Characterization of peroxisome proliferator-activated receptor at different stages of development of intestine, liver, spleen, pancreas and posterior kidney of the loach (*Misgurnus anguillicaudatus*)

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ABSTRACT

The loach is one of the most important fish species in aquaculture in China. The study of peroxisome proliferator-activated receptors (PPARs) will be essential for the determination of the functional role of the PPARα and PPARγ in loach fish. We used immunohistochemistry for PPARα and PPARγ at some stages of development beginning from the age of 1 month till the age of 15 months. Our result indicated that, the expression of PPARα and PPARγ was found in; columnar cells lining intestinal folds, hepatocytes, blood sinusoids in liver, pancreatic islets, pancreatic acini, the whole spleen and epithelial cells lining renal tubules of the kidney. The expression pattern of both PPARα and PPARγ was low at the age of one month, then reach its highest level at the age of 2 months. Then it decline at the age of 5 months. Then it re-increase at the age of 7 months and became low in older ages. The expression of PPARα was higher in comparison with PPARγ.

INTRODUCTION

The intestine of the loach (*Misgurnus anguillicaudatus*) at the age of 20 days post-hatch can be divided into anterior, mid, and posterior parts. The posterior portion was characterized by very thin wall and a large number of blood capillaries, which function in air-breathing property (**Zhang *et al*., 2016**). The intestinal air-breathing function was initially observed at about 10 days post-hatch in Dojo Loach (*Misgurnus anguillicaudatus*). In adults, it was easy to differentiate between anterior and posterior intestine. As anterior intestine was more extensive and showed interconnecting arrangement with the presence of several columnar cells and goblet cells in its lining

epithelium. Meanwhile, posterior intestine characterized by several blood capillaries under mucosal epithelium, which responsible for gas exchange (**Luo *et al*., 2016**). The stomach is absent in zebrafish like other Cyprinids and the anterior intestine its lumen is more extensive than the lumen of the posterior intestine (**Wallace *et al*., 2005**). There are many functions for intestinal epithelium, including; absorption of nutrients, osmoregulation, and protection from toxicants (**Grosell *et al*., 2010; Minghetti *et al*., 2017**). There are four kinds of cells lining intestinal epithelium; columnar absorptive type cell, goblet cells, some endocrine and immune cells. The intestinal crypt (the area between two intestinal villi) acts as, the site of intestinal stem cells which responsible for epithelium self-renewal process (**Clevers, 2013**).

The main component of the liver in fish is the hepatocytes, which appear in the form of cords or tubules. In some kinds of fish species, the pancreas is found intra-hepatic (**Sales *et al*., 2017**). Unlike mammals, the liver in most types of fishes is characterized by the absence of hepatic lobules and portal triad. The bile canaliculi are dispersed between hepatocytes, they open into bile duct, which usually lined with simple cuboidal epithelium which become columnar in large ducts (**Vicentini *et al*., 2005; Nejedli and Gajger, 2013; Faccioli *et al*., 2014**). The liver of Mugil cephalus fish was found to express PPARα and PPARγ. The PPARα was high while, PPARγ was very weak in expression (**Ibabe *et al*., 2004**).

The spleen in fish formed from white and red pulps and plays a vital role in immune mechanism against the invasion of pathogens (**Press and Evensen, 1999**). The nephron which is the central functional unit of the kidney for re-absorption of water and solutes and excretion of the waste product in mammals was proved to have the ability to regenerate in fish after its damage. While mammals lack this property as they can only restore damaged epithelium but not whole nephron (**Davidson, 2014; Drummond and Wingert, 2016**).

Peroxisome proliferator-activated receptors (PPARs) are three subtypes PPARα, PPARβ and PPARγ. They are a family of nuclear hormone receptors responsible for metabolic process and energy homeostasis (**Tyagi *et al*., 2011**). The peroxisome proliferator-activated receptors play a vital role in energy production via controlling lipid metabolism (**Kersten *et al*., 2000; Cajaraville *et al*., 2003**). PPARα has a vital role in energy production for body organs via fatty acid oxidation in the liver. It also can be found in kidney, retina, heart, skeletal muscles and brown adipose tissue (**Lefebvre *et al*., 2006; Rigamonti *et al*., 2008; Hussein *et al*., 2020**). PPARγ was found to play an essential role in stimulating the expression of genes responsible for glucose and lipid metabolism (**Kubota *et al*., 2006**). The gene cloning and expression analysis of PPARα1 and PPARα2 in loach (*Misgurnus anguillicaudatus*) indicated that, their expression increased with high lipid diets (**Liang *et al*., 2016; Li *et al*., 2018**).

This study aimed to show histological observations and determination of the functional role of PPARα and PPARγ in intestine, liver, pancreas, spleen, and kidney of loach fish (*Misgurnus anguillicaudatus*) at some developmental stages.

MATERIALS AND METHODS

1. Ethics

In this study we applied strictly all the recommendations in the guide for the use and care of laboratory animals of huazhong agricultural university.

2. Loach samples

Thirty five female loaches (five from each different age) were collected from the aquaculture base located in Huazhong agricultural university, Wuhan, China.

3. Histology

The samples from intestine, liver, spleen, and kidney were collected from fishes at different developmental stages and preserved in 4% paraformaldhyde and kept in refrigerator at 4℃ for 24 hours. Then tissue samples were dehydrated, cleared and embedded in paraffin wax. Serial sectioning by microtome at 5µm was obtained and stained with hematoxylin and eosin for observation of general histological structure according to **Hussein *et al*., (2020)**. Then slides were examined by a Zeiss light microscope for taking photos.

4. Immunohistochemistry

The tissue sections were cleared with xylene, washed three times in phosphate buffer saline (PBS) for about 5 seconds per wash and were immersed in 3% H_2O_2 in PBS (pH 7.4) at room temperature for 20 minutes (min) to remove endogenous peroxidase activity, for the elimination of non-specific binding.

Then to induce antigen retrieval, the tissue sections were treated with citrate buffer (pH 6) for 40 min. in steamer. Then the sections were incubated at 4 °C overnight with the following antibodies: Rabbit polyclonal anti-PPAR alpha antibody (ab126285) and Rabbit polyclonal anti-PPAR gamma antibody (ab45036). Antibodies were diluted 1/1000 in goat serum. Then the sections were washed by PBS for removing excess primary antibodies. Then the sections were incubated at room temperature for 1 hour with secondary goat anti-rabbit IgG antibodies H&L (HRP) (ab205718) for PPARα and PPARγ immunohistochemistry staining separately. The secondary antibodies were diluted 1/300 in goat serum. The positive reaction was developed by using a freshly prepared solution of 3.3-diaminobenzidine tetrahydrochloride for 5-10 min. at room temperature. The haematoxylin was used as a counter stain for staining of nuclei. Then were washed by PBS then coverslip was mounted, then were observed with a Zeiss light microscope for taking photos. For quantification of PPARα and PPARγ immunostaining at different developmental stages we used image J software **(Schindelin *et al*., 2012)**.

For the negative controls, we substituted the primary antibodies with normal rabbit IgG. There was complete absence of immunostaining in all negative controls.

5. *Statistical analysis*

Data were examined for normality and homogeneity of variance. All data were analyzed using one-way ANOVA followed by Duncan post hoc test. Difference between means was tested at 5% probability level. All statistical analysis was done using SPSS program Version16 (SPSS, Richmond, USA).

RESULTS

1. *General histological observations*

The intestine could be divided into three parts were anterior intestine, middle intestine, and posterior intestine. In general; the intestine of loach formed from lamina epithelialis containing simple columnar and goblet cells, lamina propria and submucosa containing connective tissue cells and red blood cells, tunica muscularis containing inner circular and outer longitudinal smooth muscles and tunica serosa externally.

At different developmental stages, it was observed that at 1.5 months of age; the anterior intestine characterized by long intestinal folds **(Fig. 1A)**. While in mid intestine the folds were moderate in length **(Fig. 1B)**, and in the posterior intestine, there were no intestinal folds **(Fig. 1C)**. At 2 months of age, the anterior intestine resembles the general structure of intestine, which previously described **(Fig. 1D)**. In the mid intestine it was observed that many blood capillaries appeared in the submucosa and several red blood cells were found in lamina epithelialis **(Fig. 1E)** and the posterior intestine its lamina epithelialis characterized by several apical protrusion from columnar cells which indicate an apocrine mode of secretion **(Fig. 1F)**. At 3 months of age, the number of goblet cells increased in the anterior intestine and both the mid and posterior intestines showed several blood capillaries under the epithelium with several intraepithelial red blood cells (data not shown). At 5 months of age, the histological observations resembled that previously described as at the age of 3 months. In adult stage at 10 months of age , at 12 months of age and at 15 months of age **(Fig. 1G)** there were several long and branched intestinal folds. At the age of 15 months, both the mid **(Fig. 1H)** and posterior intestines **(Fig. 1I)** contain several blood capillaries and red blood cells.

At one month of age, the liver of loach fish was formed from hepatocytes arranged in the form of cords and several blood sinusoids were observed between these cords **(Fig. 2A)**. At 1.5 months of age the pancreas in loach was found between liver and anterior intestine **(Fig. 2B)**, and also some locations inside liver contain intra-hepatic pancreatic tissue. The pancreas formed from exocrine portions contained pancreatic acini which their cytoplasm is highly enriched with acidophilic zymogen granules and the endocrine parts. The pancreas at the age of one month didn't reach full maturity. The exocrine portion of pancreas contains darkly stained acidophilic cells with a central spherical nucleus. These cells were more darkly stained and larger than hepatocytes and very few cells contained zymogen granules (lightly stained acidophilic granules) in their cytoplasm were observed at this age. The endocrine portion of the pancreas was tiny and consisted of two kinds of cells one with a spherical nucleus and the second one with the elongated nucleus. From the beginning of 2 months of age, the pancreas became well developed with pancreatic acini contained zymogen granules **(Fig. 2C)**.

A thin connective tissue capsule was surrounding the parenchyma of the spleen, which contains distinct white pulps and red pulps **(Fig. 2D)**. The white pulp was rich in lymphocytes and leukocytes, while the red pulp consisted of red blood cells few melanomacrophage centers were observed in the spleen. Both white pulp and red pulp were distinct beginning from the age of 1.5 months but were indistinct at one month of age.
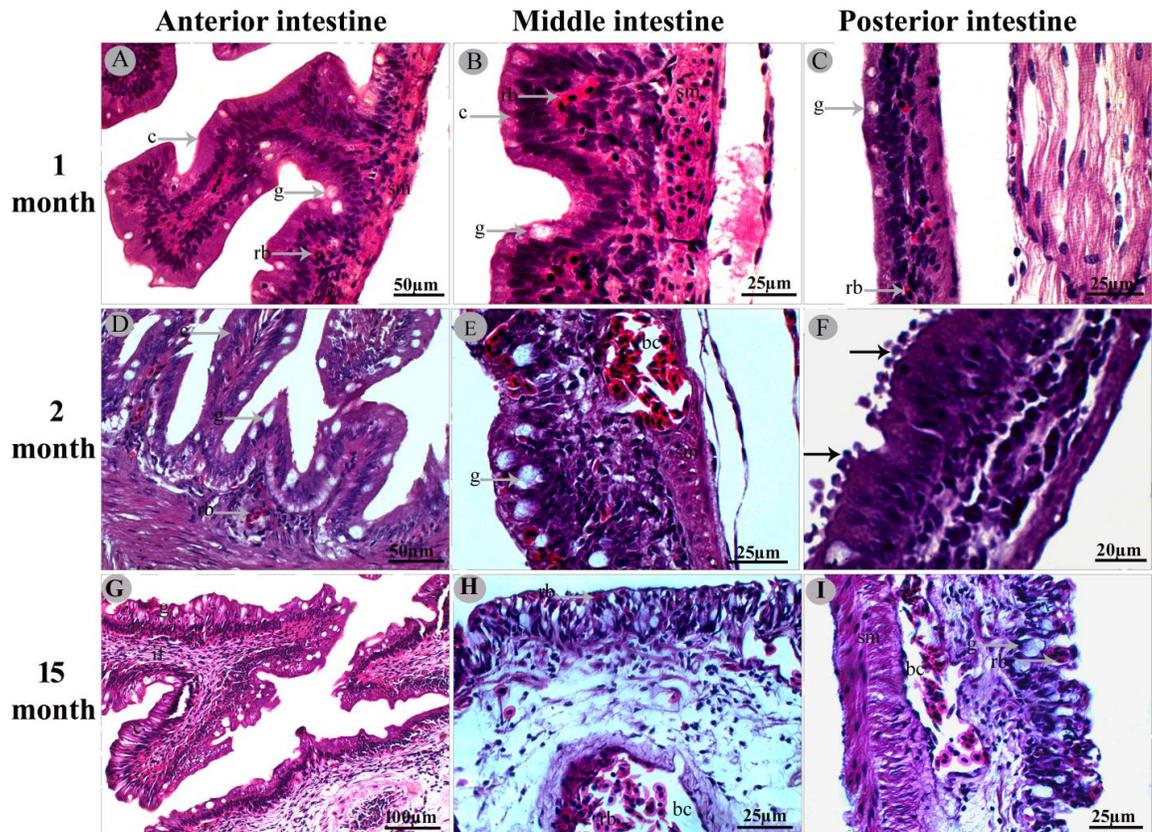
Fig. 1 Shows the general histological observations during the intestine development in loach fish (*Misgurnus anguillicaudatus*) with H&E stain. (c) columnar cell, (g) goblet cell, (rb) red blood cells, (sm) smooth muscle, (bc) blood capillary, (IL) intestinal lumen, (if) intestinal furrow, apical protrusions (black arrows).

The posterior kidney in loach fish from the age of one month consisted from renal corpuscles (RC), proximal convoluted tubules (PCT), distal convoluted tubules (DCT), collecting ducts (CT) and interrenal tissue. The PCT were lined by simple columnar epithelium and with a narrow lumen. The DCT were lined by cuboidal epithelium and have a wide lumen. However, the collecting ducts were lined by a simple cuboidal epithelium. . The inter-renal tissue consisted of red blood cells and lymphocytes **(Figs. 2E, F)**.
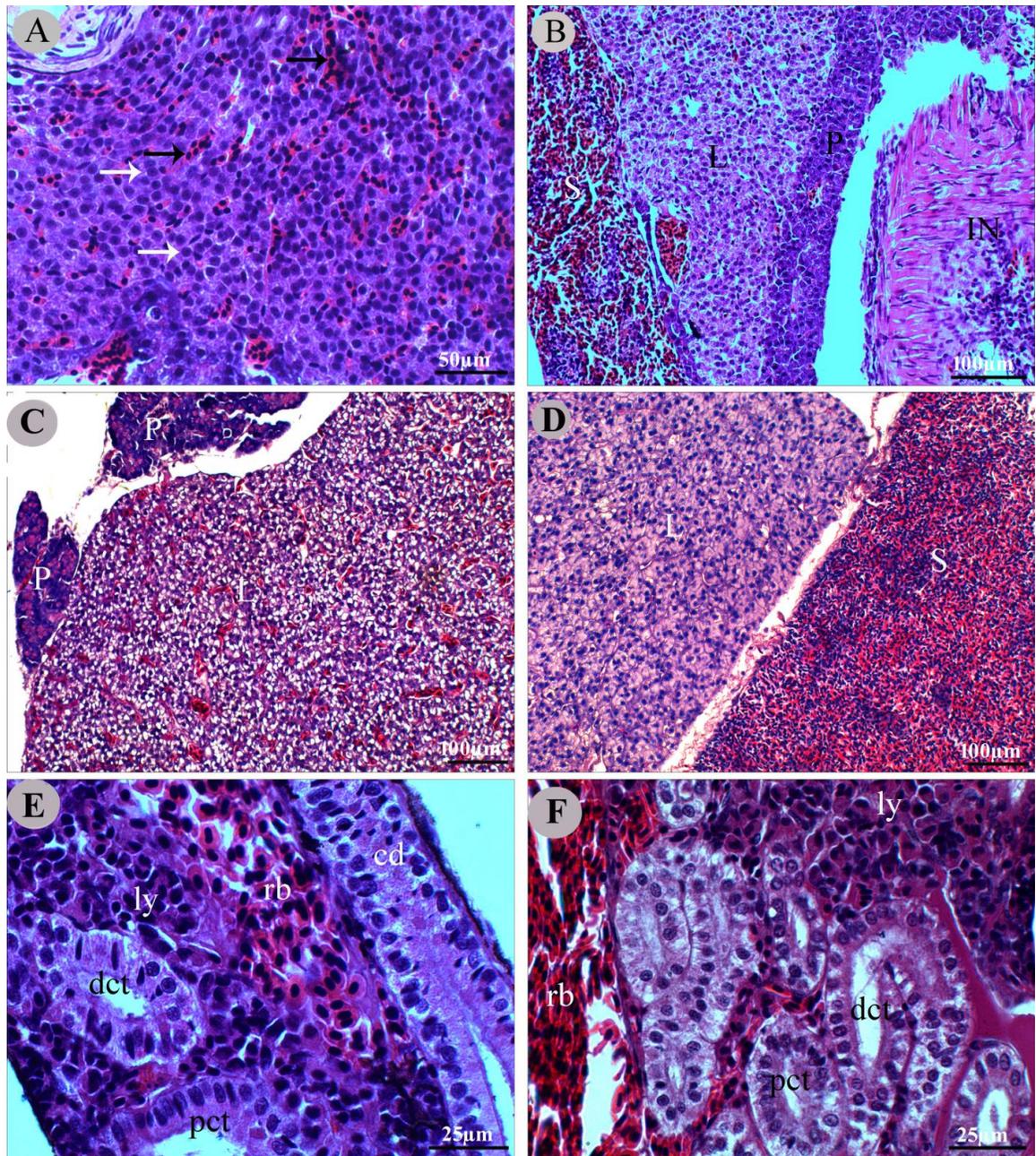
Fig. 2 Shows the general histological observations of liver, pancreas, kidney, and spleen of loach (*Misgurnus anguillicaudatus*) with H&E stain. (A) The liver at the age of one month age showing hepatocytes arranged in the form of cords (white arrow), and several blood sinusoids (black arrow). (B) The liver (L), spleen (S), intestine (IN) and pancreas (P) at the age of 1.5 months. (C) the liver and pancreas at the age of 2 months. (D) the liver and spleen at the age of 5 months. (E) the kidney at the age of one month and (F) the kidney at 2 months age, both containing, proximal convoluted tubule (pct), distal convoluted tubule (dct), collecting duct (sd), red blood cells (rb) and lymphocytes (ly).

2.   Immunohistochemistry

2.1.   PPARα at different stages of development

At 1.5 months of age, the anterior intestine, mid and posterior intestine showed a positive reaction with PPARα in the free border of columnar cells lining epithelial folds **(Figs. 3A, B, C)**. At 2 months of age, high positive reaction to PPARα was observed in the anterior intestine, while the middle and posterior intestine parts showed a weak positive reaction **(Figs. 3D, E, F)**. At 5 months of age, the positive reaction to PPARα became weak in the anterior intestine, middle and posterior intestine **(Figs. 3G, H, I)**. While at 12 months of age the positive reaction to increase slightly than that at 5 months of age **(Figs. 3J, K, L)**. At 15 months of age, the positive reaction to PPARα became very weak all over the intestine **(Figs. 3M, N, O)**.

PPARα showed significantly (P < 0.05) decreased from the fifth month in the anterior intestine **(Table 1)**. While its levels in the mid and posterior intestine was significantly (P<0.05) reduced by the third month of the fish age **(Table 1)**.

At one month of age, a moderate positive reaction to PPARα was found in hepatocytes and blood sinusoids of the liver while cells forming the exocrine portion of the pancreas were low in positive reaction. At 2 months of age, the positive reaction was very high in the cytoplasm of hepatocytes **(Fig. 4A)**. While the pancreas at 2 months of age showed a moderate reaction in pancreatic acini and pancreatic islets. At 5 months of age, both the liver and the pancreas showed a low positive reaction to PPARα **(Fig. 4B)**. At 7 months of age the liver showed a moderate positive reaction to PPARα **(Fig. 4C)**. Moreover, at 10 months of age, a moderate positive reaction in the liver was noticed **(Fig. 4D)**. The pancreas showed a moderate positive reaction to PPARα at 7 and 10 months of age.

As well as, the spleen showed a high positive reaction to PPARα at 2 months of age **(Fig. 4E)**. While at 5 months of age turn low **(Fig. 4F)** and became moderate at 7 and 10 months of age **(Figs. 4G, H)**.

The posterior kidney of the loach at one month of age showed a negative reaction to PPARα as the kidney till this age didn't reach complete maturity **(Fig. 5A)**. While at 1.5 and 2 months of age a very high positive reaction was observed in the cytoplasm of epithelial cells lining renal tubules **(Figs. 5B, C)**.

PPARα scores in the organs was not stable in the first three month, but by the fifth month significant (P < 0.05) decrease in its score was observed in the examined organs (liver, pancreas, spleen and kidney) as presented in **(Table 2)**.
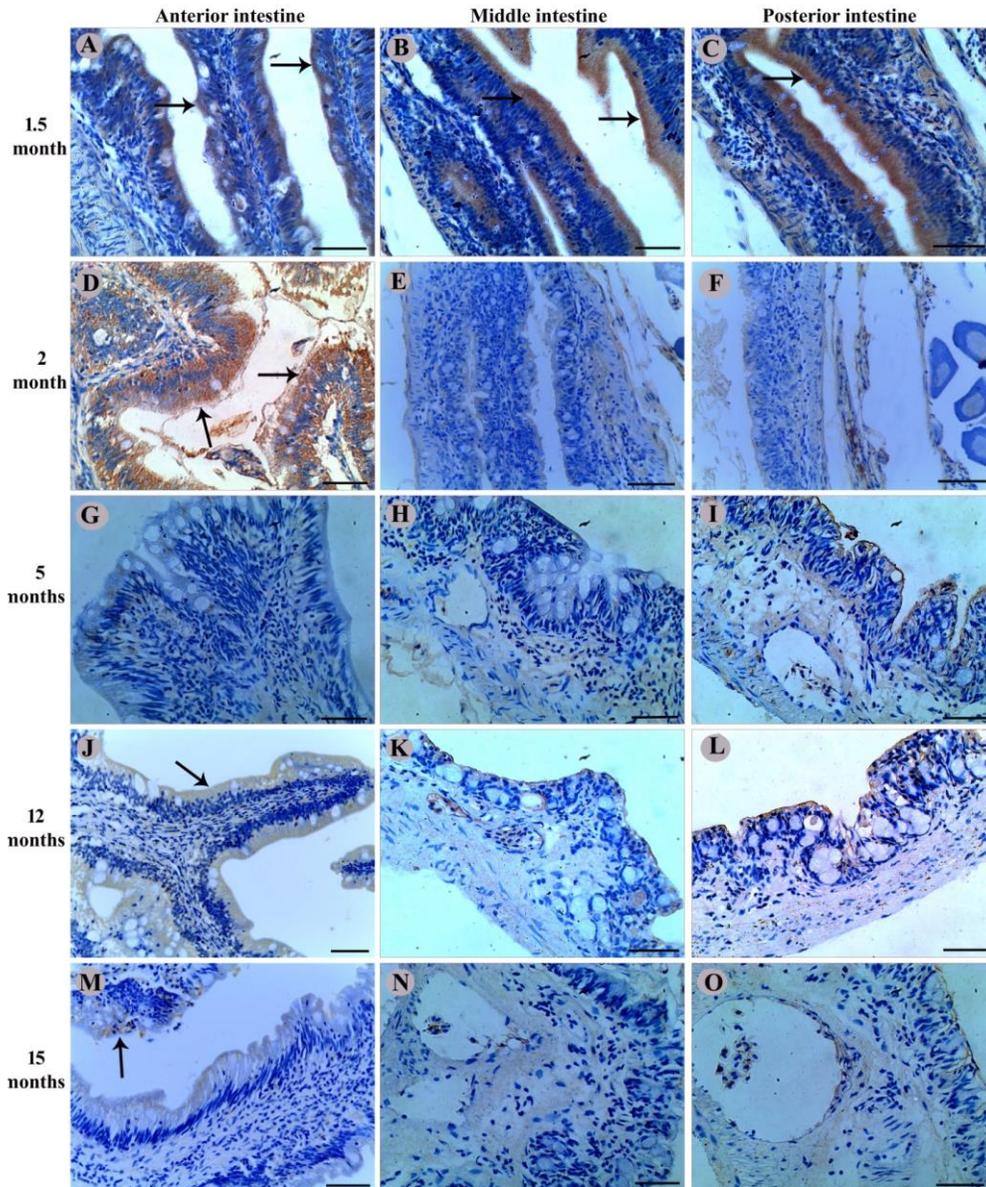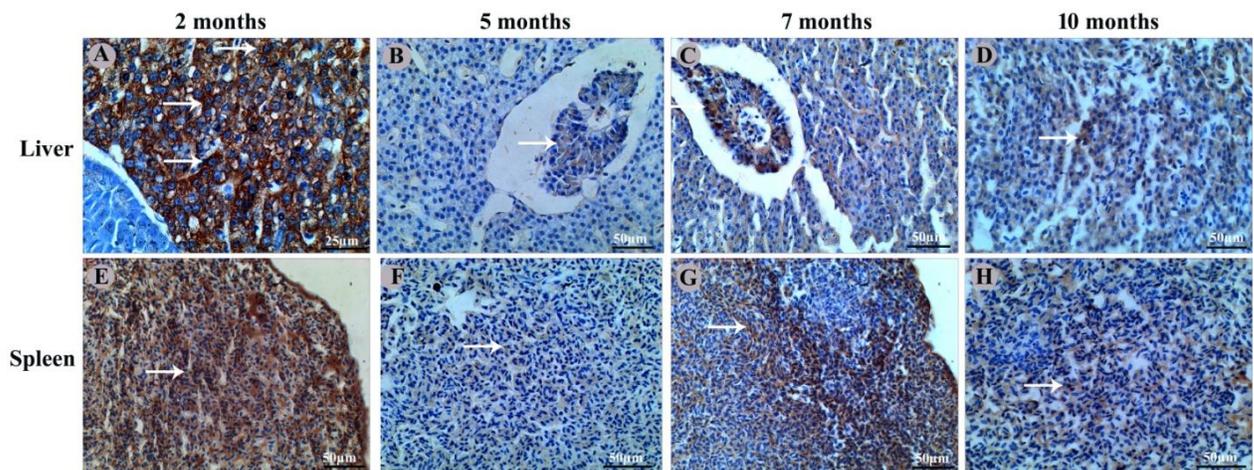
Fig. 3 The arrows shows the PPARα immunohistochemistry at different stages of the intestine development in loach (*Misgurnus anguillicaudatus*)**.** scale bar indicated, 50µm.

Table 1 Immunostaining scores for Peroxisome proliferator-activated receptor alpha (PPARα) in the intestine of loach fish (*Misgurnus anguillicaudatus*) during different developmental stages.

Age	Optical density (nm)		
	Anterior intestine	Mid intestine	Posterior intestine
Two months	1.31 ±0.21[a]	1.37 ± 0.03 [a]	1.44 ±0.20[a]
Three months	1.59 ±0.20[a]	1.18 ±0.01[b]	0.99 ± 0.06[c]
Five months	0.67 ±0.20 [b]	0.35 ±0.02[d]	1.12 ±0.04[b]
Ten months	0.3 ± 0.06 [bc]	0.73 ±0.09[c]	0.40 ±0.02[d]
Fifteen months	0.005 ±0.003[c]	0.29 ±0.01[d]	0.30 ±0.02[d]

Data are presented as mean ± SEM. Means with the different letters at the same column are not significantly different at *P<0.05*, using Duncan post hoc test.
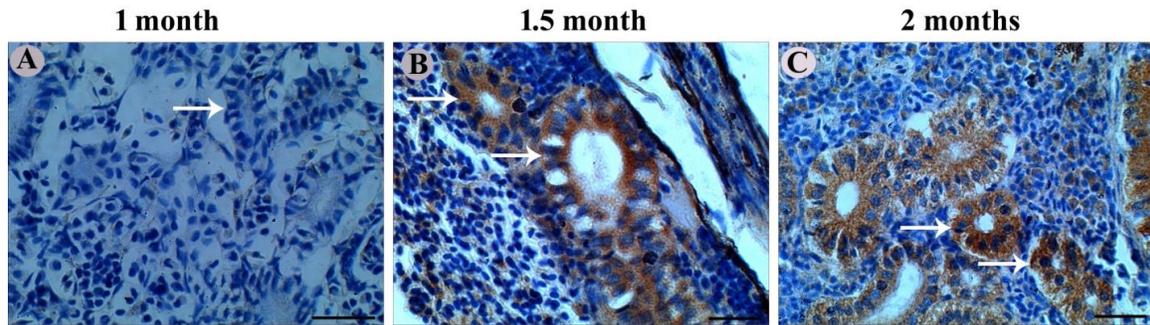

Fig. 4 The arrows shows the reactivity to the PPARα immunohistochemistry in liver, pancreas and spleen of loach (*Misgurnus anguillicaudatus*) at different developmental stages.

Table 2 Immunostaining scores for PPARα in liver, spleen, pancreas and kidney of loach fish (*Misgurnus anguillicaudatus*) during different developmental stages.

Age	optical density (nm)		
	Liver and pancreas	Spleen	Kidney
One and half month	1.68 ±.04 [b]	1.16 ±0.3[c]	1.56 ±0.03[b]
Two months	1.97 ± 0.03[a]	2.04 ±0.02[a]	1.76 ±0.03[a]

Five months	1.28 ± 0.09^{d}	1.33 ± 0.11^{bc}	1.27 ± 0.02^{d}
Seven months	1.50 ± 0.06^{c}	1.62 ± 0.08^{b}	1.46 ± 0.02^{c}
Ten months	1.49 ± 0.05^{c}	1.07 ± 0.19^{c}	1.47 ± 0.01^{c}

Data are presented as mean ± SEM. Means with the different letters at the same column are not significantly different at *P<0.05,* using Duncan post hoc test.

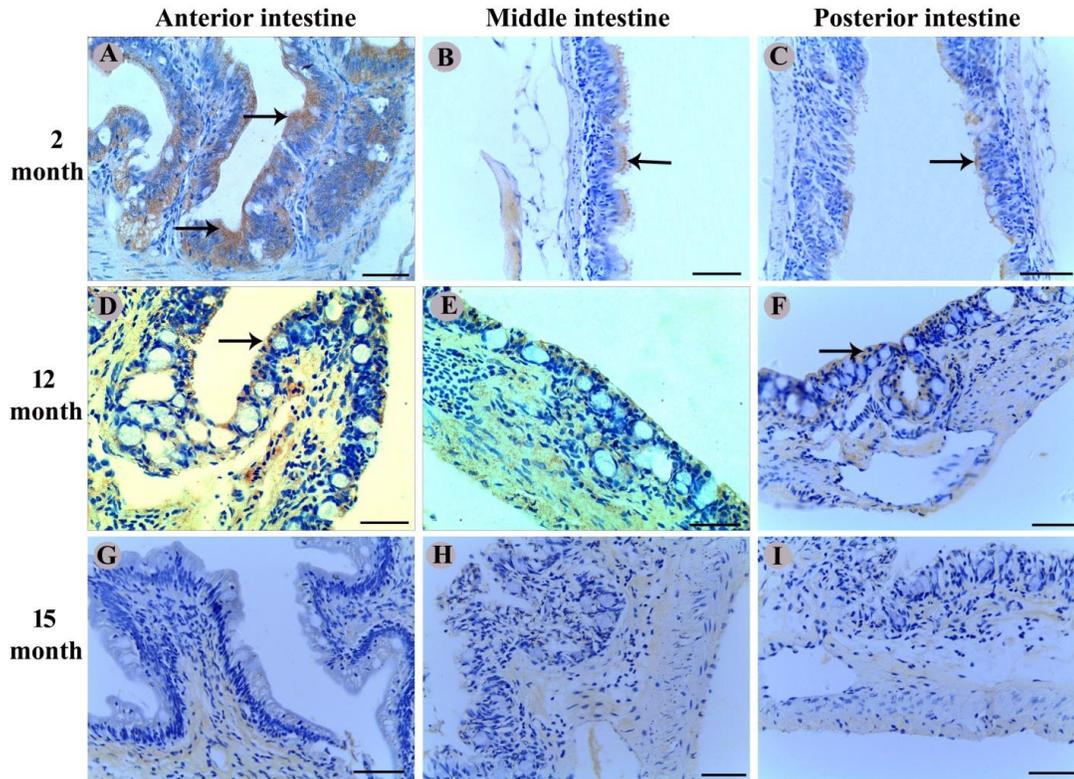**Fig. 5** The arrows show the reactivity to PPARα immunohistochemistry in kidney of loach (*Misgurnus anguillicaudatus*). scale bar indicated, 25µm.

2.2.   *PPARγ at different stages of development*

The positive reaction to PPARγ was observed in the cytoplasm of columnar cells lining intestinal folds and intestinal gland. At 1.5 months of age, the positive reaction to PPARγ was low in anterior intestine and not detected in both mid and posterior intestine. At 2 months of age, the positive reaction to PPARγ was moderate in the anterior intestine and low in both mid and the posterior intestine **(Figs. 6A, B, C)**. At 12 months of age was low in the anterior, mid and the posterior intestine **(Figs. 6D, E, F)**. While at the 15 months of age was very low all over the intestine and mainly concentrated in lamina propria submucosa and the tunica serosa **(Figs. 6G, H, I)**.

PPARγ score in the anterior intestine, middle and posterior intestine was significantly (P < 0.05) the lowest by the fifteen month **(Table 3)**.

Fig. 6 The arrows show the positive reactivity to PPARγ immunohistochemistry at different stages of intestine development in loach (*Misgurnus anguillicaudatus*). scale bar indicated, 50μm.
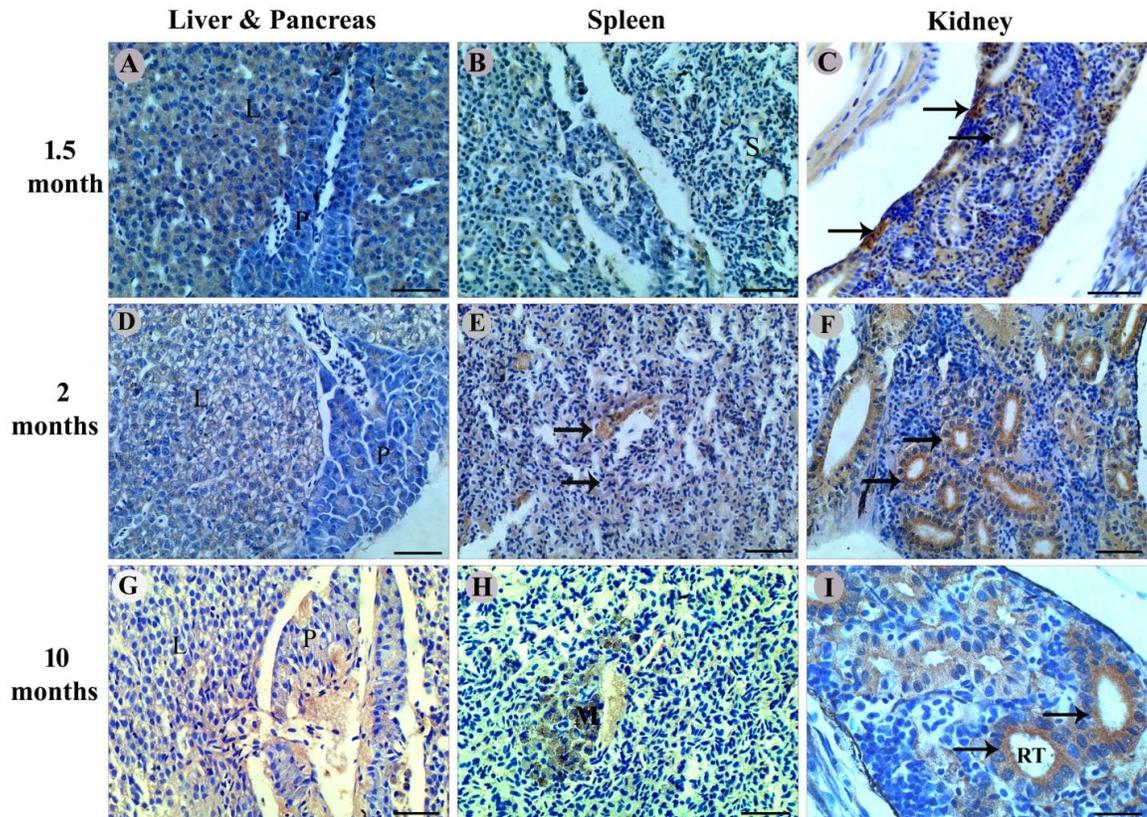
Table 3 Immunostaining scores for Peroxisome proliferator-activated receptor gamma (PPARγ) in the intestine of loach fish (*Misgurnus anguillicaudatus*) during different developmental stages.

Age	Optical density (nm)		
	Anterior intestine	Mid intestine	Posterior intestine
Two months	0.93 ± 0.02^b	0.45 ± 0.12^b	0.41 ± 0.03^c
Three months	1.20 ± 0.06^a	0.65 ± 0.04^a	0.53 ± 0.01^a
Five months	0.34 ± 0.5^d	0.19 ± 0.009^d	0.15 ± 0.01^d
Ten months	0.65 ± 0.09^c	0.30 ± 0.03^c	0.47 ± 0.01^b
Fifteen months	0.43 ± 0.006^e	0.00 ± 0.00^e	0.00 ± 0.00^e

Data are presented as mean ± SEM. Means with the different letters at the same column are not significantly different at *P*<0.05, using Duncan post hoc test.

At one month of age the positive reaction toward PPARγ was low in all examined organs, including liver, spleen, pancreas and kidney. At 1.5 months age, low positive reaction was noticed in liver, pancreas and spleen of loaches **(Figs. 7A, B)**. While the kidney showed moderate positive reaction **(Fig. 7c)**. At 2 months of age, the positive reaction was moderate in liver, pancreas, and spleen **(Figs. 7D, E)**. Meanwhile in posterior kidney was high and mainly concentrated in the cytoplasm of epithelial lining renal tubules **(Fig. 7F)**. At 5 months of age, the negative reaction was found in the liver except for few hepatocytes showed a low positive reaction also in spleen only melanomacrophage centers showed a low positive and a low positive reaction also found in the kidney, while the pancreas showed a negative reaction. At 7 months of age, a moderate positive reaction was found in the liver, kidney and spleen, while the pancreas showed a negative reaction. At 10 months of age, the positive reaction was low in liver and spleen and was moderate in pancreas and kidney **(Figs. 7G, H, I)**.

PPARγ score in liver, pancreas, spleen and kidney was significantly reduced starting from the fifth month (P < 0.05) as recorded in **(Table 4)**. The negative control staining for PPARα and PPARγ was applied in liver, pancreas, spleen and kidney tissues of loach fish **(Fig. 8)**.


Fig. 7 The arrows show the positive reactivity to PPARγ immunohistochemistry in liver, pancreas, kidney, and spleen of loach (*Misgurnus anguillicaudatus*). Liver (L), pancreas (P), spleen (S), melanomacrophage center (M), renal tubules (RT). Scale bar indicated, 50μm.

Table 4 Immunostaining scores for PPARγ in liver, spleen, pancreas and kidney of loach fish (*Misgurnus anguillicaudatus*) during different developmental stages.

Age	optical density (nm)		
	Liver and pancreas	Spleen	Kidney
One and half month	0.78 ± 0.01^{a}	0.66 ± 0.02^{c}	1.27 ± 0.01^{b}
Two months	0.71 ± 0.01^{b}	1.57 ± 0.02^{a}	1.56 ± 0.02^{a}
Five months	0.12 ± 0.01^{c}	0.11 ± 0.01^{d}	0.17 ± 0.01^{d}
Seven months	0.68 ± 0.03^{b}	0.81 ± 0.08^{b}	1.27 ± 0.04^{b}
Ten months	0.65 ± 0.01^{c}	0.57 ± 0.04^{c}	0.88 ± 0.03^{c}

Data are presented as mean ± SEM. Means with the different letters at the same column are not significantly different at *P<0.05,* using Duncan post hoc test.
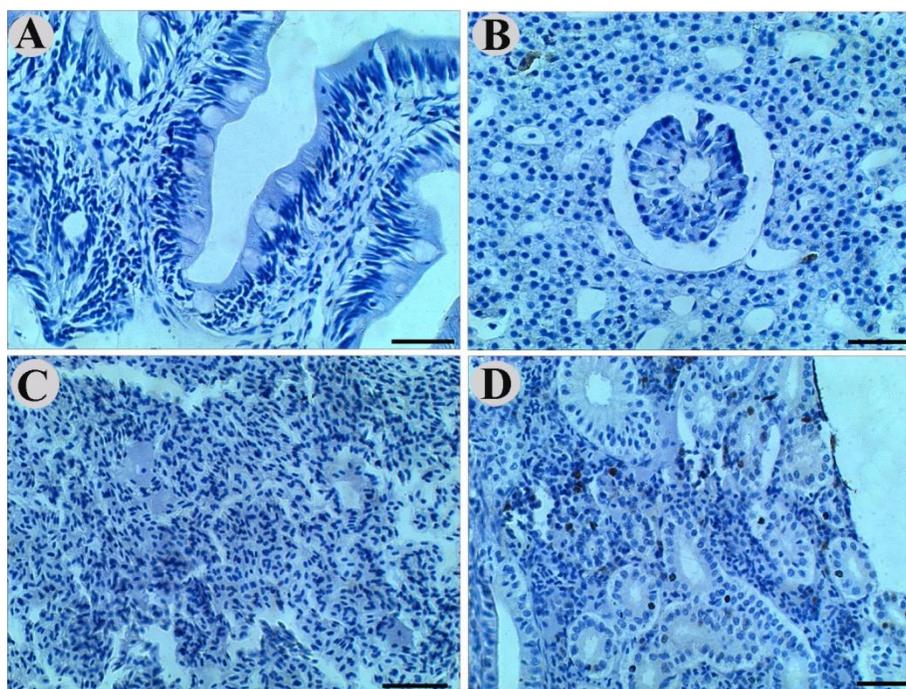
Fig. 8 Shows the negative control results for PPARα and PPARγ in different tissue sections. (a) intestine and spleen. (b) liver and intrahepatic pancreas. (c) spleen. (d) kidney. Scale bar indicated 50μm.

DISCUSSION

In the present study we investigated the expression pattern of peroxisome proliferator-activated receptor in various organs of loach fish at different developmental stages. In accordance with **Zhang *et al*., (2016)** The histological observation of intestine in loach indicated that the height of epithelial folds is high in the anterior intestine,

moderate in mid intestine and there were shallow folds in posterior intestine. The intestinal air-breathing property was first observed in the mid intestine at the age of 2 months loach fish (*Misgurnus anguillicaudatus*). However, the posterior intestine showed the apocrine mode of secretion from columnar cells, which indicate that these columnar cells have secretory property rather than absorptive property. **Luo et al., (2016)** observed the intiation of air breathing property in dojo loach at 10 days post hatching. The liver of loach formed from several hepatic cords separated by blood sinusoids and there were lack of hepatic lobules with some intra-hepatic pancreatic tissue, these results in accordance with **Faccioli et al., (2014); Sales et al., (2017)**. The pancreas in loach traditionally associated with hepatic tissues. Some blood sinusoids were observed between pancreas and liver through which the pancreatic secretions pass from pancreatic islets toward the liver blood sinusoids.

In this study, we showed the expression pattern of PPARα and PPARγ in the intestine during some developmental stages of loach fish. The peroxisome proliferator activated receptors have essential role in fat metabolism and energy production (**Li et al., 2018**). Both PPARα and PPARγ were expressed in the cytoplasm of columnar cells lining intestinal folds, as well as, in lamina propria submucosa, musculature, and serosa. There was a relationship between their expression and age development. The previous investigation on PPARs in zebrafish detected that developmental stage affects their expression in tissue in a different manner which indicates that, PPARs expression decline with an increase in age, while gender doesn't have a role in their expression (Ibabe et al., 2005). Though their expression notably increased in the gills epithelium of one year old Gray mullet than those at younger ages (**Hussein and Cao, 2018**).

Our results in accordance with **Ibabe et al., (2004)** who stated that, PPARα was strongly expressed in the liver of Mugil cephalus and found in hepatocytes, melanomacrophage centers, blood sinusoids and connective tissue capsule. Moreover, PPARγ was very weak in expression and only found in melanomacrophage centers. In our results we found that, PPARγ is expressed in hepatocytes and there were no melanomacrophage centers noticed in liver of loach. As well as, our results investigated that, the PPARα expression in the liver of loach fish was high at the age of 1.5 months. While at the age of 2 months reached the top and was strongly expressed then decline at the age of 5 months and increased slightly at the age of 7 and 10 months. These results may indicate an essential role of PPARα at the age of 2 months in fatty acid oxidation in liver for providing the energy needs of loach fish during development (**Fucci et al., 2012**).

The PPARα expression in the pancreas was high at the age of 2 months only. Previously obtained results indicated that, the beta cell which synthesis insulin was produced via replication of pancreatic islets (**Yesil and Lammert, 2008; Stanger and Hebrok, 2013**). This replication process was observed at early larval stages and late life stages (**Moro et al., 2009**). Therefore, the fluctuation of positive reaction of PPARα in

pancreatic islets with relation to age would indicate an essential role of PPARα in providing the energy needed for beta cell proliferation. Whereas, PPARα and PPARγ have an essential role in glucose homeostasis **(Fruchart *et al*., 2001; Singh, 2011)**.

In spleen PPARα expression was moderate at all examined ages except at the age of 2 and 7 months was strongly expressed and was low at the age of 5 months. The PPARα has a crucial role in spleen function more than PPARγ, as it's highly expressed in all cells of the spleen of loach. Previous investigation detected a beneficial role of PPARs as anti-inflammatory agents as they expressed in macrophage/mononcyte **(Singh, 2011)**.

In kidney PPARα expression was negative at the age of 1 month, then became strongly expressed beginning from the age of 1.5 months and older ages, which indicate an essential role of PPARα in providing energy needed for kidney function as it was expressed in epithelial lining renal tubules **(Hussein *et al*., 2020b)**. The PPARα **(Ibabe *et al*., 2004; Ibabe *et al*., 2005; Raingeard *et al*., 2009)** and PPARγ **(KissTóth and Rőszer, 2008)** have essential roles in the function of normal kidney via their roles in controlling mitochondrial and peroxisomal β-oxidation.

The PPARγ expression was weak in liver, pancreas, and spleen in all examined ages except at age of 2 months was moderate. It was expressed in hepatocytes, melanomacrophage centers of the spleen and pancreatic acini. While, in kidney PPARγ expression was moderate, and was found in the cytoplasm of epithelial cells lining renal tubules. This may indicate an essential role for PPARγ in fatty acid re-absorption from cells lining renal tubules. PPARγ was found to play an essential role in regulation of lipid biosynthesis **(Grygiel-Górniak, 2014)**.

CONCLUSION

This study is important for the characterization of the expression pattern of PPARα and PPARγ at some developmental stages of loach fish. The obtained results indicated that the highest level of expression of PPARα and PPARγ was at the age of 2 months and the lowest level of their expression was at the age of 5 months. So we can conclude that age development reversely affect PPARs expression in loach.

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