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Biochemical effects of commercial feedstuffs on the fry of climbing perch (*Anabas testudineus*) and its impact on Swiss albino mice as an animal model

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ABSTRACT

This study assesses the biochemical effects of commercially available fish feedstuffs on the fry of climbing perch (*Anabas testudineus*). Subsequently, its impact on experimental animal, Swiss albino mice, is also examined. In order to access the impact of commercial fish feed and feed consumption fish on the experimental animal, the proximate, biochemical and histopathological analyses were done using standard methods. The proximate composition as well as the concentrations of Pb, Ni, Mn, As, Zn, and Cd in the fish feed, different parts of the *A. testudineus* fish and different parts of the *A. testudineus* fish-treated experimental mice liver, were all determined using Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry. The highest levels of Cr, Pb and As were observed in the liver of Swiss albino mice treated with FFT2 and FFBB2 and their concentrations were 0.156, 0.491, 0.172 μg/g and 0.166, 0.771, 0.157 μg/g respectively. No significant changes of protein, fat, crude fiber, moisture and ash contents were observed after proximate composition analysis of fish feeds, *A. testudineus* and *A. testudineus* treated experimental mice.

Significant amounts of heavy metals (Cr, Mn, Zn Cu, Ni) were found in fish feed, different parts of *A. testudineus* fish and in the experimental mice. However, remarkably high amounts were observed in the *A. testudineus* fish's head and bone with body parts. Biochemical analysis of blood samples of *A. testudineus* fish treated experimental mice indicated that the cholesterol, TG, LDL and glucose levels were significantly higher. Yet no significant alteration in the HDL level was observed when compared to the control. In histopathological analysis, a remarkable degeneration was observed in the liver and kidney of *A. testudineus* treated mice. It can therefore be concluded that although *A. testudineus* has nutritional benefits the quality of this fish may be compromised as a consequence of contamination through various anthropogenic activities. This analysis suggests the commercial fish feed producers must take special caution to reduce the toxic metals in various fish feed products and make it nutritionally rich and safe for fish to eat. Finally, it needs to be safe for human consumption as well.

1. Introduction

Fish play a critical role in the Bangladeshi diet, providing more than 60% of animal-source food, representing a crucial source of micro-nutrients, and possessing an extremely strong cultural importance to people. Subsequently, the consumption of fish has significant implications for national food and nutrition security, poverty and growth. Official Department of Fisheries (DOF) statistics estimate total fish production of 2.56 million tons per year of which aquaculture accounts for 39% [1]. In Bangladesh, fish constitutes an irreplaceable animal-source food in the diet of millions of people, both in terms of: firstly, quantity, accounting for approximately 60% of animal protein intake at 18.1 kg consumed per person per year; and secondly, frequency of consumption, far exceeding that of any other animal-source food.

Bangladesh currently possesses diverse and abundant aquatic resources with 267 freshwater fish species and an annual production of 3.1 million tones. The south Asian nation is also a developing country experiencing an expansion in its aquaculture industry, to the extent that it is now the world's fastest growing food production sector. This is also a period witnessing a decline in capture fisheries. While it remains largely successful in increasing supply to meet the demands of a growing population, the extent to which the growth of aquaculture has been able to mitigate reduction in dietary diversity and micronutrient intake from the diverse but waning capture fisheries sector, is questionable. The fisheries and aquaculture industry sector has been recognized as a key resource in tackling food and nutrition security issues and features prominently in the country's national development agenda [2].

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The species known as climbing perch (*Anabus testudineus*, Bloch) and locally as koi is a well-known member of the Anabantoidie family. This species is indigenous to South and Southeast Asia including Bangladesh, Pakistan, Nepal, Sri Lanka, Myanmar, Thailand, Vietnam, Indonesia, Singapore and China. It is found in fresh and brackish waters but mostly in these regions' ponds, lakes and swamps [3]. It was introduced into Bangladesh from Thailand in 2002 but what hinders the culturing of this species are the availability of quality seeds and appropriate culture techniques [4]. This fish is locally known as koi, is highly esteemed for its high degree of nourishment and prolonged freshness out of water, and has proved to be a valuable addition to the diet for sick and convalescing people. *A. testudineus* has medicinal properties such as disease prevention and slowing down the ageing process for females. This fish is suitable for cultivation in ponds, reservoirs and rice fields [3]. However, the feedstuffs, disinfectants, and antibiotics employed for the rapid growth of fish are hazardous. For instance, in 2011 and 2012, a survey on the use of chemical and biological products was conducted using structured questionnaires administered to operators of nine farm groups including homestead ponds of carps, tilapias, koi fish, shrimps, prawns, and pangas fish.

Forty-six chemical and biological products were reported as being applicable in aquaculture-related activities. In comparison with other farm groups the use of disinfectants and antibiotics reached its highest level in intensive koi and pangas farms. The study identified a large number of compounds that are currently in use, and requiring further regulation and evaluation for their potential environmental and human health impacts [5].

In aquaculture, feedstuffs can be a source of hormones or persistent pollutants which act as potential endocrine disruptors (EDs). The endocrine system's normal functioning is vital for the proper development and reproduction of animals throughout the world. Substances interfering with its homeostasis are known as endocrine disruptors (EDs) and may represent a serious risk to the health of an organism. One of the mechanisms of endocrine disruption that has attracted great attention in recent year's concerns alterations in the normal functioning of the estrogen receptor (ER), yet far less attention have been paid to those substances interfering with the thyroid axis. This demonstrates that thyrogenic activity occurs in commercially available fish diets commercially and is widely used in aquaculture. Given that maintaining the homeostasis in the endocrine system is critical for the proper development and reproduction of fish [6].

Apart from these factors, fish feeds contain significant concentrations of contaminants, many of which can bioaccumulate and bioconcentrate in fish. Organochlorine (OC) contaminants are present in fish oils and fish meals utilized in feed manufacture, leading some researchers to speculate that all fish feeds contain measurable levels of some contaminants. To determine the concentration of contaminants in feeds used in the US Fish and Wildlife Service's National Fish Hatcheries, samples of feed from 11 cold-water fish hatcheries were systematically collected. At least one polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated dibenzofuran (PCDF), polychlorinated biphenyl (PCB) congener, or dichlorodiphenyltrichloroethane (DDT) metabolite were found in all samples. Overall, while contaminant concentrations were low, the ecological impacts cannot be determined without measuring the level of bioaccumulation of these compounds in the fish and the fate of these compounds after the fish are released [7].

In the Eastern Mediterranean Sea, trace element concentrations in sediment were investigated at four fish farms. Fish feed is richer in P, Zn and Cd than reference and impacted stations. An assessment among impacted stations and the respective reference stations reveals that, in anoxic sediments, all elements had higher concentrations at the impacted stations than at reference stations. Other elements (Cr, Pb, Mn) can also trigger unwanted outcomes when compounded with elevated background levels [8].

As these perilous heavy metals are bioaccumulated and bioconcentrated in fish and by rotation entered into food chain, so human being will be distressed by these heavy metals. Therefore, special concern needs to diminish these problems otherwise these persistent pollutants will devastate human health by causing damages in kidney, liver, brain and so on. The objective of this study was to conscious people about the available and highly growing commercially cultured *A. testudineus* which accumulate heavy metals in their body in turns human being consume it as protein source.

2. Methods and materials

2.1. Sample collection and preparation

Two commonly fish feed treated *A. testudineus* were collected from a particular fish farm. The collected samples were washed with distilled water to remove any contaminated particles. This was followed by the samples being cut into small pieces using a clean ceramic knife, and then they were washed with distilled water several times Samples were dried in an oven at 100 °C until a constant weight was achieved. After drying the samples were ground into a fine powder using a ceramic mortar and stored in polyethylene bags until required for heavy metal analysis, biochemical analysis and for the preparation of the diet for experimental mice.

2.2. Preparation and design of the animal experiment

The work was carried out at the Department of Genetic Engineering and Biotechnology, Jessore University of Science and Technology (Bangladesh). To study the effect of fish feed on the biochemical composition of *A. testudineus* and its impact on lipid profiles, liver and kidney functions of Swiss albino mice, eighteen male Swiss albino mice (weighting between 35 and 40 g) were collected from ICDDRB (Dhaka, Bangladesh). The mice were fed ad libitum on a basal diet (BD) and water for 15 days as an adaptation period. They were housed individually in stainless steel cages and divided into six groups of 3 animals in each group. Two different types of commercial fish feed were purchased from a local market and served as a diet for the experimental *A. testudineus* fish. The used fish feeds were designated as FF1 and FF2. These feeds were used as a diet for the same age group of *A. testudineus* fish in the two different ponds; feeding continued for 30 days. A total of 30 kg of fish feed were provided in each pond three times a day.

To observe the effects of different parts of the *A. testudineus* on experimental mice, healthy, disease-free fry climbing perch (*A. testudineus*) aged 45–50 days (65–85 gm) and 75–80 days (225–250 gm) and their respective feeds (collected randomly from the local farm) were used as diet for the experimental mice. Only the head, body with bone, body without bone and tail parts of the *A. testudineus* (45–50 days old and 75–80 days old) were taken to prepare the experimental mice's diet. The fish was cut into small pieces and sun dried for seven days before crushing. The ground samples were stored in sampling bottles. The samples FFH1, FFB1, FFBB1 and FFT1 (45–50 days old *A. testudineus* fish feed with FF1 fish feed) and FFH2, FFB2, FFBB2 and FFT2 (75–80 days old *A. testudineus* fish feed with FF2 fish feed) were used as experimental diets for mice throughout the study period. Eighteen Swiss albino mice from 4 to 5 week sold with an initial weight of 35–40 g were randomly divided into six groups of 3 animals in each one and fed: powdered *A. testudineus* fish samples (FFH1, FFB1, FFBB1,FFT1 and FFH2, FFB2, FFBB2, FFT2); and fish feed samples (FF1 and FF2) of selected amounts (6 gm of body weight). Three mice were in the control group and they were fed normal parched rice, cereal, etc. Their food intake was monitored daily and all the mice fasted before blood sampling. The mice were anesthetized using diethyl ether. The increase of weight in mice was recorded weekly.

2.3. Proximate composition analysis

Proximate compositions of the various parts of the *A. testudineus* fishes were determined using AOAC methods (2005). All analyses were

conducted in triplicate. Moisture content was measured by weighing differences before and after oven drying at 100–105 °C for 16 h. Lipid determination was carried out using the modified Bligh and Dyer procedure (Bligh et al., 1959), and the ash content was determined by igniting the sample at 550 °C for 5–6 h until the sample was completely free from carbon particles in a carbolite muffle furnace. The total nitrogen was determined by the Kjedahl method as described by AOAC (1995) and a factor of 6.25 was used for converting the total nitrogen to crude protein of the different *A. testudineus* fish parts examined here.

2.4. Analysis of heavy metals

The heavy metals (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Pb) contents in different parts of the *A. testudineus* and in various mice organs treated with *A. testudineus* as a feeds were determined using Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry.

2.5. Sample preparation and method validation

The feed samples, fish samples and treated mice organs with *A. testudineus* were dried in an oven at 60 °C until a constant weight was obtained, and the dried samples were finely pulverized in a carbide mortar with a pestle, and preserved in desiccators. To prepare the samples for analysis, the dried material was transformed into pellets by applying hydraulic pressure of approximately 3 t for 25 min. The powdered material (0.1 g) was pressed to make a pellet using a pellet maker (Specac Ltd., UK), preserved in polyethylene bags, and retained in desiccators for subsequent analysis using Energy Dispersive X-ray Fluorescence (EDXRF) Spectrometry. EDXRF analysiswas carried out on the basis of the direct comparison methods, where standards were used to construct the calibration curves. The main concern in this method is that both standard and sample should have a similar matrix so that they can produce identical sensitivity and hence nullify the matrix effects. To make this possible, three fish standards (Tuna-1, Tuna-2, and Tuna-3) were used to construct calibration curves for the elemental analysis.

DORM-2 dogfish muscle (National Research Council, Canada) was used as certified reference materials (CRM) to check the accuracy and precision of the constructed calibration curve or to validate the instrument. This CRM was prepared and analyzed under the same conditions as the real samples were prepared for analysis. Results obtained for the elements of interest and the certified values for their corresponding elements are documented in Table 1. The obtained values were found to agree well with certified values; in fact the recoveries ranged from 86.66% to 109.29%. The resulting relative error and coefficient of variance (CV) were less than 10% for most of the elements tested, thus suggesting that the proposed method was valid for determining the heavy metals in the feed samples, *A. testudineus* fish samples and *A. testudineus* fish-treated organs of mice.

2.6. Blood sampling biochemical analysis

Establishing the biochemical parameters of Swiss albino's blood was performed by feeding of different parts of *A. testudineus*. Blood sample was collected in a tube after mice were fed by *A. testudineus* for 30 days and then centrifuged at 3000 rpm for 20 min. to obtain serum, which was kept frozen until required for analysis. After collecting the serum from mice, serum concentrations of total cholesterol (TC), triglyceride (TG) and HDL-cholesterol (HDL-c) were measured by enzyme-assay using commercial kits (Randox lipid reagents; Randox Laboratories Ltd., United Kingdom). According to Kikuchi et al. (1998), LDL – cholesterol was calculated as consisting of total cholesterol – (HDL cholesterol + triglycerides/5 [9]. Plasma glucose was measured by enzyme-assay using commercial kits (Randox glucose reagents; Randox Laboratories Ltd., United Kingdom). Photoelectric colorimeter was used to undertaken these biochemical analyses (AP-101; Apel Co., Ltd, Japan).

2.7. Statistical analysis

The recorded data were subjected to two-way analysis of variance (ANOVA) to assess the influence of different variables on the concentrations of heavy metals in the broiler feed, broiler meat, and different organs of the experimental mice. ANOVA for each group of tested samples were performed separately using variables such as sites. All the statistical analyses were computed with STATS software version 8.

2.8. Histopathological examination

For the histopathology of the vital organs like liver and kidney of mice, tissue specimens from liver and kidney were collected from all experimental groups at the end of the experiment. They were subsequently fixed in 10% neutral buffered formalin, dehydrated in ascending concentration of ethanol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned into thicknesses of 4–5 μm, then stained with hematoxylin and eosin (H&E) method [10]. Then the sections were examined under light microscopy at 400× magnification (DP72, Olympus). The results were compared to the control group.

3. Results

Above in the result section, (Table 1) shows the concentration of heavy metals and trace element recoveries from the standard reference materials (DORM-2) were close to the provided certified values by the manufacturers. Recoveries for DORM-2 ranged from 86.66 to 109.23%. The measured values of SRM 1640 employed as an ICP check solution were also close to the certified values reported by NIST (data not reported).

In the result, (Fig. 1) presents the composition of the two different types of commercial farm fish feeds (FF1 and FF2) bought from a local market. The percentages of crude fat, crude fiber, sodium, iron, crude protein, vitamin C, moisture and Ash contents of FF1 and FF2 farm fish feed are shown in (Fig. 1). Results indicated that the crude protein of FF1 feeds were double that of the FF2 feed, while the crude fat contents in FF2 were also almost double that of FF1. However, the values of other parameters of the FF1 and FF2 were virtually the same.

A summary of heavy metals and trace element concentrations (mg/kg dry wt.) in two commercial farm fish feeds (FF1 and FF2) analyzed in this study was compared with published values (Table 2). Indicated herewas a higher concentration of Cr, Mn, Fe, Ni and Sr in FF2 compared to FF1. The values of Cr, Mn, Fe, Ni and Sr in FF2 were 15.03, 39.12, 199.96, 3.31 and 42.20 μg/g, respectively, whereas in FF1 these

Table 1
Comparison between experimental results and certified values (mg kg^1, dry weight, Dorm-2).

Element	Results obtained	Certified values	Relative error (%)	CV (%)	Recovery (%)
As	15.80 ± 0.569	18.0	5.89	5.84×10^{-5}	93.72
Cr	30.87 ± 0.9863	34.70	9.34	3.8×10^{-4}	86.66
Pb	0.0503 ± 0.1184	0.065	−7.69	4.04×10^{-4}	109.23
Hg	4.916 ± 0.825	4.64	−4.56	4.08×10^{-4}	104.95
Co	0.253 ± 0.0375	0.182	1.89	3.43×10^{-4}	104.40

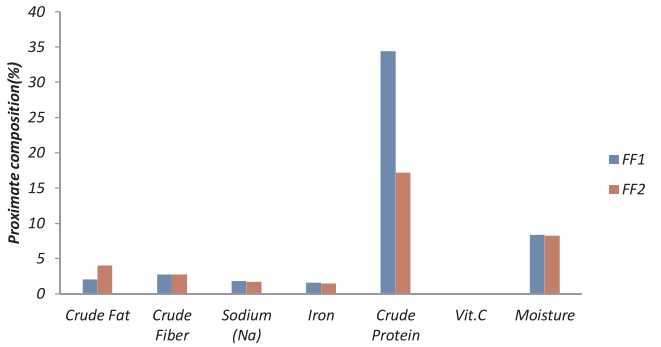


Fig. 1. Graphical representation of the proximity composition of two types of fish feedstuffs used in the experimental study. The blue bar FF1 represents the fish feedstuffs for*A. testudineus* starter crumble and the red bar FF2 represents the fish feedstuffs for grower pellet. The longest blue bar FF1 indicates the highest amount of protein present in the FF1 feedstuffs.

were 10.58, 30.28, 194.62, 2.10 and 6.49 μg/g, respectively. The V and As concentrations were only detected in the FF1 sample and these were 1.18 and 7.29 μg/g, respectively. However, the Cu (2.53 μg/g) and Pb (0.28 μg/g) concentration were almost same in the FF1 and FF2 samples.

The proximity value (crude fat, crude fiber, sodium content, iron, crude protein and vitamin C and moisture contents) in different parts of two commercial fish feed-treated *A. testudineus* has been shown in (Table 3). The level of the moisture contents of the fish without bone part (FFBB1 and FFBB2) were higher than fish feed with bone part or head/tail parts (FFB1 and FFB2), these percentages being 65.98%, 68.033%, 56.92, and 63.93%, respectively. No significant differences regarding fat content were found in the different parts of the two different feed-treated mice. The amounts of protein contents found in the FFBB1 and FFBB2 samples were 24.58% and 22.513%, respectively. Table 3 also reveals that highest amount of Fe present in the FFT1 sample when compared to the other parts of the fish was 3.726%. Na contents of the FFBB1 and FFBB2 samples were 2.03% and 1.646%, respectively, and these were comparatively higher than the other samples.

The crude fiber concentrations were very low in almost every sample but the high amount of crude fiber was detected only in the FFBB1 sample (45–50 days and 65–85 gm) group and FFBB2 sample (75—80 days and 225 250 gm) group; 1.16% and 1.256%, respectively. The higher levels of crude fats were found in FFH2, FFH1, FFBB2 and FFBB1 and their values were 16.13%, 14.31%, 12.203%, and 11.07% respectively. Based on what is presented in (Table 3), it is observed that the ash contents were significantly higher in FFB2, FFH2, FFB1 and 8.370%, 7.483%, and 7.276 respectively. It is also observed that vitamin C contents in all groups of fish sample were very low, ranging from 0.0237% to 0.038%.

The heavy metals and trace element contents in the different parts of the fish samples treated with two groups of fish feed (FF1 and FF2) in (Table 4). Heavy metals and trace elements in the FFH1, FFB1, FFBB1 and FFT1 samples were found to be: 80.13, 81.05, 98.73 and 111.06 μg/g for iron, 1.38, 0.74,1.70, and 2.25 μg/g for copper; 90.97,90.86, 89.70, and 97.64 μg/g, for zinc; 8.04, 6.13, 5.64, and 5.28 μg/g, for selenium; 120.31, 75.09, 32.21, and 124.52 μg/g, for Sr; and 0.35, 0.47, 0.43, 0.44 for Pb respectively. In the FFH2, FFB2, FFBB2 and FFT2 samples, the heavy metals and trace elements contents were 52.10, 75.18, 85.73, and 69.38 μg/g, for iron; 0.62, 0.75, 1.40, and

Table 2
Analysis of heavy metals and trace elements (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Pb) in fish feed samples on dry weight basis (μg/g).

Elements	FF1 (Commercial Feed) Concentration μg/g (M ± SE)	FF2 (Commercial Feed) Concentration μg/g (M ± SE)	Certified value μg/g
V	1.18 ± 0.57	<	–
Cr	10.58 ± 7.95	15.03 ± 7.88	–
Mn	30.28 ± 5.77	39.12 ± 5.77	–
Fe	194.62 ± 6.86	199.96 ± 6.73	–
Co	<	<	–
Ni	2.10 ± 1.83	3.31 ± 1.81	–
Cu	2.53 ± 0.43	2.15 ± 0.42	25
Zn	154.37 ± 7.01	140.37 ± 3.86	250
As	7.29 ± 5.00	<	15
Se	5.26 ± 1.62	3.09 ± 1.54	2
Sr	6.49 ± 1.61	8.20 ± 1.43	–
Pb	0.28 ± 0.05	0.36 ± 0.05	10

FF1-Commercial Fish Feed 1 (Kai Starter Crumble); FF2-Commercial Fish Feed 1 (Kai Grower Pellet); EU: European Union; AFCO: Association of Feed Control Officials. M = Mean; SE = Standard Error.

Table 3
Proximity in the different parts of the fish feed-treated *Anabas testudineus.*

Sample code	FFH1	FFB1	FFBB1	FFT1	FFH2	FFB2	FFBB2	FFT2	Certified value
Crude fat	14.31 ± 0.77	7.34 ± 0.59	11.07 ± 0.361	9.31 ± 0.740	16.13 ± 0.23	8.15 ± 0.125	12.203 ± 0.28	10.99 ± 0.03	
Crude fiber	0.60 ± 0.215	1.070 ± 0.01	1.16 ± 0.012	0.560 ± 0.040	0.65 ± 0.172	1.080 ± 0.03	1.256 ± 0.137	0.683 ± 0.02	
Crude protein	6.393 ± 0.71	13.07 ± 0.11	24.58 ± 0.602	10.13 ± 0.470	7.20 ± 1.00	14.29 ± 0.15	22.513 ± 0.31	10.27 ± 0.31	13.9 ± 0.3
Ash	6.523 ± 0.14	7.276 ± 0.15	4.86 ± 0.380	6.563 ± 0.139	7.483 ± 0.13	8.370 ± 0.12	5.456 ± 0.280	6.546 ± 0.24	0.7 ± 0.41
Moisture	59.95 ± 0.27	56.92 ± 0.24	65.98 ± 0.489	62.27 ± 0.790	60.74 ± 0.11	63.93 ± 0.46	68.033 ± 0.49	64.08 ± 0.38	70.3 ± 1.7
Sodium (Na)	0.543 ± 0.04	0.636 ± 0.04	2.03 ± 0.476	0.536 ± 0.017	0.660 ± 0.03	1.386 ± 0.02	1.646 ± 0.017	1.453 ± 0.09	60.5 ± 8.5
Iron (Fe)	1.663 ± 0.33	1.273 ± 0.10	2.32 ± 0.552	3.726 ± 0.043	1.806 ± 0.04	1.530 ± 0.20	1.67 ± 0.015	1.693 ± 0.14	2.0 ± 0.15
Vitamin C	0.037 ± 0.09	0.032 ± 0.00	0.0260 ± 0.00	0.0237 ± 0.00	0.031 ± 0.01	0.025 ± 0.00	0.024 ± 0.002	0.038 ± 0.02	

FFH1 = Head part; 45–50 days; 65–85 gm; FF1 fish feed; *Anabas testudineus*, FFB1 = Body with bone part; 45–50 days; 65–85 gm; FF1 fish feed, FFBB1 = Body without bone part; 45–50 days; 65–85 gm; FF1 fish feed, FFT1 = Tail part; 45–50 days; 65–85 gm; FF1 fish feed, FFH2 = Head part; 75–80 days; 225–250 gm; FF2 fish feed, FFB2 = Body with bone part; 75–80 days; 225–250 gm;FF2 fish feed, FFBB2 = Body without bone part; 75–80 days; 225–250 gm;FF2 fish feed, FFT2 = Tail part; 75–80 days; 225–250 gm; FF2 fish feed.

Table 4
Analysis of heavy metals (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Pb) present in different parts of the fish feed-treated *A. testudineus*.

Sample code	FFH1 Conc. µg/g (M ± E)	FFB1 Conc. µg/g (M ± E)	FFBB1 Conc. µg/g (M ± E)	FFT1 Conc. µg/g (M ± E)	FFH2 Conc. µg/g (M ± E)	FFB2 Conc. µg/g (M ± E)	FFBB2 Conc µg/g (M ± E)	FFT2 Conc. µg/g (M ± E)	Standard Conc. µg/g (M ± E) (M ± E) µg/g (M ± E)	Certified value µg/g (M ± E)
V	BDL	0.73 ± 0.5	0.76 ± 0.6	BDL	BDL	BDL	1.00 ± 0.6	BDL		
Cr	18.30 ± 7.9	BDL	BDL	24.0 ± 8.3	25.4 ± 8.1	BDL	BDL	17.1 ± 8.1	0.1	0.1
Mn	15.94 ± 5.9	11.5 ± 5.7	BDL	BDL	15.9 ± 5.6	7.1 ± 5.60	BDL	BDL		20
Fe	80.13 ± 5.9	81.1 ± 6.1	98.7 ± 6.1	111 ± 6.3	52.1 ± 7.7	75.2 ± 6.0	85.7 ± 6.0	69.4 ± 5.9		
Co	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL		
Ni	3.60 ± 1.81	5.43 ± 1.9	5.66 ± 1.8	8.09 ± 1.9	BDL	7.02 ± 1.4	3.26 ± 1.8	3.69 ± 1.8		
Cu	1.38 ± 0.42	0.74 ± 0.4	1.70 ± 0.6	2.25 ± 0.4	0.62 ± 0.2	0.75 ± 0.41	1.40 ± 0.4	2.00 ± 0.4	10	30
Zn	91.0 ± 3.50	90.9 ± 3.5	89.7 ± 3.6	97.6 ± 3.7	81.3 ± 3.4	87.83 ± 3.5	85.6 ± 3.6	83.6 ± 3.5	30	30
As	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.0	3.0
Se	8.04 ± 1.58	6.13 ± 1.6	5.64 ± 0.6	5.28 ± 1.7	3.98 ± 1.5	8.84 ± 1.67	1.86 ± 1.6	5.84 ± 1.6	2. 0	0.3
Sr	120.3 ± 1.8	75.1 ± 1.6	32.2 ± 1.4	125 ± 1.3	94.2 ± 2.1	92.67 ± 1.7	35.3 ± 1.4	93.0 ± 1.8		
Pb	0.35 ± 0.05	0.47 ± 0.1	0.43 ± 0.1	0.44 ± 0.1	0.31 ± 0.1	0.39 ± 0.05	0.54 ± 0.1	0.41 ± 0.1	0.5	0.5

M = Mean; SE = Standard Error, BDL: Below detection limit, Standards: FAO /WHO, FAO [12], Turkish guidelines.

2.00 µg/g for copper; 81.30, 87.83, 85.61, and 83.64 µg/g, for zinc; 3.98, 8.84, 1.86, and 5.84 µg/g, for selenium 94.21, 92.67, 35.31 and 92.96 µg/g, for Sr; and 0.31, 0.39, 0.54, and 0.41 µg/g for lead respectively.

Concentration of V (Vanadium was too low for the system to detect in the FF1 and FF2 samples. On the other hand, 0.73 µg/g, 0.76 µg/g and 1.00 µg/g were found in FFB1, FFBB1 and FFBB2, and mean concentrations of Cr (Chromium) were 18.30, 25.42, 24.01and 17.10 µg/g in FFH1, FFH2, FFT1 and FFT2 respectively. Mn (Manganese) was only present in FFH1, FFH2, FFB1 and FFB2 with mean concentrations of 94, 15.94, 11.46, and 7.10 µg/g respectively. Ni (Nickel) was not detected in sample FFH2 but it was observed in FFH1, FFB1, FFBB1, FFT1, FFB2, FFBB2 and FFT2 samples and these were 3.60, 5.43, 5.66, 8.09, 7.02, 3.26 and 3.69 µg/g respectively. No detectable amounts of arsenic and cobalt were observed in different fish sample groups although arsenic was evident in fish feedstuffs, FF1 sample but cobalt was not detected in either the fish feedstuffs FF1 and FF2.

The results obtained for the heavy metal analysis of the *A. testudineus* fish treated experimental mice liver are reported in (Table 5). The highest concentration of Pb was observed in the FFBB2 and FFT2 samples, i.e. 0.771 and 0.735 µg/g, whereas in control the value obtained was 0.07 µg/g respectively. It is observed that As was present in all the samples and the concentrations were almost the same (Table 5). The highest concentrations of As were observed in FFB1 (0.172 µg/g) and FFBB1 (0.169 µg/g) while in the control it was 0.154 µg/g. Cd concentration was virtually the same in all the samples but the highest amounts of Cd were present in FFB2 and FFB1. The values recorded for these samples were 0.12 µg/g and 0.09 µg/g respectively, whereas the control was 0.016 µg/g. The highest amount of Cr was found in the FFT2 and FFT1 samples and these were 0.166 and 0.156 µg/g respectively. The smallest amount of Cr was observed in the FFBB1 and FFBB2 samples and their values were nearly same as that of the control. The concentrations of Cr in FFBB1 and FFBB2 samples were 0.052 and 0.054 µg/g, and the control was 0.051 µg/g respectively.

The biochemical investigation results in the experimental mice treated with *A. testudineus* fish as a diet is shown in (Table 6). Results indicated that the blood cholesterol levels of all groups of samples were higher in comparison to the control. The cholesterol level of the control was 3.83 mmol/L, where the desirable blood cholesterol is 3.31 m mol/L. The maximum value of 12.37 m mol/L and minimum value of 4.48 m mol/L were observed in the FFB2 and FFT1 samples respectively.

The triglycerides level of all types of samples-treated experimental mice were almost the same but high levels of triglyceride were observed in the FFB2, FFBB2 and FFB1 samples; the correlating values were 3.47, 2.41 and 2.36 mmol/L respectively. A minimum level of triglyceride was observed in the FFT1 sample-treated experimental mice and it was 1.64 m mol/L, which is slightly higher than the control. The triglyceride level of the control was 1.46 ± 0.14 mmol/L but the desirable triglyceride was < 1.25 mmol/L. The blood serum HDL concentrations of almost all groups of samples treated Swiss albino mice exceeded the control (1.32 m mol/L). The results for HDL values were 1.48 mmol/L in FFB2, 1.47 mmol/L in FFBB1, 1.42 mmol/L in FFT1, 1.41 mmol/L in FFT2 and 1.40 mmol/L in FFH2 and 1.33 mmol/L in FFBB2.

The highest levels of LDL were found in the FFB2 sample (8.67 mmol/L) but nearly the same concentrations of LDL existed in the FFBB1, FFT1 and FFH2 samples, these being 3.72, 3.74 and 3.77 mmol/L, whereas in the FFB1, FFBB2 and FFT2 samples they were 5.56, 4.51 and 6.45 mmol/L respectively. The lowest levels of LDL were observed in the FFH1 sample (2.77 ± 0.29 mmol/L) and the control was 2.15 ± 0.11 mmol/L. The blood glucose analysis outcome indicated that the maximum levels of glucose were found in FFB2 (6.84 m mol/L) while the levels of glucose in FFT1, FFBB2 andFFBB1 sample-treated mice were 6.36, 5.38 and 5.04 mmol/L respectively. When compared to

Table 5
Analysis of heavy metals (Cr, Pb, As) present in liver of the mice treated with *A. testudineus*.

Sample code	FFH1 Conc. μg/g (M ± E)	FFB1 Conc. μg/g (M ± E)	FFBB1Conc.μg/g (M ± E)	FFT1 Conc. μg/g (M ± E)	FFH2 Conc. μg/g (M ± E)	FFB2 Conc. μg/g (M ± E)	FFBB2 Conc. μg/g (M ± E)	FFT2 Conc. μg/g (M ± E)	Control	Certified value
Cr	0.15 ± 0.1	0.07 ± 0.0	0.05 ± 0.0	0.156 ± 0.0	0.15 ± 0.0	0.06 ± 0.0	0.05 ± 0.0	0.17 ± 0.0	0.051 ± 0.0	0.3
Pb	0.42 ± 0.0	0.47 ± 0.1	0.49 ± 0.1	0.34 ± 0.0	0.49 ± 0.0	0.62 ± 0.1	0.77 ± 0.1	0.74 ± 0.0	0.07 ± 0.01	0.470 ± 0.024
As	0.16 ± 0.1	0.17 ± 0.1	0.17 ± 0.0	0.16 ± 0.1	0.16 ± 0.1	0.16 ± 0.1	0.15 ± 0.0	0.16 ± 0.0	0.15 ± 0.05	

M = Mean; SE = Standard Error.

Table 6
Determination of lipid profile and glucose level in the blood serum of *A. testudineus* for fish-treated experimental Swiss albino mouse.

Biochemical Parameter (mmol/L) (M ± SE)	Sample code									Certified value
	Control	FFH1	FFB1	FFBB1	FFT1	FFH2	FFB2	FFBB2	FFT2	
Cholesterol	3.8 ± 0.1	5.4 ± 0.6	8.6 ± 1.1	5.8 ± 1.0	4.5 ± 0.8	9.3 ± 0.1	12.4 ± 2.8	7.4 ± 0.4	4.6 ± 0.4	< 11
Triglycerides	1.5 ± 0.1	1.8 ± 0.1	2.4 ± 0.2	2.2 ± 0.1	1.6 ± 0.2	2.3 ± 0.3	3.5 ± 1.2	2.4 ± 0.2	1.9 ± 0.3	< 8.325
HDL	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.4	1.5 ± 0.3	1.4 ± 0.5	1.4 ± 0.5	1.5 ± 0.4	1.3 ± 0.5	1.4 ± 0.5	> 3.33
LDL	2.2 ± 0.1	2.8 ± 0.3	5.6 ± 0.1	3.7 ± 0.2	3.7 ± 0.2	3.8 ± 0.3	8.7 ± 0.3	4.5 ± 0.3	6.5 ± 0.4	< 7.21
Glucose	3.7 ± 0.2	3.7 ± 0.1	3.8 ± 0.4	5.0 ± 0.9	6.4 ± 0.2	4.0 ± 0.1	6.8 ± 0.7	5.4 ± 0.6	4.0 ± 0.2	3.5-5.5

M = Mean; SE = Standard Error, HDL = High density lipoprotein, LDL = low density lipoprotein.

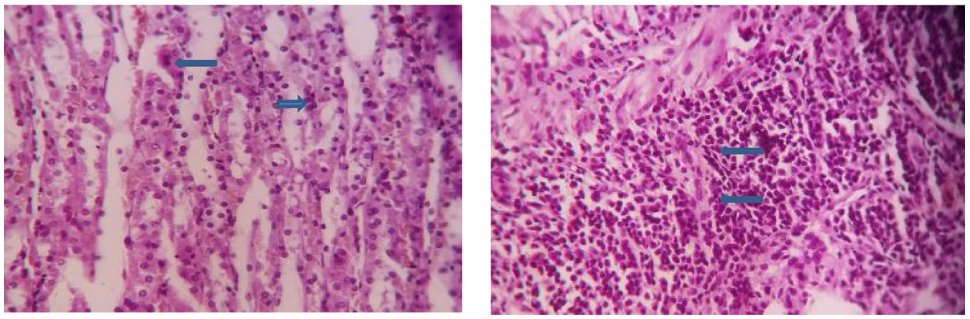
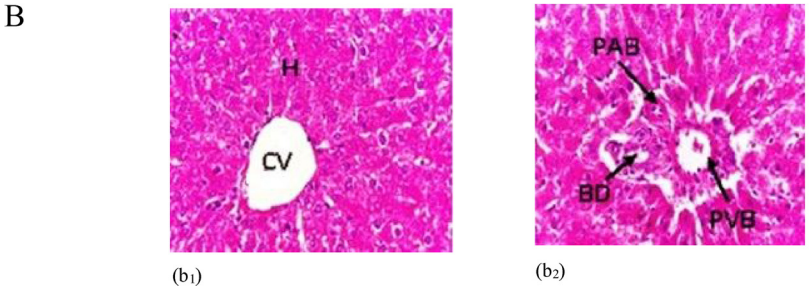


Fig. 2. Effect of heavy metal content fish meat on the liver of the mice; (a) A photomicrograph of a liver section of a mice (treated with 5 g of FFT2 fish sample as diet for 5weeks) showing hydropic degeneration predominantly in mid-zone. Arrow indicates this.
(b) A photomicrograph of liver section from control mice (treated with 5 g of normal parched rice as diet for 5weeks). (b_1) Control reveals normal liver parenchyma with central vein (CV). (b_2) portal triad (HAB : Hepatic artery branch; PVB: Portal vein branch; BD: Bile duct).



(b_1) (b_2)

the control (3.66 mmol/L), nearly the same amounts of glucose were observed in the FFB1, FFT2 and FFH2 samples and these values were 3.67 mmol/L, 3.99 and 4.00 mmol/L respectively.

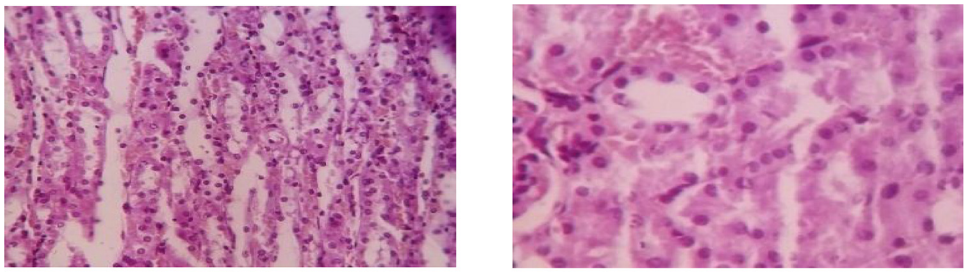
The photomicrographical histopathology results of experimental mice liver and kidney are shown in Figs. 2 and 3 , respectively. The heavy metals (Cr, Ni, Zn, Pb, Sr) were found in different parts of the *A. testudineus* fish, but especially high amounts were detected in the FFT1 fish sample. This group of sample-treated experimental mice liver and kidney served for the observed histopathological analysis. Histopathological changes were observed in the liver and kidney of experimental mice (Figs. 2a and 3a) respectively. In (Fig. 2a) atropic degeneration was observed in the liver's mid-zone. However, a mild degenerative change was observed in the kidney section (Fig. 3a). No significant changes emerged in the control group but when treated with parched rice, degenerative changes were observed in the liver and kidney of the *A. testudineus* fish–fed experimental mice (Figs. 2b and 3b).

4. Discussion

The purpose of this study is to observe the potential of *A. testudineus* on to cause health hazards; Swiss albino mice were used as an experimental model for this analysis. This research was conducted as: firstly, proximity analysis of fish feed, *A. testudineus* fish; and secondly, a biochemical analysis to determine the concentration of heavy metals in different body parts, i.e. head, body with bone, body without bone and tail of *A. testudineus* fish.

The proximity analysis of the two different types of fish feeds used in this study was illustrated in (Fig. 1). It is indicated that the highest concentration of crude protein was observed in the FF2 sample compared to the FF1. However, the fat, Na and Fe contents of the FF1 were relatively higher than those reported for the FF2 sample.

The high concentrations of Cr, Mn, Fe, Ni and Sr in FF2 as compared to FF1 have shown in (Table 2). It is interesting to note that the As and V are present in the FF1 sample but not present in FF2. The mean values obtained for Cu and Pb in the feed sample analyzed in this study were lower than the respective maximum tolerable value (ppm) recommended by the Association of Feed Control Officials (AFCO) guidelines for Cu: 25 and Pb: 30 [9]. The mean values observed for As, Cu, Zn and Pb in feeds (FF1 and FF2) in this study were below the European Union (EU) upper tolerable limits. Furthermore the mean values observed for V, Cu, Mn, Ni, Fe and Pb in feeds (FF1 and FF2) were lower than the corresponding values reported for feeds from the national fish hatcheries of Bangladesh [7]. Again, the mean values for Mn, Fe and Cu in this study were consistently lower than the corresponding values reported for other feeds (Mn: 35.9 mg/kg, Fe: 404 mg/kg, Cu: 88.0 mg/kg) [11]. However, the mean values for Cr, Zn As, Se in FF1 fish feed and for Cr, Ni, Se in FF2 fish feeds were higher than the corresponding values reported for feeds from the national fish



(a) (b)

Fig. 3. (a) A photomicrograph of a kidney section of a mice (treated with 5 g of FFT2 fish sample as diet for 5 weeks) showing mild degenerative changes in the straight proximal tubules. Arrow indicates nuclei with inclusions. (b) A photomicrograph of the kidney section from control mice (treated with 5 g of normal parched rice as diet for 5 weeks) showing no degenerative changes.

hatcheries [7]. These values remained consistently higher than the corresponding values reported for other feeds [10]. Cu and Zn are important factor for the growth of fish but one good thing to emerge is that both the Cu and Zn concentrations observed in both fish feed samples (FF1 and FF2) were the same as the recommended values [12].

Standards for the requirements of fish failed to be met by some commercial feed producers, principally because the source of raw materials for the manufacture of the feeds tended to be contaminated with heavy metals. Important information is lacking in regard to the heavy metals load of fish feeds used in aquaculture in Bangladesh. Although it is very difficult to avoid contamination of animal feeds by toxic metals, nonetheless it is vital for such contamination to be minimized. Doing so will reduce both direct impacts on animal health and indirect impacts on human health [13].

Therefore, feeds may contain varying amounts of toxic elements that can potentially affect fish health and also contribute to biomagnifications of metals in fish, thus representing various trophic levels in an ecosystem. The proximity analysis of different parts of *A. testudineus* treated with fish feed (Table 3) and it is observed that the biochemical composition of *A. testudineus* fish varies among the different parts of the two different types of fish feed-treated *A. testudineus* fish. The highest protein content (24.58) was found at the time of 75–80 days (2–2.8 kg) group, *A. testudineus* FFBB1 (body without bone or edible part of the FF1 feed-treated) sample. Large amounts of moisture contents were also observed in FFBB2 (body without bone or edible part of the FF2 feed-treated) and FFBB1 (body without bone or edible part of the FF1 feed-treated) samples. The respective percentages were 68.033% and 65.98. It has been reported that the edible part of the fish species *P. typus* contains 16.17% protein [14].

In this study, it is observed that the edible part of the FFBB1 and FFBB2 samples contained 24.58%, 22.51% protein, respectively, which were higher than the *P. typus* fish protein. It is also reported that the fish contents above 15% protein belonged to the high protein category fish [15,16] and therefore FFBB1 and FFBB2 samples can be considered as high category protein. This may be due to the treating of the FF1 fish-fed sample with high levels of protein (Fig. 1). The levels of moisture were also higher in the FFBB1 and FFBB2 samples as compared to others, i.e. 68.033% and 65.98% respectively, which is identical to the values found in *P. senegalensis* at 68% [17]. No significant changes in ash and Vit. C content was found in all groups of samples. Na and Fe contents in the most of the samples were within the acceptable level. High levels of Fe were observed in FFT1 (3.726%) and FFBB1 (2.32%) samples, whereas high levels of Na occurred in FFBB (12.03), FFBB2 (1.646), FFT2 (1.453), and FFB2 (1.386%). The high amounts of Na and Fe contents in *A. testudineus* samples were similar to those concerning *P. elongates* [14].

The heavy metals and trace metals contents in the different parts of the *A. testudineus* fish treated with FF1 and FF2 fish feed was summarized in (Table 4). Concentrations of manganese, chromium, nickel, iron and zinc concentration were high in most of the studied samples but the highest amounts of iron, nickel, chromium and zinc were observed in the tails of45–50 days old *A. testudineus* fish (FFT1) sample and these concentrations were 111.06, 8.09, 24.01 and 97.64 μg/g respectively. The FF1 (7.29 μg/g) sample has more fish feed than that in the fish hatcheries [7] but interestingly the levels of As in the FFH1, FFB1, FFBB1 and FFT1 samples were below the detectable level (< 0.002 μg/g). Furthermore it is worth noting that although, Ni is detected inFF1 and FF2fish feed and it is also detected in the different parts of theFF1 and FF2 fish feed *A. testudineus* except FFH2.

Cr, Zn and Se contents in different parts of the *A. testudineus* fish samples exceeded the respective limits of 0.1, 30 and 2 μg/g and these values (17.10–25.42 μg/g, 81.30–97.64 μg/g and 3.98–8.04 μg/g) were above the MAFF and FAO [11] limits for fish muscle. The exception was Se (FFBB2) which was 1.86 μg/g and the US EPA draft recommends a selenium whole body criterion of 7.9 μg/g dry wt. Lead and Cu levels in this study were below the Australia and New Zealand food standards of 0.5 and 10 μg/g respectively [18]. The results obtained from this analysis for Mn, Fe, Ni, Zn and Pb in all types of studied samples showed that they were higher in comparison to the respective limits yet the As and Co contents could not detected. Chromium was only present in the head and tail parts of the studied samples. Trace elements found in various parts of the *A. testudineus* fish from the fish farm pond showed strong and moderate correlations with one another in some cases.

Monika et al. (2012) indicated that metal absorption in fish is carried out via two uptake routes: digestive tract (dietary exposure) and gill surface (waterborne exposure). The correlations of trace elements in fish tissues observed may be related to environmental conditions and physiological needs [19]. While these trace element contents of fish tissues across geographical areas are important, it should be remembered that considerable variations may exist across fish species because of their nature and habits. Cr, Ni and Zn levels in the different edible parts (Table 4) of the *A. testudineus* fish are higher than the standard level. Although the As is below the detectable level and Pb is lower than the standard level, it has been reported in fish liver the Zn concentrations are correlated with As, Pb and Cr [20]. Therefore, the present study also focused on determining the accumulated heavy metals like Cr, Pb and As in liver of the experimental mice treated with different parts of the fish samples (Table 5).

The high levels of Cr observed in the FFT2, FFT1, FFH2 and FFH1 samples had values of 0.166, 0.156, 0.15 and 0.15 μg/g respectively, while the standard level is 0.10 μg/g. The Pb and As levels in the liver of the experimental mice were slightly higher than the control but lower than that of respective standard level, excepting FFBB2 (0.771 μg/g), FFB2 (0.619 μg/g) and FFT2 (0.735 μg/g) samples. These were treated mice liver and the relative standard values are 0.5 and1.0 μg/g respectively. Although As was not detected in differing parts of the fish samples, those were treated with fish feed samples FF1 and FF2. However, the As levels were detected in the liver of the mice treated with all groups of fish samples – even the control – but proved to be less than the standard value. As was only detected in the FF1 fish feed sample but not in the FF2 feed or other fish samples. It is assumed that there is a possibility to enter it into mice liver via water or environmental contamination, although its level is not enough to pose any risk to consumers.

Chromium and lead levels were above their respective threshold limits in the liver of mice, especially FFH1, FFH2, FFT1, FFT2 and FFB2, FFBB2, FFT2 samples which were used as feed for mice. It is indicated that the head and tail parts of the *A. testudineus* fish were responsible for high levels of chromium in mice liver. Conversely, the large *A. testudineus* fish muscle and tail parts are responsible for the high lead levels in mice liver. It can be concluded that the head and tail parts of farm *A. testudineus* fish could pose a threat to the health of humans if too much is consumed.

Biochemical analysis of the different groups of fish feed samples are recorded (Table 6) and it shows that there is no significant change in the blood cholesterol level among the different groups of treated mice. A high level of cholesterol was observed in FFB2, FFH2, FFB1 and FFBB2 samples than that of control. Unlike cholesterol, TG of FFB2 was higher as compared to others groups. The TG in the FFBB1, FFBB2 FFB1and FFH2 samples were slightly higher than the control group but in the FFT1 and FFT2 group, it was almost similar to the control. It is interesting that no significant changes in HDL level were observed in the all groups of samples compared to the control. In the case of LDL, the highest concentration was observed in FFB2 (8.67 mmol/L). It is surprising that the lowest LDL observed in the FFH1 was two times lower than the control. In this study, the results obtained from the biochemical analysis indicated that the cholesterol, TG and LDL levels are remarkably higher in FFB2, FFH2 and FFB1 than the control group. It is interesting to note that no significant changes in the HDL level were observed in all groups of sample-treated experimental mice as compared to the control. The blood glucose level analysis results reveal that no significant difference emerged in the experimental mice treated with

different groups of fish samples was shown in (Table 6).

Higher levels of blood glucose were found in the FFB2, FFT1 and FFBB2 groups of treated mice compared to the control. The highest level was observed in the FFB2 group of sample-treated mice, this amount being 6.84 mmol/L. It is assumed that this may have occurred due to the high crude fact content, Mn, Se and Sr evident in the FFB2, FFB1 and FFH2 sample groups. It is interesting that FFB2, FFB1 and FFH2 all belong to the body with bone and head parts of the fish. The biochemical analysis results indicated that the high amounts of crude fat and some trace elements like Mn, Se and Sr present in body with bone and head parts of the fish samples could be causative agents for high levels of cholesterol, TG and LDL in the fish-treated mice. No specific data have been reported on the effect of lipid profile and blood glucose level in the experimental mice after being fed with fish. However, it has been reported that high levels of triglyceride and HDL have been found in the mice that were fed a diet supplemented with broiler and ostrich meat [21,22]. In a similar experiment Das et al. [24] observed that the blood cholesterol levels were also significantly higher. Therefore these results are consistent with the findings in the present study [23].

The liver is the site of detoxification of materials and higher levels of contaminants in liver relative to muscles due to the presence of a cysteine-metallothionein complex and lipophilic biomembranes (liver: muscle ratio $>$ 1) that have already been reported [24]. Liver is a vital animal organ that controls the important biochemical and metabolic reactions occurring in the body. It is observed that chromium and lead levels were above their respective threshold limits in the liver of mice (Table 5). The mechanism of cadmium toxicity is not understood clearly but its effects on cells are known. When cadmium binds to cysteine-rich protein such as metallothionein, it increases 3,000-fold. In the liver, the cysteine-metallothionein complex causes hepatotoxicity and then it circulates to the kidney and is accumulated in the renal tissue, subsequently causing nephrotoxicity. As well, cadmium has the capability to bind with cysteine, glutamate, histidine and aspartate ligands and can lead to an iron deficiency [24].

Acute inhalation exposure to cadmium can cause death in humans and animals. Respiratory (kidney damage), cardiovascular (high blood pressure), gastrointestinal, hematological (lowered hemoglobin concentrations and decreased packed cell volumes have been observed in some studies), musculoskeletal, hepatic, and immunological outcomes have been documented [25].

This study emphasized the feeding impact of *A. testudineus* fish on the liver and kidney of the experimental mice (*Swiss albino*) as shown in (Figs. 2 and 3). The photomicrographical histopathology results for liver and kidney revealed no significant changes in the control group when treated with normal parched rice and cereal (Figs. 2b and 3b). In contrast, referring to the *A. testudineus* fish treat experimental mice, a tropic degeneration was observed in the mid-zone of liver shown in (Fig. 2a) and mild degenerative change did occur in the kidney shown in (Fig. 3a). The degenerative changes found in the liver and kidney may be the result of the accumulation of heavy metals (Cr, Ni, Zn), when fed *A. testudineus* fish that had these in large quantities. However, the control group that received a normal diet showed normal central vein, hepatocytes, nucleus and sinusoid shown in (Figs. 2b and 3b). Previously, these findings such as degenerative changes were observed in mice after their treatment with heavy metal. Shrinkage to mild shrinkage of glomerulus was recorded in of the kidneys of mice and infiltration of the liver parenchyma with lymphocytes was observed when treated with heavy metals. In another study it was reported that concentration of As in rats' kidneys could cause glomerular swelling in 9% of rats [26–32].

5. Conclusion

Fish feeds used at a farm fish pond in the Jessore region of Bangladesh revealed high concentrations of Cr, Ni and Zn (average: 12.80 μg/g, 2.70 μg/g, 147.37 μg/g dry wt., below the European Union guideline value) which can potentially affect the health of *A. testudineus* fish and result in bioaccumulation in tissues. Since most of the people in Bangladesh eat *A. testudineus*, this study is committed to understanding if *A. testudineus* is actually safe to eat. Finding the answer meant evaluating available feeds in the market for heavy metals and trace element toxicity and bioaccumulation in *A. testudineus* tissues. Some heavy metals that accumulated in the liver and kidney emerged as being causative agents for the degeneration of the liver and kidney in experimental mice. These heavy metals could enter the mice through A. testudineus fish, although limited amounts of heavy metals were found in the fish feedstuffs. Consequently, certain environmental factors (water, water sediments, dirt, air, etc.) could be responsible for the level of heavy metals contamination in fish feedstuffs as well as the fish themselves. This study may also be useful in developing nutrient-balanced, healthy safe and cost-effective diets for consumers.

Aquaculture practices at the local fish farm may need to be evaluated to reduce contaminants in fish tissues. Nonetheless, high concentrations of chromium, nickel and zinc accumulated in the heads and tails of *A. testudineus* fish; these metals were correlated with As, Cd and Pb. As a result of the high levels of Cr and Pb observed in the experimental mice's livers that were fed heads and tails of the *A. testudineus* fish, degeneration of liver and kidney in them was observed according to the histopathological studies. As the quantity of fish produced by the aquaculture industry increases, it is clear that safe guards for the health of humans must be maintained. Moreover, these heavy metals persist in fish, feedstuffs and also in the environment even after cooking. Thus, future direction of this study is to achieve awareness by reducing the levels of toxic elements often associated with fish feedstuffs. Finally, this study suggested the fish feeds producers and fish farmers being the origins of heavy metals contamination in their products, should take all the necessary measures during the production of feedstuffs or during the culture of the fish in the ponds. Finally, extra precaution should be taken to increase the nutrient value of fish feedstuffs by removing some ingredients that elevate the harmful cholesterol level or other toxic heavy metals.

Conflict ofinterest

The authors declare they have no competing interest.

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