rohu (Gangadhara et al., 1997; Labh, 2015) and welleye (Barrows et al., 1988).

Hence, it can be concluded that the dietary protein level influences the haemato-biochemical composition and nucleotide concentration of *O. bimaculatus,* which showed an increasing trend with increase in dietary protein to optimum level (P35) and then decreased beyond that optimum level.

Acknowledgements

The authors are thankful to the Vice-Chancellor, Central Agricultural University, Imphal, India for their encouragement and providing facilities to carry out the research.

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