Journal of Veterinary Science & Technology

Khalil, et al., J Veterinar Sci Technol 2016, 7:1
DOI: 10.4172/2157-7579.1000278

Research Article Open Access

Effect of Feeding with Different Dietary Protein Levels and Starvation on the Health, Nonspecific Immune Parameters, Behavior and Histoarchitectures of Fantail Goldfish (*Carassius auratus* L.)

ALshimaa AHM Khalil[1], Walaa EL-Husseiny[1], Azhar F Abdel Fattah[2] and Wael AM Ghonimi[3*]

[1]*Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt*

[2]*Department of Veterinary public health, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt*

[3]*Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt*

[*]**Corresponding author:** Wael AM Ghonimi, Department of Histology and Cytology, Faculty of Veterinary Medicine, Zagazig University, 44519 Zagazig, Egypt, Tel: 00201222498246; Fax: +2-055-2283683; E-mail: drwael_histology@yahoo.com

Rec date: Sep 02, 2015; **Acc date:** Dec 02, 2015; **Pub date:** Dec 04, 2015

Abstract

The present investigation was conducted to assess the effect of different dietary protein percentages and starvation on the health, behavior, blood chemistry, immune response and histoarchitectures of fan tail gold fish *Carassius auratus* L. Experiment was carried out using 80 *Carassius auratus* with an average body weight 18 ± 2 g which were divided into four groups in duplicate in which, group 1 feed with diet A; 28% Crude Protein (CP). Fish in group 2 was fed on diet B (17% CP) and fish in group 3 was feed on diet C (45% CP) and group 4 kept as fasted group. The experimental period was 8 weeks. The current study clarified that significant high growth rate, improved welfare; decrease aggressive behavior, improved biochemical serum parameters and immune response were observed in group feed with diet containing 45% crude protein. Starvation is directly affected health, immunity and welfare as well as histoarchitectures of all selected organs. Histologically, there is no any significant changes on the histoarchitectures of the all selected organs; liver, spleen, intestine, head kidney as well as muscle of groups 1, 2 and 3 feed with diet A (28% CP), diet B (17% CP) and diet C (45% CP) respectively. Meanwhile, the fasting of group 4 had the most effective changes on the histoarchitectures of all selected organs.

Keywords: Dietary protein; Starvation; Health behavior; Histoarchitectures; Fantail goldfish; *Carassius auratus* L

Introduction

Ornamental fish have growing importance at the present time. Cichlids, which are considered the most popular ornamental fish, constitute approximately 95% of all 4000 species and varieties [1].

There are different factors that affect ornamental fish welfare, including for example the physical and chemical environment, feeding, social interaction and the occurrence of fish pathogens [2]. Nutrients have an important role in keeping the health condition, normal behavior and in improvement the external appearance and color of ornamental fish [3]. Protein is considered the biggest part of the cost of the unit of feed, while fat and carbohydrates are important to supply the energy required by fish [3].

The changes in the metabolic and immunological profiles are one of the tools that evaluate the fish performance and its ability to withstand the different dietary conditions [4-6].

Histological analysis of the digestive system is considered a good indicator of the nutritional status of fish [7-9]. The intestine and liver are the most important organs in digestion and absorption of nutrients from food, and therefore monitoring of these organs is considered necessary [10].

Starvation is one of the important causative of fish mortality in nature and in aquaculture [11]. Long-term starvation can cause severe deformity in vital organs [12] and even mortality of fish. Starvation also exhibits a widespread histological degeneration in the haemopoietic organs of fish which can bring about alterations in their cellular architecture. Haemopoietic organs (liver, head kidney and spleen) have been reported to be the most sensitive tissues to be affected by starvation [13]. Structural alterations/degeneration of haemopoietic organs impairs its functional capacity (haemopoiesis) which may prove even fatal for the survival of fish. Thus histopathological alterations in haemopoietic organs can be utilized as tools in order to get a clear idea about the extent an organism is affected at tissue or cellular level [14].

The aim of our work is to investigate the effect of different dietary protein levels and starvation on fish growth performance, health, behavior as well as histoarchitectures of liver, spleen, intestines, head kidney and muscle of fantail goldfish *(Carassius auratus)*.

Material and Methods

Fish and aquaria

A total number of 80 fantail goldfish (*Carassius auratus* L.) with an average body weight 18 ± 2 g were collected alive from Zagazig fish market at Sharkia province and transported alive immediately to the laboratory in large plastic bag. Fish were kept in experimental aquaria with 60 liter capacity and kept for 10 days acclimatization period before the beginning of the experiment. Water in the aquaria was aerated permanently and the temperature was regulated by using thermostatically controlled heaters. Water was completely changed four times weekly.

Fish diets and feeding

Diets with different protein levels were prepared in fish research unit, Faculty of Veterinary Medicine, Zagazige University, Egypt. The chemical analysis of feed stuffs used in the experimental diets is shown in Table 1. The diets included different crude protein levels as following: Diet (A) in which, contains 28% crude protein; diet B, containing 17% crude protein, diet C, containing 45% crude protein and group D acts as a fasting group. The fish in groups A, B, C were fed diets 2 times daily (09:00 AM and 03:00 PM) at rate of 3% of body weight for 8 weeks. Periodical evaluation of growth was done each 15 days. The chemical composition of the experimental diets are shown in Table 2. The growth Performance Parameters carried out by evaluation of average body weight which calculated by dividing the total weight of fish by the number of fish in each group. Body gain, body gain percent, specific growth rate % and daily gain rate were determined according to Pouomonge and Ombredane [15].

Ingredient	Nutrient (% as fed basis)					
	DM	CP	EE	CF	Ash	NFE(calculated)
Yellow corn	88.80	8.75	3.60	2.10	1.20	73.15
Wheat flour	89.00	12.80	2.50	1.60	1.60	70.50
Soybean meal	90.00	43.70	1.80	6.10	6.50	31.90
Fish meal	94.60	63.40	8.70	0.7	20.50	1.30
Poultry by-product meal	92.60	60.30	12.70	2.10	14.70	2.80

Table 1: Chemical analysis of feed stuffs used in the experimental diets. (DM= Dry matter, CP=Crude protein, EE=Ether extract, CF=Crude fiber and NFE (calculated)=Nitrogen free extract). DM, CP, EE and Ash were chemically analysed according to procedures of AOAC (2000).*Calculated according to tables of NRC (1993).

Ingredients	Experimental diets		
	CP % in diets		
	A	B	C
Yellow corn	39.00	50.00	14.00
Wheat flour	12.00	23.00	6.00
Soybean meal	16.00	7.00	22.00
Fish meal	13.00	6.00	27.00
Poultry by-product meal	13.00	5.00	27.00
Vegetable oil	5.50	7.50	2.50
Vitamins and Minerals mixture*	1.50	1.50	1.50
DM, %	84.14	81.57	88.19
CP, %	28.02	17.20	45.01
EE, %	10.04	10.89	9.16
CF, %	2.38	2.02	2.50
Ash, %	6.30	3.40	11.25
NFE, %	42.63	55.24	22.60
DE, Kcal/ kg diet**	2919.40	2915.49	2944.55

Table 2: Chemical composition of the experimental diets. *Vitamin and Mineral mixture (alfakema):- Each 1 kg contains:-Vit. A 580000 I.U, vit.D3 8600 I.U, vit.E. 720 mg, vit. K3 142 mg, vit C 0.1 mg, vit B1 58 mg, vit B2 34 mg, vit. B6 34 mg , vit.B12 58 mg , Folic acid 86 mg, Pantothenic acid 8 mg , Manganese sulfate 65 mg , Zinc methionine 3000 mg , Iron sulfate 2000 mg , Copper sulfate 3400 mg , Cobalt sulfate 572 mg , Sodium selenite 25 mg, Calcium iodide 25 mg, Calcium carbonate (Carrier substance) till 1000 gm. **Digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm (Santiago et al. 1982). (DM=Dry matter, CP=Crude protein, EE=Ether extract, CF=Crude fiber and NFE=Nitrogen free extract).

Experimental design

Fish are divided into four groups in duplicate in which, group 1 feed with diet A. Fish in group 2 was fed on diet B and fish in group 3 was fed on diet C. while fish in group 4 kept as fasting group. The experimental period was 8 weeks.

Behavioral observation

Carassius auratus identified by short plastic strips applied in dorsal fin of fish, behavior recorded in the period between 09:00 Am till 03:00 Pm for 8 weeks by using focal sample technique for 15 sec. intervals during one hour daily. Visually by using a note book for recording behavior, a stop watch, multipurpose counter and video camera according to Altuman [16]. The following behaviors were recorded according to Stephan [17]:

Feeding: Frequency and duration (Sec.) spent in feeding.

Swimming: Frequency and duration (Sec.) spent in swimming.

Aggression: Frequency and duration (Sec.) spent in attacking each other.

Rest: Frequency and duration (Sec.) in which fish completely immobile and rest on the bottom of their aquaria.

Arousal: Frequency and duration (Sec.) in which fish has a locomotors activity.

Fish coming to surface of aquaria: Frequency and duration (Sec.) in which fish hanging around the top of aquaria.

Blood sample

At the end of the experimental periods, blood samples were collected from caudal vessels and centrifuged at 3000 rpm for 10 minutes for obtaining serum samples and stored in freezer at -0°C then analyzed 24 hrs post collection. Blood samples should be collected 17 h after the final feeding for the plasma glucose assay according to Cheng et al. [18].

Biochemical analysis

Biochemical analysis for glucose which was determined colorimetrically according to Trinder [19], cholesterol [20], total protein [21] and creatinine [22] were determined.

Immunological assessment

Immunological response of fish was evaluated through determination of serum lysozyme levels [23] and IgM [24].

Statistical analysis

Data were collected, organized and analyzed using one-way analysis of variance (ANOVA) through the general linear models (GLM) procedure of the Statistical Package for Social Sciences version 21.0 (SPSS for Windows 21.0, Inc., Chicago, IL, USA). The comparison of means was carried out with Duncan's multiple range tests (DMRT). Results were recorded as mean ± standard deviation (SD). The value of $P<0.05$ was used to indicate statistical significance.

Histological analysis

At the end of experiment, small slices of liver, spleen, intestine, head kidney and skeletal muscle were taken and fixed immediately in neutral buffered formalin 10%. The fixed specimens were processed using the usual histological techniques; dehydrated in ascending grades of ethanol series, cleared in benzene and embedded in paraffin. 5-7 µm thick sections were prepared and mounted on glass slides. These are dewaxed in xylene, hydrated in descending grades of ethanol series and stained with Harris's hematoxylin and Eosin (H&E) for routine histopathological studies according to Bancroft and Gamble [25]. The microphotographies were taken using a digital Dsc-W 130 super steady cyper shot camera (Sony, Japan) connected to an Olympus BX 21 light microscope.

Results

The effect of different levels of dietary protein on the growth performance of *Carassius auratus* is summarized in Table 3 in which there is a high significant increase in all nutritional parameters of group C "fish feed on high dietary protein" followed by control then low protein groups.

Parameters	Group		
	A	B	C
Initial body weight (g)	18.60 ± 0.28a	18.83 ± 0.20a	18.88 ±0.19a
Final body weight (g)	34.72 ± 0.20b	29.67 ± 0.17c	35.79 ± 0.16a
Weight gain (g)	15.89 ± 0.20b	11.07 ± 0.23c	16.91 ± 0.22a
weight gain %	84.54 ± 1.69a	59.77 ± 1.99b	89.69 ± 1.84a
Specific growth rate SGR	1.02 ± 0.02a	0.78 ± 0.02b	1.07 ± 0.02a
Daily gain rate (DGR)	0.26 ± 0.00b	0.18 ± 0.00c	0.28 ± 0.00a

Table 3: Effect of dietary protein levels on mean nutritional Parameters of *Carassius auratus*. Means within the same row carrying different superscripts are sig. different at $P<0.05$ based on Duncan's Multiple Range Test (DMRT).

Concerning the behavioral alteration of *Carassius auratus exposed to* different dietary protein levels and starvation stress is shown in Table 4. The feeding frequency and duration in which there was a significant increase in group C (high protein in diet) when compared with groups B (low protein in diet) and D (fasting group). Regarding the swimming frequency and duration, there was a significant increase in group B when compared with group D. Table 4 also showed the effect of dietary protein levels and starvation on aggressive frequency and duration. In which there was a significant increase in group B when compared with other groups. The rest and arousal behavior and duration have a significant increase in fasting group (D), while fish coming to the surface of aquaria behavior showed a significant increase in group B.

Behavioral patterns		Group		
	A	B	C	D
Frequency of feeding	3.05 ± 0.14b	2.19 ± 0.16c	4.73 ± 0.23a	0.04 ± 0.02d
Feeding time (Sec.)	76.01 ± 3.07b	25.62 ± 2.84c	82.30 ± 2.84a	5.00 ± 2.84d
Frequency of swimming	2.38 ± 0.15b	5.45 ± 0.61a	1.86 ± 0.4b	0.75 ± 0.15c
Swimming time (sec.)	27.28 ± 1.86b	43.95 ± 3.89a	28.34 ± 3.33b	29.02 ± 4.89b
Frequency of aggression	0.84 ± 0.10b	12.8 ± 2.77a	3.04 ± 0.26b	0.45 ± 0.12b
Aggression time (sec.)	4.23 ± 0.94b	21.59 ± 2.52a	5.08 ± 1.99b	7.15 ± 1.65b
Frequency of rest	0.19 ± 0.46b	3.72 ± 0.94ab	0.25 ± 0.05b	4.09 ± 2.32a
Rest time (sec.)	11.87 ± 3.19b	11.11 ± 3.96b	14.37 ± 3.53b	89.93 ± 5.62a
Frequency of arousal	1.00 ± 0.00a	0.94 ± 0.02a	0.97 ± 0.00a	0.36 ± 0.05b
Arousal time (sec.)	108.88 ± 3.19a	108.12 ± 3.56a	105.62 ± 3.53a	33.40 ± 5.85b
Frequency of fish coming surface	1.70 ± 0.13b	2.34 ± 0.17a	0.83 ± 0.10c	0.47 ± 0.10c

Frequency of feeding	3.05 ± 0.14b	2.19 ± 0.16c	4.73 ± 0.23a	0.04 ± 0.02d

Table 4: Effect of nutritional factors on the behavioral patterns of *Carassius auratus*. Means within the same row carrying different superscripts are sig. different at P<0.05 based on Duncan's Multiple Range Test (DMRT).

Table 5 showing the effect of different dietary protein levels and starvation on some blood serum biochemical Parameters of *Carassius auratus*. As shown in this table, there was a significant increase in serum cholesterol level in group feeding on 28% and 45% CP., when compared with low and fasting groups. The serum total protein considers a nutritional indicator that gives information about fish metabolism in which there was a significant increase in serum protein in groups feeding on 28% and 45% CP. Concerning the glucose level a significant decrease in glucose level in group feeding high protein in addition there was a significant increase of creatinine in group feeding 45% cp in diet when compared with other groups.

Parameters	**Group**			
	A	**B**	**C**	**D**
Cholesterol(CHO) (mg/dl)	259 ± 4.05^{a}	244 ± 6.39^{b}	260 ± 9.11^{a}	220 ± 2.17^{c}
Total Protein	4.90 ± 0.33^{a}	3.80 ± 0.21^{b}	5.10 ± 0.53^{a}	3.42 ± 0.29^{c}
Glucose	22 ± 2.03^{b}	23 ± 0.91^{b}	18 ± 1.20^{c}	29 ± 2.02^{a}
Creatinine	0.3 ± 0.02^{c}	0.39 ± 0.02^{b}	0.41 ± 0.03^{a}	0.28 ± 0.01^{b}

Table 5: Effect of different nutritional factors on some blood serum biochemical Parameters of *Carassius auratus*. Means within the same column carrying different superscripts are sig. different at P<0.05.

Regarding the effect of dietary protein levels and starvation on mean IgM and Lysozyme levels as an important immunological parameters of fish blood, in which we observed that the highest IgM lysozyme level was in group C (high protein fed group) (Table 6).

Groups	**Parameter**	
	IgM	**Lysozyme**
Cholesterol(CHO) (mg/dl)	259 ± 4.05a	244 ± 6.39b
Total Protein	4.90 ± 0.33a	3.80 ± 0.21b
Glucose	22 ± 2.03b	23 ± 0.91b
Creatinine	0.3 ± 0.02c	0.39 ± 0.02b

Table 6: Effect of different nutritional factors on mean IgM and Lysozyme levels. Means within the same column carrying different superscripts are sig. different at P<0.05.

Regarding the histological examination, our result revealed that there are no any significant changes on the histoarchitectures of the all selected organs; liver, spleen, intestine, head kidney and muscle of groups 1, 2 and 3 feed with diet A (28% CP), diet B (17% CP) and diet C (45% CP) respectively. Meanwhile, the fasting of group 4 had the most effective changes on the histoarchitectures of all selected organs.

Liver of fantail goldfish (*Carassius auratus* L.) groups 1, 2 and 3 exhibited a normal, homogenous, intact hepatic parenchyma with normal central vein and there were no any pathological abnormalities. Hepatocytes were arranged as plates or cords that were dorsally radiated from the central vein toward the periphery of the hepatic lobules. Hepatic lobulations in fan-tailed goldfish was indistinct as they were separated by a very delicate loose connective tissue (Figure 1A). With the higher magnification, liver demonstrated the sponge-like appearance of the parenchyma which is primarily composed of large irregular polygonal hepatocytes with typically large single central or subcentral spherical nucleus with prominent nucleoli and sometimes binucleated. Nucleus is associated with a pale or vacuolar area. Hepatocytes cytoplasm is pale and homogenous as a lot of glycogen. Furthermore, the hepatic cords were laterally separated by normal sinusoidal architectures that were filled with erythrocytes. Moreover, some kupffer cells and melanomacrophage centres (MMCs) were observed (Figure 1B). Meanwhile, liver of fasted fantail goldfish (*Carassius auratus* L.) group 4 showed focal to diffuse areas of vacuolar degeneration with mild necrotic areas characterized by focal necrotic cells with pyknosis and karryorhexis of their nuclei. Disorganization of the hepatic cords with sever congestion of the central vein were observed (Figure 1C). Massive distension, vacuolations and micro vesicular fatty degeneration of hepatocytes were also demonstrated in some examined sections as well as overall degeneration of cellular architecture of liver tissue (Figure 1D). Some extravasated RBCs were observed in between the hepatocytes within the hepatic parenchyma (Figure 1C, 1D). Focal areas of chronic inflammation with fibrous connective tissue proliferation (Figure 1E, 1F) infiltrated with chronic inflammatory cells were clarified (Figure 1F, 1G). Sever thickening of the bile duct with fibrous tissue proliferation were also observed (Figure 1H, 1I).

Spleen of fantail goldfish (*Carassius auratus* L.) groups 1, 2 and 3 exhibited a normal, intact splenic parenchyma; white pulp and red pulp as well as the dark staining melanomacrophage centres in the splenic tissues (MMCs) without any pathological abnormalities (Figure 2A, 2B and 2C). Meanwhile, spleen of fasted fantail goldfish (*Carassius auratus* L.) group 4 showed mild necrosis of the splenic parenchyma in both white and red pulps with even initiation of vacuolations and some loss of normal architecture with diffused patches of haemosiderin pigments (Figure 2D). Sever haemosidriosis; haemolysis of RBCs and escape of large amount of haemosidrin pigment that accumulated

forming large golden yellow patches of haemosiderin pigments within the splenic parenchyma were observed (Figure 2E and 2F).

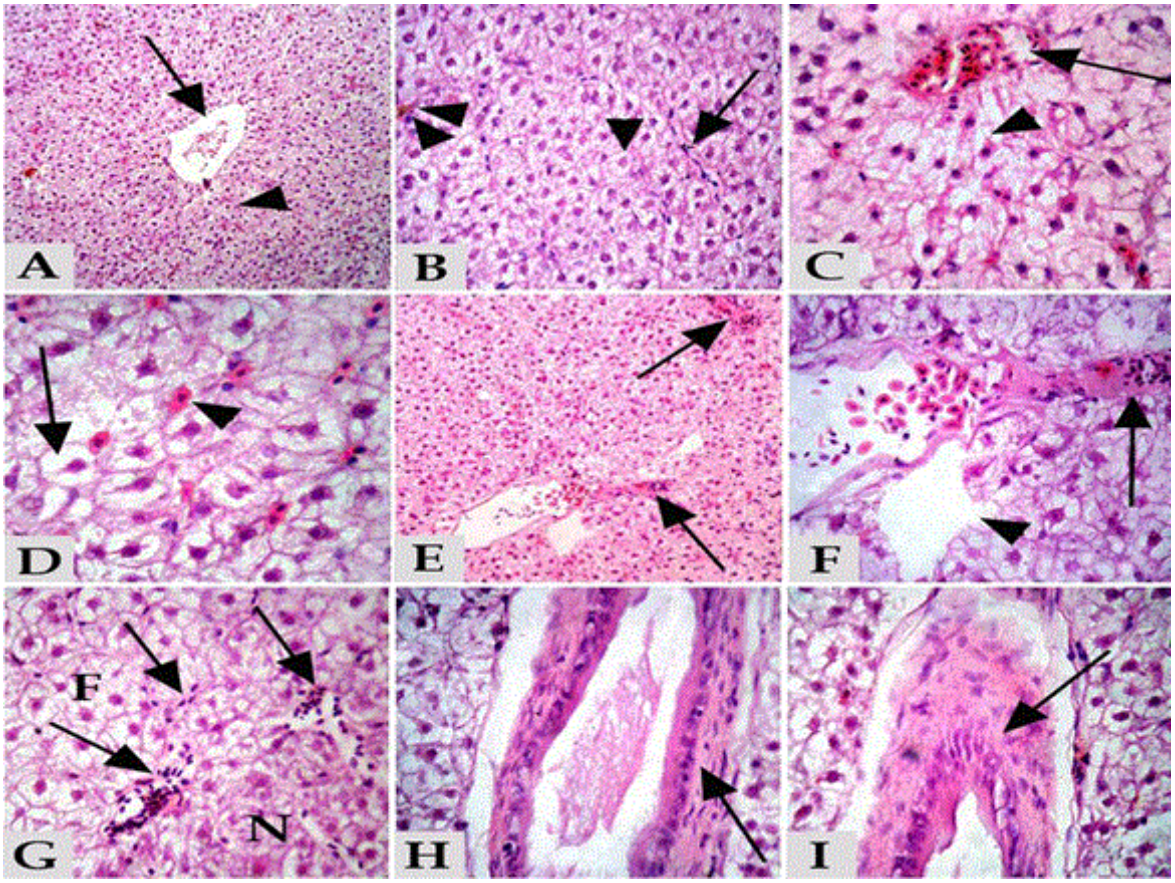

Figure 1A: Section of fantail goldfish (*Carassius auratus* L.) liver of group 1, 2 and 3 feed with diet A (28% CP), diet B (17% CP) and diet C (45% CP) respectively showing normal, homogenous, intact hepatic parenchyma with normal central vein (arrow) and distinct radiated hepatic cords from the central vein to the periphery (arrow head) where there is no any significant changes on the histoarchitectures of all selected organs in these three different groups. **Figure 1B:** Section of *Carassius auratus* L. liver of group 1, 2 and 3 showing normal hepatic sinusoids with normal hepatocytes of irregular polygonal shaped cells with single, central, large vesicular nucleus and pale cytoplasm with slightly vacuolations (arrow head), large like kupffer cells (arrow) and melanomacrophage centres (MMCs) (double arrow heads). **Figure 1C:** Section of *Carassius auratus* L. liver of group 4 (fasted group) showing diffuse degeneration, necrosis of hepatocytes infiltrated with numerous lymphocytes and few extravasated erythrocytes, with disorganization of the hepatic cords (arrow head) with sever congestion of central vein (arrow). **Figure 1D:** Section of *Carassius auratus* L. liver of group 4 (fasted group) showing diffuse microvesicular fatty degenerations , pyknotic central nuclei and pale cytoplasm with sever vacuolaions (arrow), and some extravasated RBCs (arrow head). **Figure 1E:** Section of *Carassius auratus* L. liver of group 4 (fasted group) showing focal areas of chronic inflammation (arrows). **Figure 1F:** Higher magnification of fig E showing area of chronic inflammation; fibrous connective tissue proliferation infiltrated with chronic inflammatory cells (arrow) with area of necrosis (arrow head). **Figure 1G:** Section of *Carassius auratus* L. liver of group 4 (fasted group) showing hepatocytes of fatty degeneration (F) with sever aggregations of chronic inflammatory cells (arrows) and area of sever necrosis (N). **Figure 1H and 1I:** Section of *Carassius auratus* L. liver of group 4 (fasted group) showing sever thickening of the bile duct (arrow).

Intestine of fantail goldfish (*Carassius auratus* L.) groups 1, 2 and 3 exhibited a normal, intact intestinal wall; tunica mucosa, submucosa and muscularis without any pathological abnormalities (Figure 3A and 3B). The intestinal mucosa is the inner most layers and has deep finger-like processes; villi that extending in the organ lumen (Figure 3A, 3B). These expansions are lined with a simple columnar epithelium comprising mainly absorptive cells and scattered mucus-secreting or goblet cells (Figure 3C). The lamina propria and submucosa are generally composed of a loose connective tissue containing blood and lymph capillaries and large numbers of wandering eosinophilic granular cells and variable quantities of lymphoid tissue. The role of the eosinophilic granular cells (EGCs) comes as containing antimicrobial peptides and their degranulation that can increase the vascular permeability and promote neutrophil adhesion, suggesting that they are intimately involved in innate immunity and inflammation (Figure 3C). The muscularis mucosa usually consists of a thin layer of smooth muscle cells, longitudinal in direction. The intestine is covered externally with tunica serosa that is mainly formed of vascularized loose connective tissue; tunica adventitia and mesothelium of simple squamous epithelium (Figure 3B). Meanwhile, intestine of fasted

fantail goldfish (*Carassius auratus* L.) group 4 showed necrosis in the intestinal villi with degeneration of intestinal mucosa along with focal detachment of the lining epithelial (Figure 3D). Severe vacuolations of the cytoplasm of the intestinal columnar cells were observed (Figure 3E) with chronic enteritis that characterized by aggregation of mononuclear cells mainly lymphocytes, macrophages and plasma cell as well as fibrous connective tissue proliferation within the propria submucosa (Figure 3F).

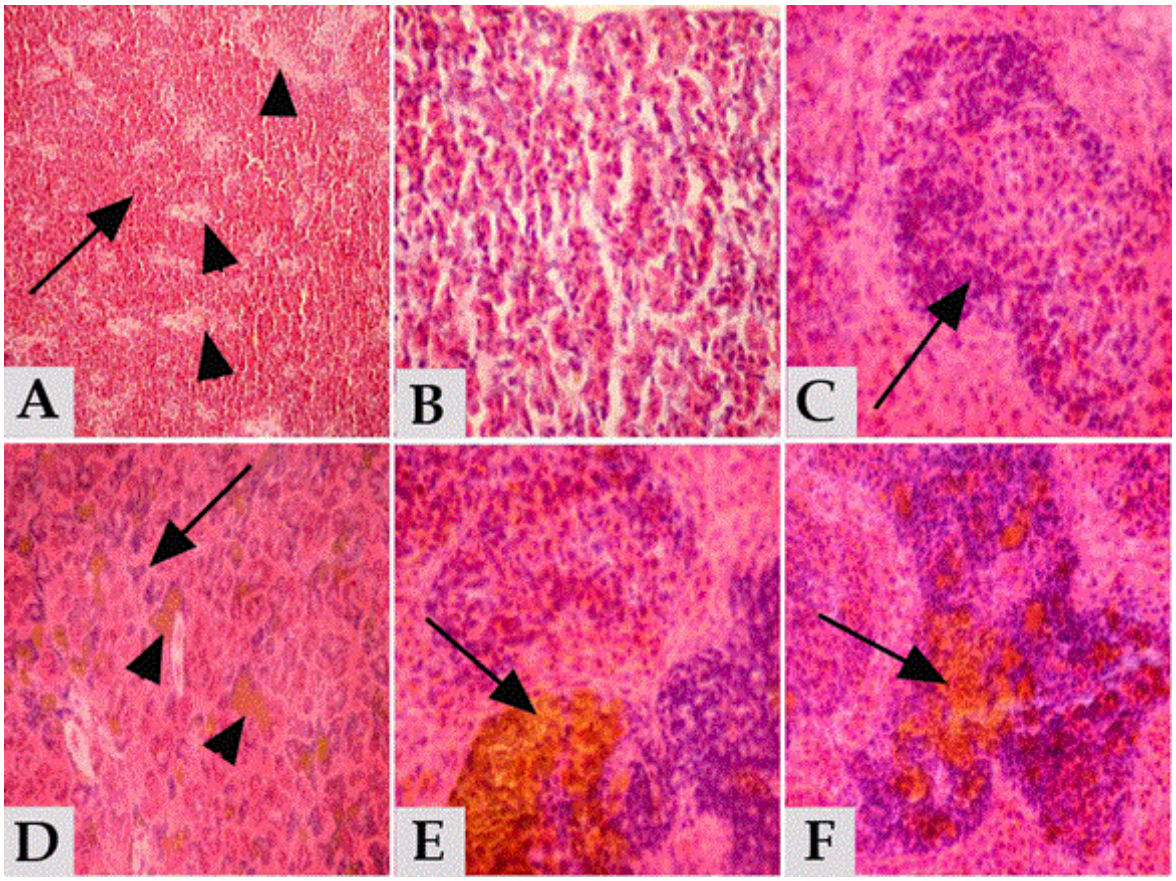

Figure 2A: Section of *Carassius auratus* L. spleen of group 1, 2 and 3 showing normal, intact splenic parenchyma; white pulp (arrow heads) and red pulp (arrow). **Figure 2B:** Section of *Carassius auratus* L. spleen of group 1, 2 and 3 showing normal red pulp. **Figure 2C:** Section of *Carassius auratus* L. spleen of group 1, 2 and 3 showing normal white pulp (arrow). **Figure 2D:** Section of *Carassius auratus* L. spleen of group 4 (fasted group) showing mild necrosis of the splenic parenchyma; white and red pulps with some loss of normal architecture (arrow) and with diffused patches of haemosiderin pigments (arrow heads). **Figure 2E and 2F:** Section of *Carassius auratus* L. spleen of group 4 (fasted group) showing large golden yellow patches of haemosiderin pigments within the splenic parenchyma (arrow).

Head kidney of fantail goldfish (*Carassius auratus* L.) groups 1, 2 and 3 exhibited a normal, intact renal parenchyma; cortex and medulla which enriched with the haemopoietic tissue, without any pathological abnormalities. Meanwhile, head kidney of fasted fantail goldfish (*Carassius auratus* L.) group 4 showed severe congestion and hemorrhage presented by extravasated RBCS within the renal parenchyma (Figure 4A, 4B, 4C and 4D). Mild degenerative changes in the renal corpuscles with loss of their normal architecture were noticed (Figure 4E and 4F), with sever tubular vacuolations (Figure 4G), and sever necrosis & degenerative changes in the renal tubules and haemopoietic tissue with loss of their normal architecture (Figure 4H). Furthermore, sever hyaline degenerative changes in the lining epithelium of the renal tubules were observed (Figure 4I).

Skeletal muscle of fantail goldfish (*Carassius auratus* L.) groups 1, 2 and 3 showed normal, intact skeletal muscle fibers with acidophilic cytoplasm that exhibited the cross striation banding pattern of alternating dark band with light band. The nuclei were multiple, peripheral and elongated (Figure 5A). Meanwhile, skeletal muscle of fasted fantail goldfish (*Carassius auratus* L.) group 4 showed necrosis in the skeletal muscle cells with loss of their nuclei (Figure 5B) with sever hyaline degeneration and edema where the muscle cells were converted to complete mass of hyaline material. The muscle cells cytoplasm became homogenous, acidophilic and devoid of any obvious striations and nuclei (Figure 5C, 5D).

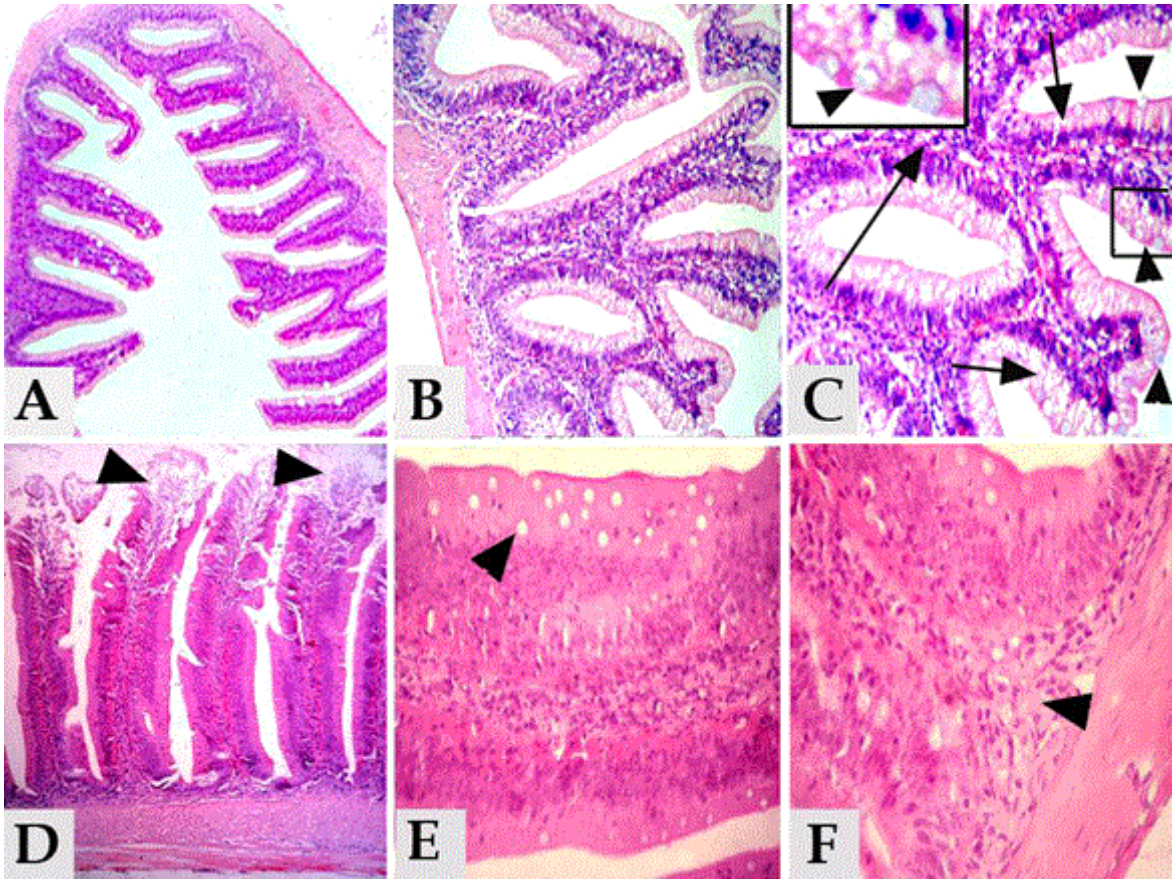

Figure 3A: Section of *Carassius auratus* L. intestine of group 1, 2 and 3 showing normal, intact intestinal wall and intestinal villi. **Figure 3B:** Section of *Carassius auratus* L. intestine of group 1, 2 and 3 showing normal, intact intestinal mucosa, submucosa and muscularis. **Figure 3C:** Section of *Carassius auratus* L. intestine of group 1, 2 and 3 showing normal lining epithelium of simple columnar cells (short arrow) with scattered goblet cells (arrow head) and normal submucosa (long arrow).Inset box, showing higher magnification of the goblet cells. **Figure 3D:** Section of *Carassius auratus* L. intestine of group 4 (fasted group) showing necrosis in the intestinal villi with focal detachment of the lining epithelial (arrow head). **Figure 3E:** Section of *Carassius auratus* L. intestine of group 4 (fasted group) showing severe vacuolations of the cytoplasm of the intestinal columnar cells (arrow head). **Figure 3F:** Section of *Carassius auratus* L. intestine of group 4 (fasted group) showing chronic enteritis characterized by aggregation of mononuclear cells mainly lymphocytes, macrophages and plasma cell as well as fibrous connective tissue proliferation within the submucosa.

Discussion

This research was conducted to know the importance of the different protein levels in diet and its impact on health and immunity, behavior and histoarchitectures of fantail goldfish *Carassius auratus* (L.) as well as the study shows how importance exposing those fish to starvation. Regarding the results of growth performance of *Carassius auratus* in which there was a high significant increase in all nutritional parameters of fish feed on high dietary protein followed by control then low protein groups. This can be attributed to the increase amount of protein in the diet and consequently it convert to body mass also some of that protein may acts as fuel as suggested by Bancroft JD et al. [26]. Also Lovell [27] mentioned that, the relative deficiency of protein in fish diet leads to lowering food intake and impairing development and growth. Our results also coordinated with that reported by Cheng AC, et al. [18] who mentioned that, the moderate dietary protein content resulted in weight gain and feed efficiency better than the low-protein diets. The results were in disagreement with that mentioned by Elangovan A, et al. [28] who reported that, protein level in diet around 50% showed a significant decrease in the body weight it may be due to the animal limitations to use the protein and their reduced feed efficiency. The fish survival was 100% in all treatments.

The behavioral patterns of *Carassius auratus* were also influenced by different dietary protein levels and starvation. As in case of feeding frequency and duration in which there was a significant increase in group in which fish fed high protein in diet. It may be attributed to that increase the dietary protein level in diet of fish increase the feed intake and growth rate which correlated with increase oxidative metabolism and protein synthesis. This result could be supported by Borge D, et al. [2] who reported that high numbers of factors including feeding affect fish welfare for maintaining homeostasis and normal development and protected against physical damages. While the effect of dietary protein levels and starvation on swimming frequency and duration, there was a significant increase in group B (low protein in diet) when compared with group and D (fasting group).

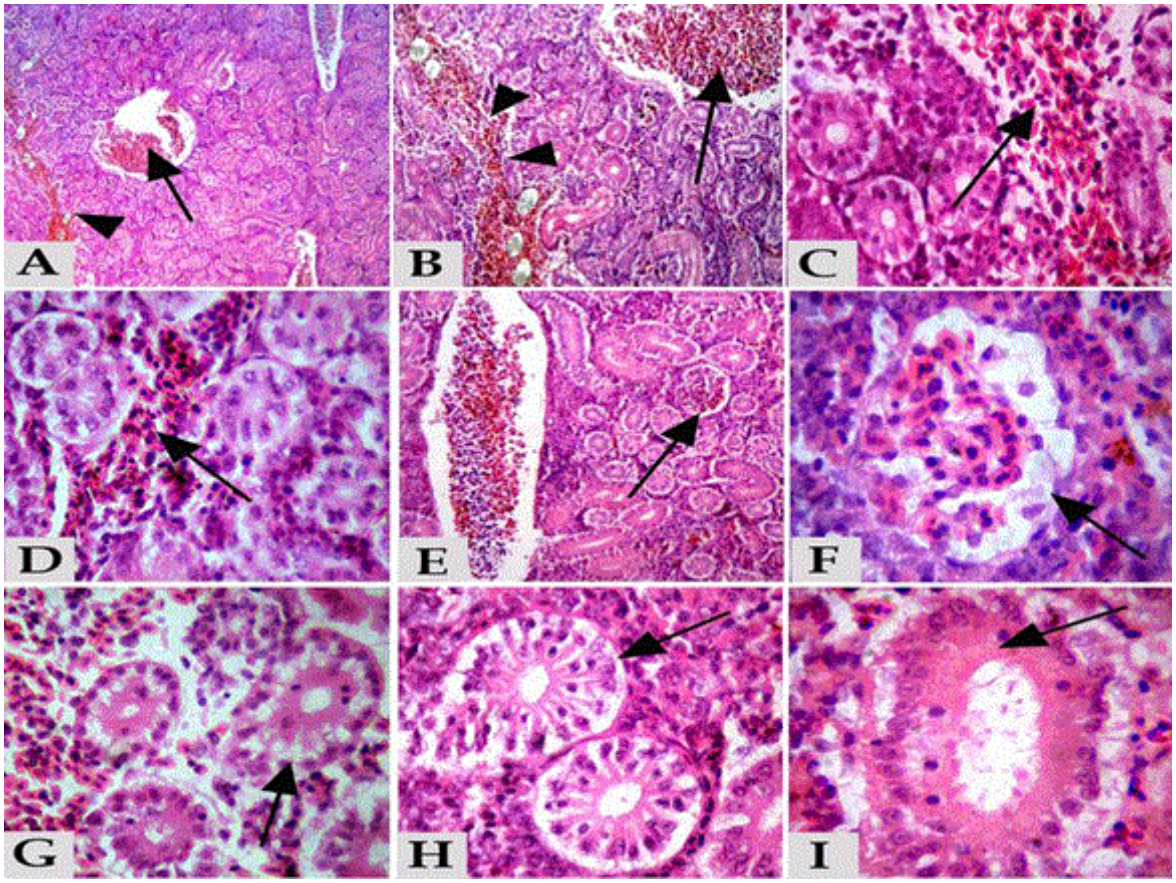

Figure 4A: Section of *Carassius auratus* L. head kidney of group 4 (fasted group) showing severe congestion (arrow) and hemorrhage (arrow head) within the renal parenchyma. **Figure 4B:** Higher magnification of fig A showing severe congestion (arrow) and sever extravasated RBCs (arrow heads) within the renal parenchyma. **Figure 4C and 4D:** Section of *Carassius auratus* L. head kidney of group 4 (fasted group) showing sever hemorrhage with numerous lymphocytes within the renal parenchyma (arrow). **Figure 4E, 4F:** Section of *Carassius auratus* L. head kidney of group 4 (fasted group) showing mild degenerative changes in the renal corpuscles with loss of their normal architecture. **Figure 4G, 4H:** Section of *Carassius auratus* L. head kidney of group 4 (fasted group) showing sever tubular vacuolations in fig G and sever necrosis & degenerative changes in the renal tubules with loss of their normal architecture in fig H. **Figure 4 I:** Section of *Carassius auratus* L. head kidney of group 4 (fasted group) showing sever hyaline degenerative changes in the renal tubules (arrow).

This result was in harmony with that mentioned by Martínez M, Alanara A, Brannas E, et al. [29-31] who illustrated that, food deprivation and deficiency in the diet leads to changes in metabolic activity and changes in territorial behavior strategies and activity pattern especially swimming.

Concerning the effect of dietary protein levels and starvation on aggressive frequency and duration, there was a significant increase in group B when compared with other groups. It may be due to decrease dietary protein levels which enhance the aggressive behavior. This result was in agreement with that of Höglund E, et al. [32] who illustrated that increase dietary levels has been shown to suppress aggressive activity. On the other hand, the effect of dietary protein levels and starvation on rest and arousal behavior and duration in which there was a significant increase in fasting group. There was a significant decrease in arousal behavior. These results agreed with Zielinski WJ [33] who suggested that the availability of food increase the activity, arousal and decrease the rest duration of fish. The results of the effect of dietary protein levels and starvation on fish coming to the surface of aquaria, showed that there was a significant increase in group B. this may be due to that the protein deficiency in diet acts as a stress factor in which fish become aggressive and try to come surface to get more food in order to be more growth rate and development as suggested by Houlihan DF, et al. [34].

Results of the effect of different dietary protein levels and starvation on some blood serum biochemical parameters of *Carassius auratus*, the serum cholesterol level, there was a significant increase in group A and C when compared with B and D groups. This result go hand with hand of that mentioned by Abdel-Tawwab M [35] who stated that, serum lipids significantly increased when the protein level increased this may be as a result of that the muscle is a pivotal compartment directly associated with amino acid turnover. It includes protein synthesis or breakdown of those molecules as energetic substrates. While, serum lipid levels decreased in case of fish starvation. As in case of fasting the stored lipids were used as an energy source. Also our results ensure that, energy homeostasis of fish during starvation confirm occurrence of energy reserves mobilization as lipids as

recorded by Sheridan MA, Navarro I [36,37] and so in case of prolonged starvation, the cholesterol level in serum is decreased.

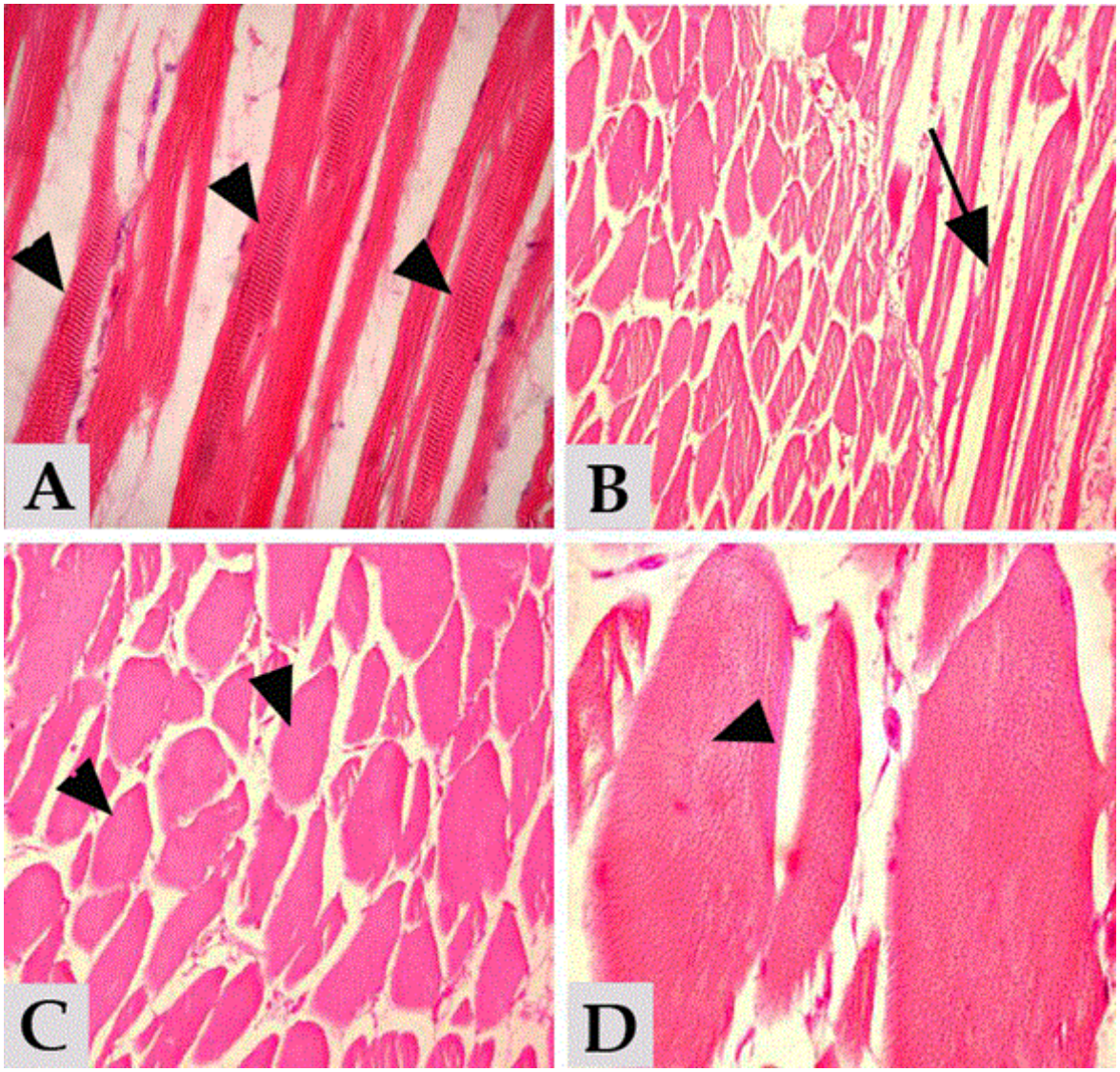

Figure 5A: Section of *Carassius auratus* L. skeletal muscle of group 1, 2 and 3 showing normal, intact skeletal muscle with acidophilic cytoplasm that showing the cross striation banding pattern of alternating dark band with light band and also multiple, peripheral nuclei (arrow head). **Figure 5B:** Section of *Carassius auratus* L. skeletal muscle of group 4 (fasted group) showing necrosis in the skeletal muscle with loss of their nucleus and cross striation (arrow). **Figure 5C, 5D:** Section of *Carassius auratus* L. skeletal muscle of group 4 (fasted group) showing sever hyaline degeneration and edema in the skeletal muscles with loss of their nuclei (arrow head).

The serum total protein considers a nutritional indicator that gives information about fish metabolism as illustrated by our results in which there was a significant increase in serum protein in groups feeding on 28% and 45% CP this may be due to increase of liver protein synthesis as supported by Hoseini SM [38]. This result was in harmony with that of Melo BJF, Abdel-Tawwab M [26,39] who reported that, serum protein increased with increased dietary protein level. Also [40] stated that, the increased protein level in diet leads to rise of serum protein which could likely be due to the enhancement of digested protein. On the other hand there was a clear tendency for serum protein to decrease in fasting fish. This could confirmed by presence of previous studies on several starved fish species, including brook trout [41], European eel [42] and Senegalese sole, Solea senegalensis [43] that ensure the decrease of serum total protein after starvation.

Serum glucose level has a significant decrease in group feeding high protein. Our results was harmony with that mentioned by [18] who reported that, there was a significant decrease of glucose levels in fish fed the diets containing the higher protein in diet. Regarding the effect of starvation on serum glucose level, we observed that, there was an elevation in the level of glucose in fasting group when compared with other groups. This result was coordinated with that of Mommsen TP

[44] who mentioned that serum glucose levels increased by fasting it may explained by fish trying to keep glucose concentrations during starvation by the increase of glycogenolysis and gluconeogenesis. Also Laiz-Carrión R, et al. [45] found an enhancement in the gluconeogenic and glycogenolytic during starvation.

Creatinine is considered a nitrogenous end product of protein catabolism and is directly increase with high levels of protein in diet that was clear in group feeding 45% cp in diet when compared with other groups. This result is harmony with that stated by Ajeniyi SA, et al. [46] who mentioned that the increased ratio of creatinine may be due to high protein blood levels.

IgM and Lysozyme levels in serum considered important immunological parameters of fish. Results showed that the highest IgM and lysozyme levels was observed in high protein fed group as supported by Kiron V, et al. [47] who found that lysozyme activity was reduced in protein deficient rainbow trout. In contrast the fasting group showed a significant decrease in IgM and lysozyme levels in serum that confirms that prolonged starvation influence the immune status of fish. The result was in agreement with that of [48] who reported that starvation stress can cause a decrease in the lysozyme level of European eel starved for 31 days.

It is well known that, the digestive system especially liver and intestines are considered a good indicator of the nutritional status of all mammals as well as fish [7-9]. The most important organs in digestion and absorption of nutrients from food are liver and intestines, and therefore monitoring of these organs is considered necessary [10]. For this, liver and intestines were used to show what changes occurred through experiments. Our results revealed that, the fasted group of fish showed various histological alterations in the liver that exhibited degenerative changes in the form of necrosis, vacuolations of hepatocytes and degeneration of cellular architecture of hepatic cords and hepatic sinusoids distention. These findings are in coincidence with the findings of [49-53] who reported that the necrosis of liver hepatocytes in different fishes is following stress of starvation.

As a result of hepatic cells vacuolations, impairing its synthetic machinery resulted in an imbalance in the rate of synthesis and release of substances in the systemic circulation. As starvation prolonged, the hepatocytes lose their integrity and hence the energy reserves in the form of glycogen and lipids stored in hepatocytes may apparently get depleted. As progressed depletion, energy decreased and landing the fish in extremely worst condition. Similar viewpoint has been put forth by [54,55] in fish liver tissue following starvation. Distension of hepatic sinusoids in our results may be due to the mechanical obstruction of blood supply to liver tissue. Such distention and vacuolation seemingly appear to result in overall degeneration of the cellular architecture of liver tissue towards the end of the starvation period and disruption of various metabolic processes. All these histopathological observations indicate that exposure of fish to prolonged starvation caused destructive changes in its liver tissue ultimately disrupting the functional efficiency of liver.

Spleen of fasted group showed mild necrosis of the splenic parenchyma with even initiation of vacuolations and some loss of normal architecture with sever haemosidriosis that appeared as large golden yellow patches of haemosiderin pigments within the splenic parenchyma. These results are in agreement with [56] who clarified that necrosis and vacuolation in splenic tissue of European eel Anguilla anguilla following stress of fasting resulting in impairment of normal physiology of fishes. Deposition of haemosiderin leads to pathological state known as haemosiderosis [57]. Hibiya [58] also stated that the main cause of haemosidrosis was the increased rate of erythrocyte destruction in the spleen. Under the influence of prolonged starvation, the histological architecture of splenic tissue manifested severe degeneration ultimately affecting its blood cell forming capacity.

Intestine of fasted fantail goldfish (*Carassius auratus* L.) group 4 showed necrosis in the intestinal villi with degeneration of intestinal mucosa with focal detachment of the lining epithelial and severe vacuolations of the cytoplasm of the intestinal columnar cell and with chronic enteritis that infiltrated with chronic inflammatory cells and fibrous connective tissue proliferations within the propria submucosa. These investigations are very close and similar to those described after [59]. The present study showed that enteritis is dependent on the percentage of crude proteins in fish meal and starvation. Lowering the proteins percentage in the fish meal as well as starvation, increase the signs of enteritis that observed in starved group. Similar results were recorded by [60].

The microscopic examination of the head kidney of starved fan-tailed goldfish revealed tubular vacuolations and tubular necrosis as well as haemopoietic tissue. The renal tissue was observed to undergo total degeneration with loss of normal architecture of renal tubules and haemopoietic tissue in some examined sections. Such results were observed in renal tissue are in parallelism with the findings of [61-63] in fishes when exposed to different periods of starvation. Degenerative changes in renal tissue as observed presently are considered the main causes of different excretory disorders. Moreover continuous decrease in the haemopoietic tissue of the kidney can lead to impairment of haemopoiesis; result in disruption in the blood forming efficiency of head kidney and hence inhibits further release of normal erythrocytes into the general circulation, thereby affecting its functional efficiency which has a direct influence on physiology of fish.

Conclusion

From our results, we can conclude that the protein level is very important metabolite of *Carassius auratus*. The ideal levels of protein required by *Carassius auratus* is 45% CP followed by 28% CP that in case of starvation there was a significant decrease in protein level, that consider a fuel source for fish. In addition, the high protein level decrease the stress on *Carassius auratus* as illustrated from low serum glucose level, while increased glucose value in case of staved fish indicates chronic stress. Prolonged starvation for 8 weeks affects health, immune response, behavior and tissue architectures of *Carassius auratus*. Histologically, there is no any significant changes on the histoarchitectures of the all selected organs; liver, spleen, intestine, head kidney as well as muscle of groups 1, 2 and 3 feed with diet A (28% CP), diet B (17% CP) and diet C (45% CP) respectively. Meanwhile, the fasting of group 4 had the most effective changes on the histoarchitectures of all selected organs.

References

1. Güroy D, Sahin I, Güroy B, Altin A, Merrifield DL (2012) Effect of Dietary Protein Level on Growth Performance and Nitrogen Excretion of the Yellow Tail Cichlid, Pseudotropheus acei. The Israeli Journal of Aquaculture Bamidgeh, IJA, 64: 684-690.
2. Borge D, Erik Juell J, Brastad B (2006) Welfare in farmed fish. Norwegian Institute of fisheries and aquaculture research p. 10.
3. Erdogan F, Erdogan M, Gümüs E (2012) Effects of Dietary Protein and Lipid Levels on Growth Performances of Two African Cichlids

(Pseudotropheus socolofi and Haplochromis ahli). Turk J Fish Aquat Sci 12: 635-640.

4. Moraes G, Bidinotto PM (2004) Digestive proteases of pacu, Piaractus mesopotamicus fed on distinct protein-starch diets. J Appl Aquac 15: 197–207.
5. Lundstedt LM, Melo JF, Moraes G (2004) Digestive enzymes and metabolic profile of Pseudoplatystoma corruscans (Teleostei: Siluriformes) in response to diet composition. Comp Biochem Physiol B Biochem Mol Biol 137: 331-339.
6. Cuesta A, Meseguer J, Esteban MA (2004) Total serum immunoglobulin M levels are affected by immunomodulators in seabream (Sparus aurata L.). Vet Immunol Immunopathol 101: 203-210.
7. Hall KC, Bellwood DR (1995) Histological effects of cyanide, stress and starvation on the intestinal mucosa of Pomacentrus coelestis, a marine aquarium fish species. Journal of Fish Biology 47: 438-454.
8. Green BS, McCornick MI (1999) Influence of larval feeding history on the body condition of Amphiprion melanopus. Journal of Fish Biology 55: 1273-1289.
9. Caballero MJ, Izquierdo MS, Kjorsvik E, Montero D, Socorro J, et al. (2003) Morphological aspects of intestinal cells from gilthead seabream (Sparus aurata) fed diets containing different lipid sources. Aquaculture 225: 325-340.
10. Roberts RJ (1989) Fish pathology. Baillière Tindall, London.
11. Margulies D (1993) Assessment of the nutritional condition of larval and early juvenile tuna and Spanish mackerel (Pisces Scombridae) in the Panama Bight. Mar Biol 115: 317-330.
12. Kjorsvik E, Vandermeeren T, Kryvi H, Arnfinnson J, Kvenseth PG (1991) Early development of the digestive tract of cod larvae, Gadius morhua L, during start feeding and starvation. J Fish Biol 38: 1-15.
13. Bisbal GA, Bengtson DA (1995) Description of the starving condition in summer flounder Paralichthys dentatus early life history stages. Fish Bull 93: 217-230.
14. Sachar A (2012) Studies on effect of organic and inorganic pollutants on haematology, blood biochemistry and immune organs in some fishes of Jammu region. Ph.D Thesis, Department of Zoology, University of Jammu, Jammu.
15. Pouomonge V, Ombredane D (2001) Effect of feeding frequency on the growth of Tilapia (Oreochromis niloticus) in earthen ponds. Tropicultura 19: 147-150.
16. Altmann J (1974) Observational study of behavior: sampling methods. Behaviour 49: 227-267.
17. Stephan GR (2008) Sleep in fishes. Universite de Moncton, Canda pp. 1-13.
18. Cheng AC, Chen CY, Liou CH, Chang CF (2006) Effects of Dietary Protein and Lipids on Blood Parameters and Superoxide Anion Production in the Grouper, Epinephelus coioides (Serranidae: Epinephelinae). Zoological Studies 45: 492-502.
19. Trinder P (1969) Determination of glucose concentration in the blood. Clinical Biochemistry 6: 24.
20. Hestrin S (1949) The reaction of acetylcholine and other carboxylic acid derivatives with hydroxylamine, and its analytical application. J Biol Chem 180: 249-261.
21. Henry RJ (1964) Clinical Chemistry Principles and Techniques. (2nd Edn) Harper and Row Publ, New York, USA pp. 160-172.
22. Henry RJ (1974) Clinical Chemistry Principles and Techniques. 2nd ed., Harper and Row Publ, New York, USA pp. 1354-1369.
23. Ellis AE (1990) Lysozyme assays. In Stolan JS, Fletcher TC, Erson DP, Roberson BS, Muiswinkel WB (Eds) Techniques in Fish Immunology, USA: SOS publications 1: 1-103.
24. Siwicki AK, Anderson DP (1993) Non-specific defense mechanisms assay in fish. II Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum. FAO-Project GCP/INT/526/JPN, IFI Olzsztyn pp. 105-112.
25. Bancroft JD, Gamble M (2001) Theory and practice of histological techniques (5th Edn) Churchill livingstone, New york pp. 281-285.
26. Melo BJF, Lundstedt LM, Metón I, Baanante IV, Moraes G (2006) Effects of dietary levels of protein on nitrogenous metabolism of Rhamdia quelen (Teleostei: Pimelodidae). Comparative Biochemistry and Physiology, Part A 145: 181-187.
27. Lovell RT (1979) Factors affecting voluntary food consumption by channel catfish. Proc World Symp. Finfish Nutr Fishfeed Technol 1: 555-564.
28. Elangovan A, Shim KF (1997) Growth response of juvenile Barbodes altus fed isocaloric diets with variable protein levels. Aquaculture 158: 321-329.
29. Martínez M, Guderley H, Dutil JD, Winger PD, He P, et al. (2003) Condition, prolonged swimming performance and muscle metabolic capacities of cod Gadus morhua. J Exp Biol 206: 503-511.
30. Alanara A, Burns MD, Metcalfe NB (2001) Intraspecific resource partitioning in brown trout: the temporal distribution of foraging is determined by social rank. J Anim Ecol 70: 980-986.
31. Brannas E, Jonsson S, Lundquist H (2003) Influence of food abundance on individual behavior strategy and growth rate in juvenile brown trout (Salmo trutta). Can J Zool 81: 684-691.
32. Höglund E, Weltzien FA, Schjolden J, Winberg S, Ursin H, et al. (2005) Avoidance behavior and brain monoamines in fish. Brain Res 1032: 104-110.
33. Zielinski WJ (1988) The influence of daily variation in foraging cost on the activity of small carnivorous. Animal behavior 36: 239-249.
34. Houlihan DF, Mathers E, Foster AR (1993) Biochemical correlates of growth rate in fish. In: Rankin JC, Jensen FB (Eds) Fish Ecophysiology, Chapman & Hall, London, pp: 45–72.
35. Abdel-Tawwab M (2012) Effects of dietary protein levels and rearing density on growth performance and stress response of Nile tilapia, oreochromis niloticus (L). International Aquatic Research 4: 3.
36. Sheridan MA, Mommsen TP (1991) Effects of nutritional state on in vivo lipid and carbohydrate metabolism of coho salmon, Oncorhynchus kisutch. Gen Comp Endocrinol 81: 473-483.
37. Navarro I, Gutierrez J (1995) Fasting and starvation. In: Hochachka PW, Mommsen TP (Eds) Biochemistry and Molecular Biology of Fishes. Elsevier, New York, pp: 393–434.
38. Hoseini SM, Ghelichpour M (2013) Effects of pre-sampling fasting on serum characteristics of common carp (Cyprinus carpio L). International Journal of Aquatic Biology 1: 6-13.
39. Abdel-Tawwab M, Ahmad MH, Khattab YAE, Shalaby AME (2010) Effect of dietary protein level, initial body weight, and their interaction on the growth, feed utilization, and physiological alterations of Nile tilapia, Oreochromis niloticus(L). Aquaculture 298: 267-274.
40. Lundstedt LM, Melo JFB, Santos-Neto C, Moraes G (2002) Diet influences proteolytic enzyme profile of the South American catfish.In: Rhamdia quelen (Ed) Proceedings of the International Congress on the Biology of Fish biochemistry and physiology advances in finfish aquaculture, Vancouver pp. 22–25.
41. Heming TA, Paleczny EJ (1987) Compositional changes in skin mucus and blood serum during starvation of trout. Aquaculture 66: 265–273.
42. Dave G, Johansson-Sjobeck ML, Larsson A, Lewander K, Lidman U (1975) Metabolic and hematological effects of starvation in the European eel, Anguilla anguilla LI Carbohydrate, lipid, protein and inorganic ion metabolism. Comp Biochem Physiol A Physiol 52A: 423–430.
43. Costas B, Aragao C, Ruiz-Jarabo I, Vargas-Chacoff L, Arjona FJ, et al. (2011) Feed deprivation in Senegalese sole (Solea senegalensis Kaup, 1858) juveniles: effects on blood plasma metabolites and free amino acid levels. Fish Physiol Biochem 37: 495–504.
44. Mommsen TP, Plisetskaya EM (1991) Insulin in Fishes and agnathans: history, structure, and metabolic regulation. Reviews in Aquatic Sciences 4: 225-259.
45. Laiz-Carrión R, Viana I R, Cejas J R, Ruiz-Jarabo I, Jerez S, et al. (2012) Influence of food deprivation and high stocking density on energetic metabolism and stress response in red porgy, Pagrus pagrus L. Aquaculture International 20: 585-599.

46. Ajeniyi SA, Solomon RJ (2014) Urea and Creatinine of Clarias Gariepinus in Three Different Commercial Ponds. Nature and Science 12: 124.
47. Kiron V, Fukuda H, Okamoto N, Takeuchi T (1995) Protein nutrition and defense mechanisms in rainbow trout, Oncorhynchus mykiss. Comp Biochem Physiol 111A: 351-359.
48. Caruso G, Denaro MG, Caruso R, Genovese L, Mancari F, et al. (2012) Short fasting and refeeding in red porgy (Pagrus pagrus, Linnaeus 1758): Response of some haematological, biochemical and non specifc immune parameters. Marine Environmental Research 81: 18-25.
49. Pal M (2006) Bioassay studies under the ecotoxicological stress of chromium tri-oxide in a freshwater food fish, Channa punctatus (Bloch). PhD Thesis, Department of Zoology, University of Lucknow, Lucknow.
50. Sarkar B, Chatterjee A, Adhikari S, Ayyappan S (2005) Carbofuran and cypermethrin induced histopathological alterations in the liver of Labeo rohita (Hamilton) and its recovery. J Appl Ichthyol 21: 131-135.
51. Camargo M, Martinez C (2007) Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream. Neotropical Ichthyology 5: 327-336.
52. Pathan T, Shinde S, Thete P, Sonawane D (2010) Histopathology of liver and kidney of Rasbora daniconius exposed to paper mill effluent. Research Journal of Biological Sciences 5: 389-39.
53. Radhakrishnan M, Hemalatha S (2010) Sublethal effects of cadmium chloride to liver of freshwater fish Channa striatus (Bloch). American Eurasian Journal of Toxicological Sciences 2: 54-56.
54. Gisbert E, Conklin D, Piedrahita R (2004) Effects of delayed first feeding on the nutritional condition and mortality of California halibut larvae. J Fish Biol 64: 116-132.
55. Rios F, Donatti L, Fernandes M, Kalinin A, Rantin F (2007) Liver histopathology and accumulation of melanomacrophage centres in Hoplias malabaricus after long-term food deprivation and refeeding. Journal of Fish Biology 71: 1393-1406.
56. Spazier E , Storch V, Braunbeck T (1992) Cytopathology of spleen in eel Anguilla anguilla exposed to a chemical spill in the Rhine River. Diseases of Aquatic Organisms 14: 1-22.
57. Zapata AG, Cooper EL (1990) The immune system: comparative histopathology. John Wiley and Sons, Chichester, England, Zool Soc Bengalis 1: 67-70.
58. Hibiya T (1982) An atlas of fish histology. Normal and pathological features. Kodansha Ltd, Tokyo pp: 147.
59. Urán PA, Gonçalves AA, Taverne-Thiele JJ, Schrama JW, Verreth JA, et al. (2008) Soybean meal induces intestinal inflammation in common carp (Cyprinus carpio L.). Fish Shellfish Immunol 25: 751-760.
60. Krogdahl Å, Bakke-McKellep AM, Baeverfjord G (2003) Effects of graded levels of standard soya bean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (Salmo salar L). Aquaculture Nutrition 9: 361-371.
61. Velmurugan B, Selvanayagam M, Cengiz EI, Unlu E (2007) The effects of fenvalerate on different tissues of freshwater fish Cirrhinus mrigala. J Environ Sci Health B 42: 157-163.
62. Mohamed F (2009) Histopathological studies on Tilapia zilli and Soleo vulgaris from lake Qarun, Egypt. World Journal of Fish and Marine Sciences 1: 29-39.
63. Prashanth M (2011) Histopathological changes observed in the kidney of freshwater fish, Cirrhinus mrigala (Hamilton) exposed to cypermethrin. Recent Research in Science and Technology 3: 59-65.