Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Hematological, Histopathological and Growth Performance Studies on Albino Rats Fed on *Mystus gulio* Fillet with Formulated Cereal

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(Received: 3 June 2018 Accepted: 10 November 2018)

KEYWORDS

Growth performance;
Toxicological effect;
Mystus gulio;
Nona Tengra

ABSTRACT: The growth and development of human body is dependent on the protein, carbohydrate, vitamins and minerals. Fishes are one of the major sources of protein, vitamins and minerals for human. This study was conducted to know the effect of *Mystus gulio* fish fillet on growth performance of albino rats after feeding different concentrations of fish fillet as a protein source with formulated cereal for 32 days. Haematological and histopathological analysis were performed between control group and examined groups of albino rats. It was noticed that the body weight of each experimental albino rat was increasing gradually with increasing fish fillet up to 15% and beyond this the value was decreased. Moreover, the highest protein efficiency ratio (PER) and calorie efficiency ratio (CER) were observed 2.53% and 13.02% respectively for 15% supplied fish fillet. The gained in body weight (GBW) of albino rats in each experimental group depended mainly on supplied fish protein concentration but not on formulated cereal. According to hematological and histopathological studies, no toxicological effect was observed between control group and experimental groups of rats treated with different fish protein concentration for 32 consecutive days. Supplement of 15% fish fillet with formulated cereal was required to produce maximum nutritive values and the *Mystus gulio* could be considered as protein rich human diet.

INTRODUCTION

For human and all animals, protein is an essential constituent of diet and source of essential and non-essential amino acids. Without ingesting the essential amino acids, death is inevitable. Inadequate protein or amino acid results in muscle loss, impaired immune system and poor hair coat quality [1]. However, high dietary protein intake is associated with increased risk of kidney disease [2, 3], increased bone loss [4] and may increase formation of calcium oxalate crystals [5] in human body. Foods with high protein concentration and imbalance of insulin sensitivity may put the human at risk for other disease related to excess nitrogen metabolism or mobilization of calcium [6] in body tissue. Any toxicological effect associated with these tissues and blood can be investigated through hematology and histopathological studies. Hematology is the study of blood, blood forming

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DOI: 10.22034/jchr.2019.545640

tissues and relative substances concerned in blood production, conservation and destruction of blood and their disorders. Histopathology refers to the microscopic examination of tissue in order to study the manifestations of disease [7-9].

Diet containing balance protein is essential for maintaining sound health. Fishes are the principle source of proteins and minerals (both micro and macro minerals) [10]. These are known to play important roles in physiological processes and in certain diseases. Nutritional studies have proved that fish protein is ranked in the same class of chicken portions but are superior to milk, beef protein and egg. Traditionally the South Asian people consume fishes of fresh water and near shore brackish water origin [11]. However, nature has endowed Bangladesh lavish sources of both marine and fresh water fishes. The people of Bangladesh, India and Srilanka are highly dependent on fish protein due to the availability and low price. *Mystus gulio*, locally known as Nona Tengra, is a brackish water fish available in South-Western coastal area of Bangladesh. This species is preferred by the people of our country for its delicious tastes, therapeutic values and availability throughout the year [10]. Among the four species (namely *Mystus gulio*, *Mystus bleekeri*, *Mystus cavasius* and *Mystus vittatus*), *Mystus gulio* contains highest amount of protein 22.2±0.94% and lipid 5.25±0.64% that can be conventionally used to improve the protein quality in our diet [10]. Considering the nutritional significance of protein content in this fish, the effect of fish protein concentration on growth performance was evaluated by determining GBW, PER and CER in the young albino rats after feeding different amount of fish fillet with formulated cereals [12]. Moreover, to assess the toxicological effect of this fish the hematological and histopathological studies were performed by determining the parameters like hemoglobin, red blood cells (RBC), erythrocyte sedimentation rate (ESR), cholesterol, white blood cells (WBC) and total count of WBC [7,13]. For this purpose, the albino rats in both control groups and experimental groups were assaulted by maintaining the all precaution and their blood, liver, heart, lung, spleen and kidney were collected and preserved in freeze for analysis. These experiments were carried out by observing any changes in the cellular structures (degradation and regeneration) of these organs of the experimental rats after received fish fillet at different fish protein concentration levels for 32 days with respect to control group. Although *Mystus gulio* is popular among the people in our country, still now there is insufficient information available on the nutritional value and toxicological effect of this species. Thus, the main objective of this study was to establish the nutritional significance and hygienic value of *Mystus gulio* as protein rich diet to the Bangladeshi people.

MATERIALS AND METHODS

Experimental animal

Twelve male albino rats (Wistar Kyoto Outbreed) having body weight 50-60 g were used in this study. The experimental animals were kept in an animal house in Biochemistry and Molecular Biology, Rajshahi University, Bangladesh and formulated cereal and water had fed them for 32 days. These animals were distributed randomly into six groups (diet-A, diet-B, diet-C, diet-D, diet-E and diet-F). Each experimental group contained two animals and they were almost in similar size and weight. Diet-A was considered as controlled group whereas diet-B, diet-C, diet-D, diet-E and diet-F were experimental group.

Diet formulation and compositional analysis of fish protein

Formulated cereal ingredients were collected from International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB). The major ingredients of formulated cereal were corn, wheat and wheat bran, gram, til oil cake, soya-44, salt, fish meal-60, rice polishing, and soybean oil. The quantitative ingredients composition and nutritive values of this formulated cereal have been shown in Table 1 and Table 2. Five experimental diets (diet-B, diet-C, diet-D, diet-E and diet-F) were prepared by supplementing fish protein concentration with formulated cereal. In these experimental diets the level of fish protein concentration (FPC) and fat content were 1.11 % to 5.55 % and 0.2625 % to 1.3125 % (Table 3). The percentage of total protein and fat in different diets were 21.00 to 21.30 and 5.00 to 5.0625 respectively.

Table 1. Compositions of formulated cereal.

Ingredients	% of ingredients	Ingredients	% of ingredients
Corn	12.76	**Sorbatox**	0.25
Wheat	30.00	**Limestone**	0.30
Wheat bran	20.00	**Dichloride phos-phate**	0.25
Til oil cake	6.00	**Choline chloride**	0.10
Gram	8.00	**Uitamine premix**	0.30
Soya-44	3.00	**Salt**	0.50
Skimoned milk powder	1.00	**Fish meal-60**	10.00
Lysin	0.09	**Soyabean oil**	1.00
Feedzine	0.05	**Rice polishing**	6.00
Sal kill	0.04		

Table 2. Nutritive values of formulated cereal.

Proximate compositions of ingredients	% in formulated cereal
Protein	21.00
Fiber	5.15
Ash	5.90
Fat	5.00

Table 3. Composition of experimental diets.

Name of diets	Composition of diets (g)	% of protein(g) in diets	% of fat (g) in diets	Total protein in diets (g)	Total fat in diets (g)
Diet A	100% FC only	21	5	21	5
Diet B	5% FF+ 95% FC	FF =1.11, FC =19.95	FF =0.2625, FC =4.750	21.06	5.0125
Diet C	10% FF+ 90% FC	FF =2.22, FC =18.90	FF =0.5250, FC =4.500	21.12	5.025
Diet D	15% FF+ 85% FC	FF =3.33, FC =17.85	FF =0.7875, FC =4.250	21.18	5.0375
Diet E	20% FF+ 80% FC	FF =4.44, FC =16.80	FF =1.050, FC =4.000	21.24	5.050
Diet F	25% FF+ 75% FC	FF =5.55, FC =15.75	FF =1.3125, FC =3.750	21.30	5.0625

Where, FF= Fish Fillet, FC= Formulated cereal

Maintenance of animals

The animals in controlled and experimental groups were housed individually in stainless steel cages with screen bottoms. The animal house was well-ventilated, hygienic and kept optimum room temperature with exposed light and dark cycle of 12 h duration each. Both controlled and experimental animals had an access to 20 g of food in every 2-4 h interval during 32 days. The mean weight of controlled and experimental animals was noted after 4 days interval.

Calculation of PER and CER

Protein efficiency ratio (PER) was defined as GBW per one gram of crude protein intake [12, 14]. Calorie efficiency ratio (CER) was calculated from gain in body weight (g) per 100 calories intake during the experiment [12].

$$\text{PER} = \frac{\text{Gained body weight}}{\text{Protein intake by each rat}} \quad (1)$$

$$\text{CER} = \frac{\text{Gained body weight}}{\text{Calorie intake by each rat}} \times 100 \quad (2)$$

Haematological and histopathological analysis

At the end of 32 days, the albino rats were starved for overnight and sacrificed under mild ether anesthesia. Blood were collected from each rat of either group by cardiac puncture over EDTA for the measurements of various hematological parameters [15]. Hemoglobin (Hb) was measured according to the acid haematin method of Shali [15]; peripheral count of RBCs and total count of white blood cells (WBCs) and differential count of WBCs were measured according to manual method with the help of hematocytometer [7]. Erythrocyte sedimentation rate (ESR) was determined by Westergren Method. In this method, the blood was mixed with a suitable anticoagulant agent (3.1% trisodium citrate solution) and was made to stand vertically [7]. Blood serum cholesterol was determined by spectrophotometrically according to Leibermann-Burchard reaction method [7]. For histopathological investigation, the liver, kidney, heart, lung and spleen of all rats were isolated, weighted and recorded. The total liver protein and total liver fat were estimated according to Bligh-Dyer method and Microkjeldahl's method respectively [16]. A small amount of these tissues were separately sliced, fixed in 10% formalin and processed for paraffin sections. After hematoxylin-eosin staining, the section of the control group and experimental groups were carefully examined under high power microscope and were recorded by photographs. The histopathological and hematological examinations were carried out at Rajshahi Medical College, Rajshahi, Bangladesh.

RESULTS AND DISCUSSION

Fed intake and body weight gain

Initial body weights, total food consumed, GBW for each rat after 32 days and weight of organs has been shown in Table 4. The table describes that the body weight of all rats increased gradually with increasing the fish protein concentration in spite of having same amount of food. Moreover, the given results also confirmed that the GBW of each rat mainly depended on supplied fish protein concentration, not on formulated cereal.

Table 4. Weight of different organs of twelve albino rats fed on FPC (*Mystus gulio*) at different protein levels supplemented with formulated cereal.

Diets	Rat No.	Initial body weight (g)	Food consumed (g)	Final body weight (g)	GBW (g)	Weight of organs (g)				
						Liver	Kidney	Lung	Spleen	Heart
Diet A	a	54.64	230.80	117.12	62.48	3.99	0.99	0.51	0.24	0.36
	b	57.50	232.87	121.06	63.56	4.37	0.64	0.49	0.25	0.31
Diet B	a	61.02	215.71	142.54	81.52	4.68	0.82	0.52	0.31	0.51
	b	55.75	225.50	134.12	78.37	5.30	0.95	0.72	0.34	0.54
Diet C	a	53.89	219.36	152.13	98.24	5.02	0.91	0.61	0.29	0.39
	b	61.67	235.85	163.97	102.30	5.49	0.93	0.81	0.34	0.43
Diet D	a	59.53	215.02	175.45	115.92	3.99	0.96	0.59	0.30	0.46
	b	56.25	220.15	172.61	116.36	5.33	1.06	0.69	0.36	0.48
Diet E	a	63.15	224.60	182.65	119.50	4.86	0.83	0.63	0.28	0.61
	b	58.17	278.36	164.52	106.35	6.17	1.09	0.78	0.38	0.65
Diet F	a	58.90	262.09	179.20	120.30	4.99	0.77	0.71	0.28	0.45
	b	59.82	290.50	177.32	117.50	4.51	0.98	0.77	0.42	0.46
Reference Values [17]						**3.51-5.35**	**0.75-1.08**	**0.56-0.81**	**0.22-0.33**	**0.29-0.54**

During the experimental period, the GBW for each rat was noted in every four days (Table 5). The mean GBW of all rats increased with increasing days and reached at maximum values of 63.02g, 79.95g, 100.25g, 116.14g, 112.93g and 118.9g respectively.

Table 5. Mean GBW at every four days interval of male albino rats after feeding FPC at different levels supplemented with formulated cereal.

Days	Mean GBW (g) at every four days interval					
	Diet A	**Diet B**	**Diet C**	**Diet D**	**Diet E**	**Diet F**
4	7.56	8.99	13.25	25.90	40.33	45.00
8	17.55	23.78	35.65	55.00	70.45	82.45
12	32.60	42.34	55.34	80.56	90.56	100.00
16	45.65	59.34	76.65	100.00	103.12	111.56
20	56.34	70.23	89.67	110.00	107.9	114.13
24	61.50	75.26	95.62	112.34	112.63	115.84
28	62.85	77.90	98.56	114.20	112.82	117.23
32	**63.02**	**79.95**	**100.27**	**116.14**	**112.93**	**118.90**

According to Table 6 it was revealed that the mean GBW per one gram of food intake, PER and CER increased along with increasing protein concentration. The maximum value of these parameters was observed 0.534g, 2.53 and 13.02 respectively in diet-D.

Table 6. The effect of feeding FPC at different levels supplemented with formulated cereal on GBW per one gram food, PER and CER

Diets	Rat No.	GBW (g) per one gram food	Mean	Protein (g) intake by each rat	PER	Mean PER	Calorie intake by each rat	CER %	Mean CER (%)
A	a	0.270	0.272	48.47	1.29	1.30	946.28	6.60	6.63
	b	0.273		48.90	1.30		954.77	6.66	
B	a	0.378	0.363	45.43	1.79	1.72	884.41	9.22	8.85
	b	0.348		47.49	1.65		924.55	8.48	
C	a	0.448	0.441	46.33	2.12	2.09	899.38	10.92	10.75
	b	0.433		49.81	2.05		966.99	10.58	
D	**a**	**0.539**	**0.534**	**45.54**	**2.55**	**2.53**	**881.58**	**13.15**	**13.02**
	b	**0.529**		**46.63**	**2.50**		**902.61**	**12.89**	
E	a	0.532	0.457	47.70	2.51	2.16	920.86	12.98	11.15
	b	0.382		59.12	1.80		1141.28	9.32	
F	a	0.460	0.432	55.83	2.15	2.03	1074.57	11.20	10.54
	b	0.404		61.88	1.90		1191.05	9.87	

When the levels of protein were further increased through FPC, the GBW per one gram of food, PER and CER were noticeably decreased from diet-E (0.457g, 2.16 and 11.15) and diet-F (0.432g, 2.03 and 10.54 respectively). Rats under diet-A had less effect on GBW (0.272g) per one gram of food intake, PER (1.30), CER (6.63) with compared to others. According to this data, it was found that diet-D had highest PER and CER followed by diet E, C, F, B, and A. It was also significant that PER values were directly proportional to their respective CER values. From the values of PER, CER and also GBW per one gram of food in Table 6, it was inferred that the effect of 15% FPC in diet-D was remarkably high.

Hematological and histopathological changes

According to Table 7, it could be noticed that hematological parameters of all experimental rats were almost similar to that of control group rat. The values of hematological parameters were within the normal range when compared to reference values provided by Mitruka and Ramsley in Table 8 [17].

Table 7. Hematological profiles of albino rats for feeding FPC (*Mystus gulio*) at different protein levels supplemented with FC after 32 days

Hematological parameters	Rat No.	Control rat with FC	Percentage of fish filet of *Mystus gulio* in diet with formulated cereal				
			Diet B	Diet C	Diet D	Diet E	Diet F
Total RBC count (10^6/µl)	a	7.5	7.9	9.2	9.8	8.2	8.1
	b	8.1	7.6	8.5	9.4	8.6	7.6
	Mean	7.8	7.75	8.85	9.6	8.4	7.85
Hemoglobin (g/dl)	a	14.3	14.89	16.43	15.61	13.14	15.77
	b	13.9	15.20	14.82	16.51	13.63	14.91
	Mean	14.1	15.04	15.6	16.06	13.38	15.34
Cholesterol (mg/dl)	a	134	140	146	150	154	160
	b	138	139	144	152	158	162
	Mean	136	139.5	145	151	156	161
Erythrocyte sedimentation rate (ESR)(mm)	a	05	03	01	02	07	03
	b	04	05	02	03	04	04
	Mean	4.5	04	1.5	2.5	5.5	3.5
Total WBC Count (10^3/µl)	a	9.20	14	12	14	09	13
	b	12.0	13	10	15.8	11	10
	Mean	10.6	13.5	11	14.9	10	11.5
Differential count a) Neutrophil (%)	a	07	16	09	20	6.5	13
	b	10	11	17	13	10.0	17
	Mean	8.5	13.5	13	16.5	8.25	15
b) Lymphocytes (%)	a	74	80	72	79	90	85
	b	76	77	86	81	83	90
	Mean	75	78.5	79	80	86.50	90
c) Monocytes (%)	a	02	04	02	03	02	02
	b	01	02	04	02	03	01
	Mean	1.5	3	03	2.5	2.5	1.5
d) Eosoniphils (%)	a	0.5	0.25	0.50	03	0.54	0.55
	b	01	0.50	0.40	02	01	0.60
	Mean	0.75	0.37	0.35	0.45	0.77	0.57

Table 8. Reference values for hematological parameters of the Sprague Dawley rats [17].

RBC ($\times10^6$/µl)	TWBC ($\times10^3$/µl)	LYMPH (%)	NEUT (%)	MON (%)	EOS (%)
6.26-8.98	9.4-14.9	72-94	4.5-23.3	0.5-3.5	0.35-0.6

For assessing histopathological parameters, the weight of liver, kidney, heart, lung and spleen of all rats were compared to the reference values for Spague Dawley rats and observed no abnormalities (Table 4). During the whole experimental period, locomotive behavior, central nervous system, excitation, weakness, reflexes, salivation, urination, diarrhea etc. of all albino rats were closely monitored and found normal. Histopathological changes in heart, liver, lung, kidney and spleen of all rats were observed normal as stated in Table 9. No morphological changes were noticed in their microscopic view in displayed Figures 1-5.

Table 9. Histopathological examination of the section of Heart, Livers, Kidney, Lung and Spleen of all rats (Both control group and experimental groups)

Observation	Liver	Kidney	Heart	Lung	Spleen
Control group	No change	Glomerulus's. No inflammatory cell was observed	Cardiac muscle, no morphological change was occurred and no congestion was found	Alveoli, no congestion was occurred	No abnormalities were detected
Experimental Groups	No abnormalities were detected	No evidence of pathological change	As same as control group	No change were occurred	Same as control group

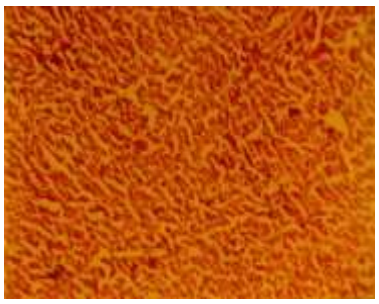

Figure 1a. Heart tissues of control rat

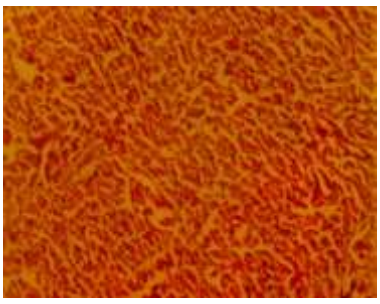

Figure 1b. Heart tissues treatment with 10 % FPC

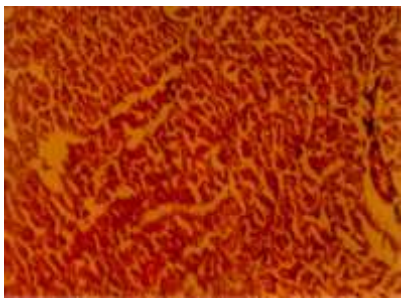

Figure 1c. Heart tissues treatment with 20% FPC

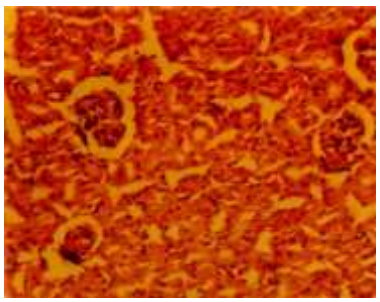

Figure 2a. Kidney tissues of control rat

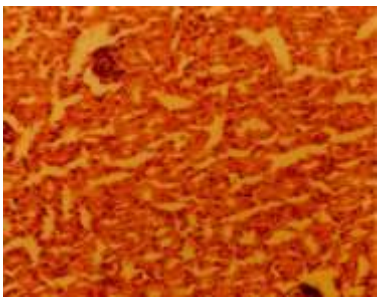

Figure 2b. Kidney tissues treatment with 10 % FPC

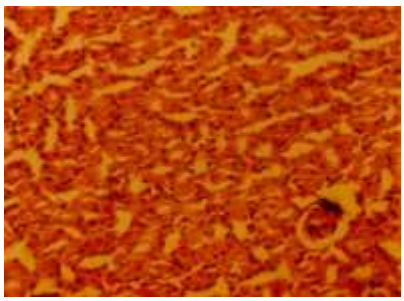

Figure 2c. Kidney tissues treatment with 20% FPC

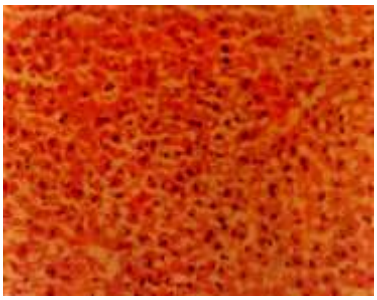

Figure 3a. Liver tissues of control rat

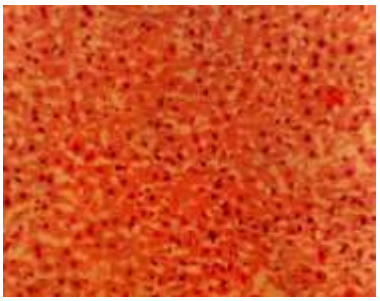

Figure 3b. Liver tissues treatment with 10 % FPC

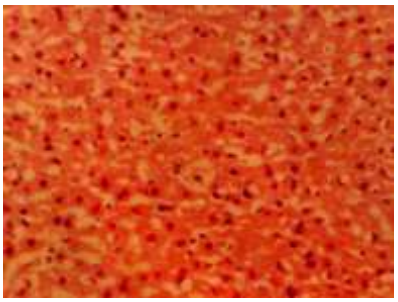

Figure 3c. Liver tissues treatment with 20% FPC

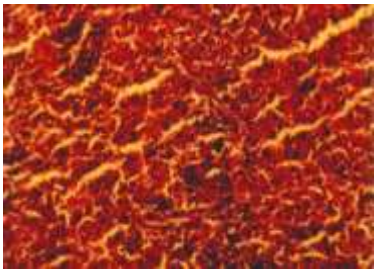

Figure 4a. Spleen tissues of control rat

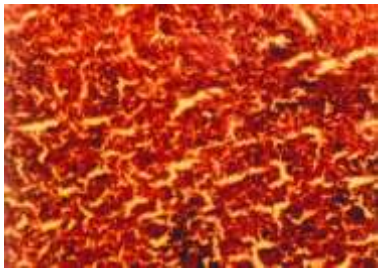

Figure 4b. Spleen tissues treatment with 10 % FPC

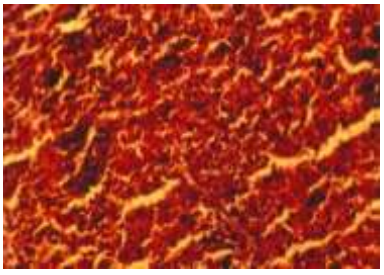

Figure 4c. Spleen tissues treatment with 20% FPC

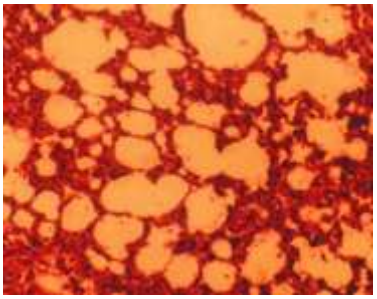

Figure 5a. Lung tissues of control rat

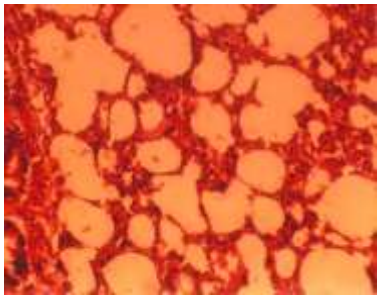

Figure 5b. Lung tissues treatment with 10 % FPC

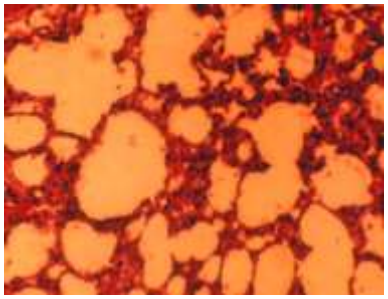

Figure 5c. Lung tissues treatment with 20% FPC

CONCLUSIONS

Hematological studies indicated no obvious differences in any one of the hematological parameters between control group and experimental groups. Microscopic examination of treated tissues in all group of rats had no abnormalities and no remarkable effect was observed. From this study it was suggested that the fish species *Mystus gulio*, the long whiskers catfish could be considered as a cheap protein rich diet as well as hygienic to the Bangladeshi people without any health risk.

ACKNOWLEDGMENTS

The authors thank to Department of Biochemistry & Molecular Biology, Applied Chemistry & Chemical Engineering, Rajshahi University, Rajshahi, Bangladesh for giving technical and financial support. The authors are also grateful to Rajshahi Medical College, Rajshahi, Bangladesh for giving bio-technical support.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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