Int. J. Aquat. Biol. (2021) 9(4): 226-233
ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Comparative study of plasma biochemical parameters in mature male and female goldfish, *Carassius auratus*

Marzieh Abbasi[1], Bahram Falahatkar[*1,2], Ali Bani[2,3], Behroz Heidari[2,3]

[1]Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Guilan, Iran.
[2]Department of Marine Sciences, The Caspian Sea Basin Research Center, University of Guilan, Rasht, Iran.
[3]Department of Biology, Faculty of Science, University of Guilan, Rasht, Guilan, Iran.

Abstract: Blood biochemical parameters are important factors that can show the changes in health offering critical feedback on physiological condition of fish. In the present study, we examined a comparative study on the blood biochemical parameters in mature male and female goldfish, *Carassius auratus*. Plasma samples of 72 male and female goldfish that have been kept at 24°C for 30 days were analyzed and their biochemical parameters levels were determined. There were significant differences in all measured parameters between genders so that, maximum concentrations of the glucose (73.45±0.68 mg/dl) and calcium (8.32±0.05 mg/dl) were found in female fish, while the highest levels of the total protein (3.14±0.01 g/dl), cholesterol (281.65±3.19 mg/dl) and triglyceride (428.31±1.17 mg/dl) were measured in males. Based on the results, the plasma biochemical parameters changes vary considerably between male and female goldfish.

Article history:
Received 3 June 2021
Accepted 23 August 2021
Available online 25 August 2021

Keywords:
Biochemistry
Blood Plasma
Reproduction
Carassius auratus

Introduction

Blood biochemical parameters are valuable, effective and sensitive indices to monitor physiological changes of animals (Satheeshkumar et al., 2012) that offer critical feedback on state of body health and condition of aquatic organisms (Edsall, 1999). Recent attention has been given to the biochemical characterization of fish blood as an internal index because these parameters supplies valuable knowledge about internal organs and metabolic parameters of both wild and cultured fishes (Edsall, 1999), thus they are used by fish physiologists.

Numerous studies have documented that several factors can influence blood biochemistry, such as diseases and toxic chemicals (Silverira-Coffigny et al., 2004), season (Dawson, 1990), stress (Morales et al., 2005), species (Langston et al., 2002), temperature (Magill and Sayer, 2004), sexual maturity (Hatami Nasari et al., 2014) and sex (Adel et al., 2016). Fish sex and maturation influence blood parameters (Gabriel et al., 2011) and sex differences is one of the most important factor that affects oscillation in blood biochemical parameters (Svoboda et al., 2001) and change the levels of various substances such as glucose, calcium, protein, cholesterol and triglyceride (Rosety et al., 1992; Pedro et al., 2005; Asadi et al., 2006; Zhang et al., 2014). On the other hand, plasma nutrient variations could be a result of gonad maturation and nutrient transportation to gonads by blood. For this reason, the levels of glucose, total protein, triglyceride and cholesterol are considered important indices of the internal milieu status of both sexes in teleosts.

Research on some species such as, Caspian Kutum, *Rutilus frisii* (Firouzbakhsh et al., 2013), Pike, *Esox lucius* (Adel et al., 2016) and Pikeperch, *Sander lucioperca* (Zakes et al., 2016) have revealed that biochemical parameters are affected by sex. Determination of variation in biochemical analysis in male and female fish might provide some useful baseline information to enhance further studies on mechanisms of sex and effects of sexual processes.

Goldfish, *Carassius auratus*, as a cyprinidae member, is widely distributed in the world and

[*]Correspondence: Bahram Falahatkar
E-mail: falahatkar@guilan.ac.ir
DOI: https://doi.org/10.22034/ijab.v9i4.1279

excellent laboratory model for study of the endocrine system and various experiments (Popesku et al., 2008; Munakata and Kobayashi, 2010; Blanco et al., 2018). In this study, we hypothesized that sex differences may exert changes on biochemical characteristics. In other words, the purpose of the present study was to characterize variations in blood biochemistry in both sexes of goldfish.

Materials and Methods

Fish and sampling: This study was carried out on 72 mature goldfish (36 males and 36 females) supplied from Agha-Seyed Sharif Aquatic Farm (Guilan Province, Iran) in December 2018. The fish were transferred to the wet laboratory at the Faculty of Natural Resources, University of Guilan (Sowmeh Sara, Guilan Province, Iran). They were maintained in a reservoir tank (150×50×50 cm) for a day before experiment, with a water circulation and filtration system. Water temperature, dissolved oxygen and pH were 24±1°C, 7.5±0.4 mg/L and 7.5±0.3, respectively. The mean weight of mature male and female goldfish was 25.12±1.10 and 27.59±1.29 g and average length was 15.10±0.17 and 16.44±0.36 cm, respectively.

Experimental design: Six 60 L aquaria were used for the experiment and 12 fish were introduced into each aquarium at ambient temperature of 24°C. The temperature was controlled by a thermostat (300 Watt, Aqua, Tehran, Iran). During the experiment, the fish were fed twice daily at a rate of 2% body weight (Priestley et al., 2006; Hafeez-ur-Rehman et al., 2015) with a commercial carp pellet (Faradaneh, Shahrekord, Iran; containing 35-38% crude protein; 4-8% crude fat; 7-11% ash; 5-11% moisture; 1-1.5% phosphorus; 4-7% crude fiber). Fish were kept under 12L:12D light regime throughout the experiment.

Sampling: A total of 72 sexually mature goldfish (36 males and 36 females) were used in this experiment. Two males and two females were sampled from each aquarium at days 0, 14 and 30. Fish were anesthetized by 200 mg/l clove powder (Abdulrahman et al., 2018), then the blood samples were quickly extracted from the caudal vein of each goldfish by a 2.5 ml heparinized syringe to measure the blood biochemical indices. Blood samples were centrifuged for 10 min at 1500 *g* at 4°C (Labofuge, HeraeusSepatch, Germany) to plasma extraction, then they were stored at -80°C. The experiment was conducted in accordance by animal ethics handling (Granstrom, 2003). To ensure the fish were mature, 6 males and 6 females at the initiation of the experiment were dissected and their gonads were removed and fixed in Bouin's solution for histological analysis.

Biochemical analysis: Blood glucose, calcium, total protein, cholesterol and triglyceride concentrations were measured using enzymatic colorimetric analysis based on Morris and Davey (2001). All parameters were measured using commercial available kits (Pars Azmun, Karaj, Iran) and spectrophotometer (UV-2100 Plus, UNICO, USA). Optical absorption of samples was measured at 25°C for all parameters using spectrophotometry method. The absorbance of glucose, total protein, cholesterol and triglyceride were recorded at 546 nm and 570 nm for calcium.

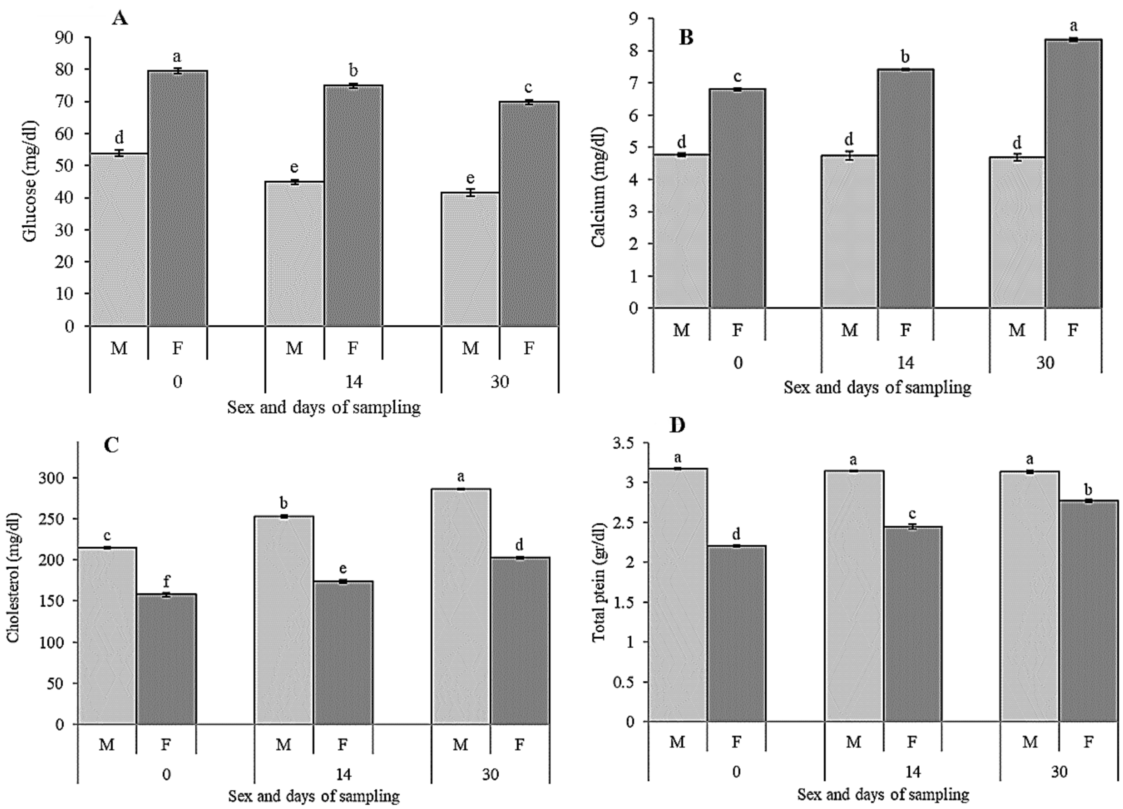
Gonad histology: After 24 h of preserving of the samples in fixative solution, the gonads were dehydrated in alcohol, embedded in paraffin wax, sectioned at 5 μm thickness, stained with hematoxylin and eosin (H&E) (Sanchez et al., 2011) and examined under light microscope. Classification of gonad maturation stages was done according to criteria defined by Brown-Peterson et al. (2011).

Statistical analysis: Data were examined for normality and homogeneity of variances using Kolmogorov-Smirnov and Levene's tests, respectively. All data were analyzed by Two-way and One-way ANOVA test. Sex and days of sampling were considered as independent variables and blood biochemical parameters were dependent factors. The accepted statistical significance level was $P<0.05$. The SPSS software (Ver. 16.0, Chicago, USA) was used for analysis and data are presented as the mean ± standard error (SE). Pearson correlation was used to detect any relationship between the analyzed parameters.

Results

The blood biochemical variables in mature fish are shown in Figure 1. Variations between sex and days of sampling were found in the blood glucose, calcium, total protein, cholesterol and triglyceride values. There were significant differences in blood glucose, calcium, total protein, cholesterol and triglyceride between male and female sampled throughout the experiment (*P*<0.05; Fig. 1; A-E). Interaction between sex and day showed that males have higher concentrations of total protein, cholesterol and triglyceride, but the glucose and calcium levels in females were higher in all days.

Tables 1 and 2 show correlation coefficient of the blood plasma biochemical parameters of mature male and female, respectively. Our results in mature male showed significant positive correlation of the triglyceride with cholesterol (Table 1; r=0.946, *P*<0.05) and significant negative correlation of the triglyceride and cholesterol with glucose (r=-0.739, P<0.05; r=-0.821, *P*<0.05, respectively). Pearson analysis revealed that there was significant positive correlation of calcium with cholesterol, total protein and triglyceride ($r_{cholesterol}$ = 0.957, $r_{total\ protein}$ = 0.904, $r_{triglyceride}$ = 0.889, *P*<0.05) in female. Furthermore, in mature female, there was a significant positive correlation of the triglyceride with cholesterol (r =0.889, *P*<0.05) and total protein (r= 0.886, *P*<0.05).



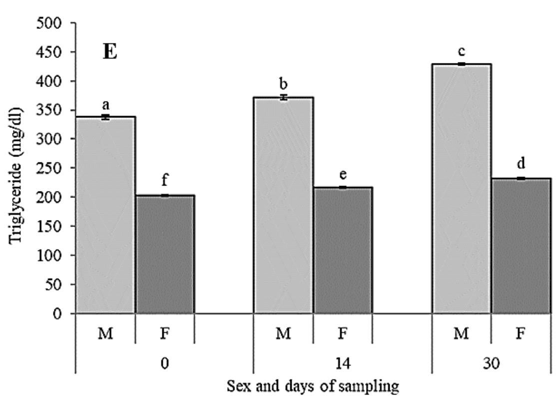


Figure 1. Glucose (A), calcium (B), protein (C), cholesterol (D) and triglyceride (E) levels in mature male and female goldfish (*Carassius auratus*) in day 0, 14 and 30. Values are reported as mean±S.E.; n=12 fish for each group, M=Male, F=Female. Numbers with different superscripts indicate significant differences among treatments (*P*<0.05).

Table 1. Correlation of blood plasma biochemical parameters in mature male goldfish, *Carassius auratus*.

Parameters	Glucose	Calcium	Total protein	Cholesterol
Triglyceride	-0.739*	0.005	-0.278	0.946*
Cholesterol	-0.821*	-0.125	-0337*	
Total protein	0.356*	0.202		
Calcium	0.119			

Table 2. Correlation of blood plasma biochemical parameters in mature female goldfish, *Carassius auratus*.

Parameters	Glucose	Calcium	Total Protein	Cholesterol
Triglyceride	-0.774*	0.889*	0.886*	0.889*
Cholesterol	-0.819*	0.957*	0.926*	
Total Protein	-0.796*	0.904*		
Calcium	-0.797*			

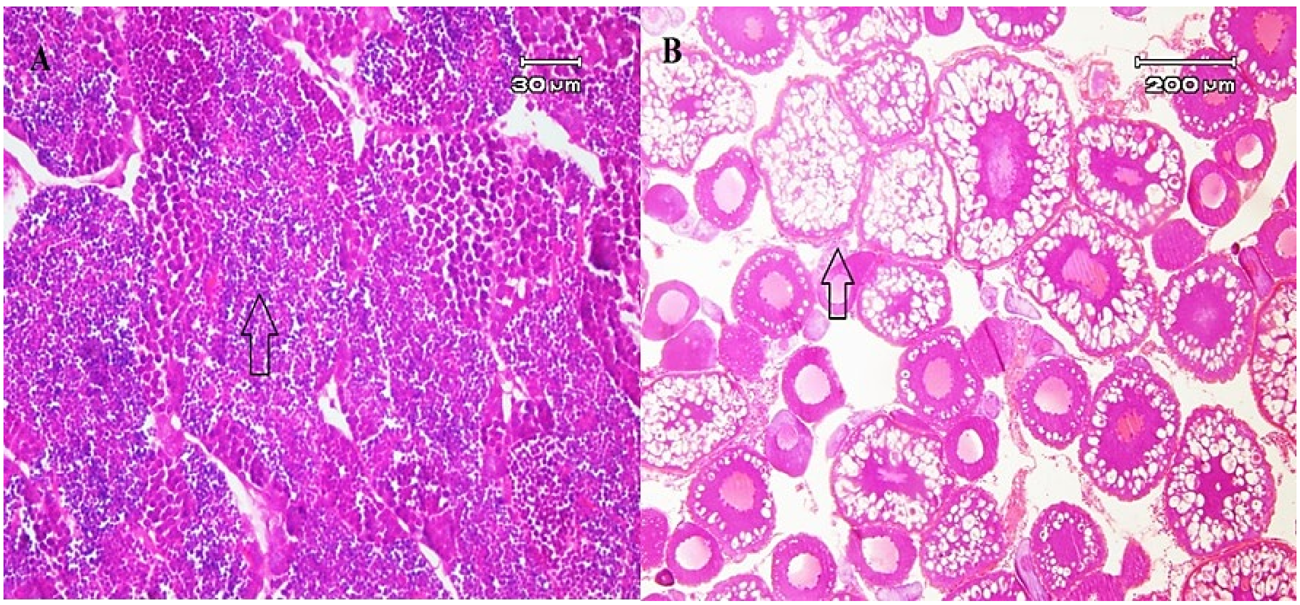


Figure 2. Histological sections of testis (A) and ovary (B) stained with Hematoxylin & Eosin in goldfish, (*Carassius auratus*) in day 0, 14 and 30. For male and female: scale bar 30 µm, 20 × magnification; scale bar 200 µm, 40 × magnification, respectively. Arrows in A and B represent spermatid (included possess dense nuclei) and vitellogenic oocyte (included lipid deposition), respectively.

A significant negative correlation between the cholesterol, calcium, total protein and triglyceride with glucose ($r_{cholesterol}$=-0.819, $r_{calcium}$= -0.797, $r_{total\ protein}$=-0.796, $r_{triglyceride}$=-0.774, P<0.05) were recorded in female mature goldfish.

Histological examination of the female and male gonads showed the vitellogenesis (vitellogenic oocyte in the ovary) and spermatogenesis (spermatids in testis) as main characteristics corresponding to the maturation stage, respectively (Fig. 2).

Discussion

Exogenous and endogenous factors such as diseases (Chen et al., 2005), sex (Adel et al., 2016) and reproduction (Suljević et al., 2017) could induce major changes in the blood compositions. In the present study, we found female's goldfish have higher concentrations of the glucose which is in consistent with previous studies on Beluga sturgeon (*Huso huso*) (Asadi et al., 2006), Atlantic Bluefin tuna (*Thunnus thynnus*) (Percin and Konyalioglu, 2008) and Sterlet sturgeon, *Acipenser ruthenus* (Akhavan et al., 2016). Glucose plays a key role during the reproductive period and is an important fuel for metabolism (Gharaei et al., 2011). Glucose levels vary considerably between species, sex, age, nutritional, size, sexual maturity and reproductive status (McDonald and Milligan, 1992). In our study, increased plasma glucose level in female goldfish might be a consequence of the gonad development and vitellogenesis processes because during vitellogenesis and ovary development glucose demand increase for energy supply and oocyte growth. In vitellogenesis, the hepatic glucose production increase due to glycogenolysis. Glycogenolysis is the process of glycogen breakdown to glucose-1-phosphate and glycogen, an important strategy to increase the blood glucose levels (Hemre et al., 2002). In the present study, the glucose in female was higher in day 0 compared with days 14 and 30. It seems that during ovary development, the glucose is utilized for energy supply of vitellogenesis process or stored in oocyte (Akhavan et al., 2016).

Changes in the blood plasma calcium of fish species occur as a result of physiological parameters such as sex, development, sexual maturity, diseases and etc. (Suljević et al., 2017). In the present study, interaction of sex and day showed that the highest level of calcium was found in female. Moreover, the calcium levels have significantly increased in female in all days. Baghizadeh and Khara (2015) in Common Carp (*Cyprinus carpio*) and Adel et al. (2016) in Pikeperch showed significant differences in the calcium concentrations between genders, and noted that females exhibited higher levels of calcium. The plasma calcium in female fish exhibits changes during the ovarian maturation (Sumpter, 1985; Tyler and Sumpter, 1990) and during this phase, the plasma calcium displays a variation and increases (Srivastava and Srivastava, 1994), whereas in male, plasma calcium levels show no change at different stages of the gonadal maturation (Balbontin et al., 1978). Calcium is essential mineral for vitellogenesis; vitellogenin binds by calcium and deliver in oocyte. There is a positive correlation between the concentrations of calcium and ovarian stages in female fish but calcium levels have no changes in male during spermatogenesis (Patino and Sullivan, 2002). Based on the results, interaction of sex and day showed that in all of days, and the blood plasma total protein increase in male compare to female goldfish. These results were in agreement with Adel et al. (2016) in Pikeperch. Plasma total protein increased during the vitellogenesis as it is necessary for vitellogenin forming and increasing demand of protein is controlled by several sex hormonal mechanisms in this process (Akhavan et al., 2016). In the present study, the accumulation of the protein in oocyte caused decreasing the total protein in female. However, due to the short time of the experiment, further studies are required to determine their reasons.

The results also showed that the plasma cholesterol and triglycerides increase in male in day 30 compare with female in day 0. Lund et al. (2000) in Striped Bass (*Morone saxatilis*), Yeganeh (2010) in Common Carp, Zakes et al. (2014) in Pikeperch and Adel et al. (2016) in Pike have revealed that cholesterol values significantly increase during maturation and reproductive cycle. In the current study, we observed cholesterol and triglyceride concentrations in females were lower than males. These finding may be caused by changing in physiological condition as a result of the oocyte maturation. The reason for the decrease of the plasma cholesterol in female is unknown, although it seems that the use of cholesterol for steroid synthesis, especially estrogenic hormones for vitellogenesis may be an explanation of the reduction in the lipid levels measured in female compared to male fish (Kusakabe et al., 2009). Because cholesterol is main precursor to the steroid hormones biosynthesis and its levels may change during the reproductive cycle between genders (Mommsen et al., 1999; Tokarz et al., 2015). In addition, cholesterol and triglycerides account important compounds during the gonadal development (Firouzbakhsh et al., 2013). Decrease in concentrations of the plasma cholesterol and triglycerides in the mature female may be due to the lipid mobilization towards oocytes for increased growth and prerequisite for gonadal development (Young et al., 2004).

In conclusion, the present study revealed that biochemical indices were different between male and female goldfish. Glucose and calcium increased in females because females have more demand of the glucose and calcium than males during ovarian development. Decrease of the blood total protein in female can be implemented that protein is stored in oocyte as main egg structure. To the best of our knowledge, changes of cholesterol levels affected by the synthesis of steroids levels that may be due to sex-specific reactions and different gonadal demanded.

Acknowledgements
This study was supported by a grant (no. 2235116) from the Caspian Sea Basin Research Center to the MA which is gratefully acknowledged. We are also thankful to M. Mehdizadeh for his help during preparation of fish.

References
Abdulrahman N.M., Hassan B.R., Salman N.A. (2018). Physiological impacts of using clove powder as f anesthetic on young common carp (*Cyprinus carpio* L.) under different levels of temperatures. Journal of Applied Veterinary Sciences, 3: 1-11.

Adel M., Safari R., Yeganeh S., Kumar P.S., Safaie P. (2016). Hematological and biochemical profile of pike breeders (*Esox lucius* Linneaus, 1758) from the Anzali Wetland, Caspian Sea. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 87: 1271-1276.

Akhavan S.R., Salati A.P., Falahatkar B., Jalali S.A.H. (2016). Changes of vitellogenin and Lipase in captive Sterlet sturgeon *Acipenser ruthenus* females during previtellogenesis to early atresia. Fish Physiology and Biochemistry, 42: 967-978.

Asadi F., Halajian A., Pourkabir M., Asadian, P., Jadidizadeh F. (2006). Serum biochemical parameters of *Huso huso*. Comparative Clinical Pathology, 15: 245-248.

Baghizadeh E., Khara H. (2015). Variability in hematology and plasma indices of common carp *Cyprinus carpio*, associated with age, sex and hormonal treatment. Iranian Journal of Fisheries Sciences, 14: 99-111.

Balbontin F., Espinosa X., Pang P.K.T. (1978). Gonadal maturation and serum calcium levels in two teleosts, the hake and the killifish. Comparative Biochemistry and Physiology, 61A: 617-621.

Blanco A.M., Sundarrajan L., Bertucci J.I., Unniappan S. (2018). Why goldfish? Merits and challenges in employing goldfish as a model organism in comparative endocrinology research. General and Comparative Endocrinology, 257: 13-28.

Brown-Peterson N.J., Wyanski D.M., Saborido-Rey F., Macewicz B.J., Lowerre-Barbieri S.K. (2011). A standardized terminology for describing reproductive development in fishes. Marine and Coastal Fisheries, 3: 52-70.

Chen Y.E., Jin S., Wang G.L. (2005). Study on blood physiological and biochemical indices of *Vibrio alginilyticus* disease of *Lateolabrax japonicas*. Journal of Oceanography in Taiwan Strait, 24: 104-108.

Dawson M.A. (1990). Blood chemistry of the windowpane flounder *Scophthalmus aquosus* in Long Island Sound: geographical, seasonal and experimental variations. Fishery Bulletin, 88: 429-37.

Edsall C.C. (1999). A blood chemistry profile for lake trout. Journal of Aquatic Animal Health, 11: 81-86.

Firouzbakhsh F., Abedi Z., Rahmani H., Khalesi M.K. (2013). A comparative study of some blood factors in male and female Caspian kutum (*Rutilus frisii kutum*) brood stock from the southern basin of the Caspian Sea. Turkish Journal of Veterinary and Animal Sciences, 37: 320-325.

Gabriel U.U., Akinrotimi O.A., Eseimokumo F. (2011). Haematological responses of wild Nile tilapia *Oreochromis niloticus* after acclimation to captivity. International Journal of Biological Sciences, 4: 225-230.

Gharaei A., Ghaffari M., Keyvanshokooh S., Akrami R. (2011). Changes in metabolic enzymes, cortisol and glucose concentrations of Beluga (*Huso huso*) exposed to dietary methylmercury. Fish Physiology and Biochemistry, 37: 485-493.

Granstrom D.E. (2003). Agricultural (nonbiomedical) animal research outside the laboratory: A review of guidelines for institutional animal care and use committees. Institute for Laboratory Animal Research, 44: 206-210.

Hafeez-ur-Rehman M., Iqbal K.J., Abbas F., Mushtaq M.M.H., Rasool F., Parveen S. (2015). Influence of feeding frequency on growth performance and body indices of goldfish (*Carrassius auratus*). Journal of Aquaculture Research and Development, 6: 1-4.

Hatami Nasari F., Kochanian P., Salati A.P., Pashazanoosi H. (2014). Variation of some biochemical parameters in female yellow fin sea bream, *Acanthopagrus latus* (Houttuyn) during reproductive cycle. Folia Zoologica, 63: 238-244.

Hemre, G.I., Mommsen, T.P., Krogdahl, A. 2002. Carbohydrates in fish nutrition: effects on growth, glucose metabolism and hepatic enzymes. Aquaculture Nutrition, 8: 175-194.

Kusakabe M., Zuccarelli M.D., Nakamura I., Young G. (2009). Steroidogenic acute regulatory protein in white sturgeon (*Acipenser transmontanus*): cDNA cloning, sites of expression and transcript abundance in corticosteroidogenic tissue after an acute stressor. General and Comparative Endocrinology, 162: 233-240.

Langston A.L., Hoare R., Stefansson M., Fitzgerald R., Wergeland H., Mulcahy M. (2002). The effect of temperature on non-specific defence parameters of three strains of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). Fish and Shellfish Immunology, 12: 61-76.

Lund E.D., Sulivan C.V., Place A.R. (2000). Annual cycle of plasma lipids in captive reared striped bass: effects of environmental conditions and reproductive cycle. Fish Physiology and Biochemistry, 22: 263-275.

Magill A.H., Sayer M.D.J. (2004). The effect of reduced temperature and salinity on the blood physiology of juvenile Atlantic cod. Journal of Fish Biology, 64: 1193-1205.

McDonald D.G., Milligan C.L. (1992). Chemical properties of the blood. In: W.S. Hoar, D.J. Randall, A.P. Farrell (Eds.). Fish Physiology, San Diego, CA, Academic Press. pp: 55-133.

Mommsen T.P., Vijayan M.M., Moon T.W. 1999. Cortisol in teleosts: Dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries, 9: 211-268.

Morales A.E., Cardenote G., Abellalin E., Garca-Rejon L. (2005). Stress-related physiological responses to handling in common dentex (*Dentex dentex* Linnaeus, 1758). Aquaculture Research, 36: 33-40.

Morris M.W., Davey F.R. (2001). Basic examination of blood, clinical diagnosis and management by laboratory methods. Journal of Animal and Veterinary Advances, 20: 479-519.

Munakata A., Kobayashi M. (2010). Endocrine control of sexual behavior in teleost fish. General and Comparative Endocrinology, 165: 456-468.

Patino, R., Sullivan, C.V., 2002. Ovarian follicle growth, maturation, and ovulation in teleost fish. Fish Physiology and Biochemistry, 26: 57-70.

Pedro N.D., Guijarro A.H., Lopez-Patin M.A., Martinez-Alvarez R., Delgado M.J. (2005). Daily and seasonal variations in haematological and blood biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. Aquaculture Research, 36: 1185-1196.

Percin P., Konyalioglu S. (2008). Serum biochemical profiles of captive and wild northern bluefin tuna (*Thunnus thynnus* L. 1758) in the Eastern Mediterranean. Aquaculture Research, 39: 945-953.

Popesku J.T., Martyniuk C.J., Mennigen J., Xiong H., Zhang D., Xia X., Cossins A.R. Trudeau V.L. (2008). The goldfish (*Carassius auratus*) as a model for neuroendocrine signaling. Molecular and Cellular Endocrinology, 293: 43-56.

Priestley S.M., Stevenson A.E., Alexander L.G. (2006). The influence of feeding frequency on growth and body condition of the common goldfish (*Carassius auratus*). The Journal of Nutrition, 136: 1979-1981.

Rosety M., Blanco M., De Canalez M.L.G., Grau A., Sarasquete M.C. (1992). Biochemical parameters during reproduction of the toad fish, *Halobatrachus didactylus*. Scientia Marina, 56: 87-94.

Sanchez W., Sremski W., Piccini B., Palluel O., Maillot-Marechal E., Betoulle S., Jaffal A., Ait-Aissa S., Brion F., Thybaud E., Hinfray N. (2011). Adverse effects in wild fish living downstream from pharmaceutical manufacture discharges. Environment International, 37: 1342-1348.

Satheeshkumar P., Ananthan G., Senthilkumar D., Khan A.B., Jeevanantham K. (2012). Comparative investigation on haematological and biochemical studies on wild marine teleost fishes from Vellar estuary, southeast coast of India. Comparative Clinical Pathology, 21: 275-281.

Silverira-Coffigny R., Prieto-Trujillo A., Ascencio-Valle F. (2004). Effects of different stressors in haematological variables in cultures *Oreochromis aureus*. Comparative Biochemistry and Physiology, 139C: 245-250.

Srivastava S.J., Srivastava S.K. (1994). Seasonal changes in liver and serum proteins, serum calcium, inorganic phosphate and magnesium levels in relation to vitellogenesis in a freshwater catfish, *Heteropneustes fossilis* (Bloch). Annales d'endocrinologie, 55: 197-202.

Suljević D., Alijagić A., Islamagić E. (2017). Temporal influence of spawning on serum biochemical parameters in brown trout *Salmo trutta* (teleostei: salmonidae). Bulgarian Journal of Agricultural Science, 23: 485-490.

Sumpter J.P. (1985). The purification, radioimmunoassay and plasma levels of vitellogenin from the rainbow trout, *Salmo gairdneri*. Current Trends in Comparative Endocrinology, 2: 355-357.

Svoboda M., Kouril J., Hamakova J., Kalab P., Savina L., Svoboda Z., Vykusova B. (2001). Biochemical profile of blood plasma of tench (*Tinca tinca*) during pre and post spawning period. Acta Veterinaria Brno, 70: 259-268.

Tokarz J., Möller G., Hrabě de Angelis M., Adamski J. (2015). Steroids in teleost fishes: A functional point of view. Steroids, 103: 123-144.

Tyler C.R., Sumpter J.P. (1990). The development of a radioimmunoassay for carp, *Cyprinus carpio*, vitellogenin. Fish Physiology and Biochemistry, 8: 129-140.

Yeganeh S. (2010). Seasonal changes of blood serum biochemistry in relation to sexual maturation of female common carp (*Cyprinus carpio*). Comparative Clinical Pathology, 21: 1059-1063.

Young G., Kusakabe M., Nakamura L., Lokman P.M., Goetz F.W. (2004). Hormones and their receptors in fish reproduction. In: P. Melamed, N. Sherwood (Eds.), Gonadal steroidogenesis in teleost fish. World Scientific Publishing, Singapore. pp: 157-159.

Zakes Z., Demska-Zakes Z., Szczepkowski M., Rozynski M., Ziomek E. (2016). Impact of sex and diet on hematological and blood plasma biochemical profiles and liver histology of pikeperch (*Sander lucioperca*). Archives of Polish Fisheries, 24: 61-68.

Zhang L.Z., Zhang T., Zhuang P., Zhao F., Wang B., Feng G. P., Song C., Wang Y., Xu S.J. (2014). Discriminant analysis of blood biochemical parameters at different developmental gonad stages and gender identification for controlled breeding of Amur sturgeon (*Acipenser schrenckii*, Brandt, 1869). Journal of Applied Ichthyology, 30: 1207-1211.