The Pharma Innovation Journal 2022; 11(11): 485-487

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(11): 485-487

www.thepharmajournal.com
Received: 11-08-2022
Accepted: 17-09-2022

Ningthoukhongjam Soranganba
Department of Fisheries, Resource Management, College of Fisheries, GBPUA & T, Pantnagar, Uttarakhand, India

Corresponding Author:
Ningthoukhongjam Soranganba
Department of Fisheries, Resource Management, College of Fisheries, GBPUA & T, Pantnagar, Uttarakhand, India

Seasonal assessment of high density lipoproteins in different age groups of Amur common carp associated with reproduction

Ningthoukhongjam Soranganba

Abstract
The present investigation was taken up to determine the seasonal change in high density lipoproteins (HDL) in blood plasma, muscle, gonadal and hepatic tissues in 1+ and 2+ year's age groups of Amur common carp, *Cyprinus carpio haematopterus* in Tarai region of Uttarakhand with physiological changes. Seasonal observations of water quality parameters of the trial ponds were carried out. Four different seasons – summer (July), autumn (October), winter (January) and spring (March) were chosen for sampling. HDL concentrations increased from the initial detection in summer until winter season. Comparison of physiological indices in 1+ and 2+ year's age groups showed two peak with spring being the highest followed by summer season. Inverse correlation of GSI and HSI was observed irrespective of age and sex in all seasons. The HDL level showed significant positive correlation with GSI with higher magnitude in 2+ than 1+ year's age group. Based on the present investigation, it can be inferred that seasonal changes in HDL level certainly plays a significant role and its availability in any form as food intake can be instrumental in reproductive success of Amur common carp, *Cyprinus carpio haematopterus*.

Keywords: High density lipoprotein, Amur common carp, seasonal, reproduction

Introduction
Assessment of the state of internal environment of broodstock and sexes during the reproduction period can be done using biochemical tools (Svoboda *et al*., 2001) [22]. Factors that affect the biochemical properties of fish includes the age of the fish (Svetina *et al*., 2002) [21], species, strain & traits (Langston *et al*., 2002) [12], environmental conditions like temperature (Magill and Sayer, 2004 [14]), reproductive & gonadal periodicity (Bayir, 2005) [4] and seasonal alterations (Sreevalli and Sudha, 2014 [18]; Soranganba and Singh 2018) [16]. Several biochemical parameters in terms of species-specific, seasonal and diurnal changes have been observed in *Tinca tinca* (De Pedro *et al*., 2005) [6]. Bastami *et al*., (2009) [3] reported significant differences between sexes in both the sexes of wild common carp and observed that only hematological characteristics were insufficient to provide the total physiological condition of the fish. Studies have shown about energetic lipids mobilization from tissues preferentially to structural lipids during starvation (Henderson and Tocher, 1987) [8]. Besides maintenance, significant quantities of lipids reserved in liver and muscles were mobilised and transferred to gonads, especially ovaries. During maturation and spawning, this lipids are transported through blood serum complexes with specific proteins (apolipoproteins) as particles, known as lipoproteins in striped bass, *Morone saxatilis* (MacFarlane *et al*., 1990) [13], carangids, *Scomberoides lysan* (Sutharshiny and Shivashanthini, 2011) [20] and Nile tilapia *Oreochromis niloticus* (Singh *et al*., 2012 [15]). Karataş *et al*., 2014 [11] observed the differences in the serum lipids of cultured rainbow trout (*Oncorhynchus mykiss*) and cultured brook trout (*Salvelinus fontinalis*) and attributed the changes due to growth, size, species, age and sexual maturity cycle of the species. Lipoprotein particles are characterized by size, density and their chemical composition as chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high density (HDL) lipoprotein. It is well understood that HDL particles have important functions in granulosa and theca cell steroid genesis, serving as the predominant source of cholesterol (Hughes *et al*., 2011) [9]. Liver synthesised HDL and is rich in terms of phospholipids and cholesterol. The most important role of HDL is to transport cholesterol from peripheral tissues to the liver (Atamanalp and Solak, 2004) [2].

And these lipoproteins made by the liver from triglycerides, cholesterol and Apo lipoproteins enable fats and cholesterol to move within the water-based solution of the bloodstream for various physiological functions.

Material and Methods

Biochemical analysis for HDL was carried out using analytical kits from Erba, Germany. Tissue samples of muscles, liver and gonads needed an extraction procedure before analysis. The lipid extraction of the target tissues was carried out using modified Folch (1957) [7] method.

a) Mixed the tissue with 10 ml (20 times the tissue volume) of 2:1 ratio dichloromethane and methanol solution. The problem associated with the used of Chloroform in Folch method was replaced by Dichloromethane (Cequier-Sánchez *et al*., 2008) [5].
b) Agitated the homogenate for 20 mins using modified digital rocker.
c) Centrifuged the homogenate at 2000 rpm for 10 mins and collected the liquid phase in centrifuged tubes.
d) Washed the solvent with 0.2 volume (2 ml for 10 ml) 0.9 % NaCl (sodium chloride) solution (9 gm NaCl in 1000 ml water) and vortexes for some few seconds.
e) After vortex, centrifuged the mixture at low speed of 2000 rpm and separated the two phases.
f) Siphoned off the upper phase and collected the lower dichloromethane containing lipid for analysis.

HDL Estimation

a) Pipette 200 µl of the test sample and mixed with 400 µl of precipitating reagent (supplied with the kit) into a 2 ml micro centrifuged tube.
b) Mixed the solution well and allowed to stand for 10 mins at normal room temperature (15-30 ºC).
c) Centrifuged the solution at 4000 rpm for 10 mins to obtain a clear supernatant and the supernatant was used to determine the concentration of HDL cholesterol in the sample.
d) Prepared the blank by mixing 50 µl of distilled water into 1 ml cholesterol reagent.
e) Similarly, prepared the standard solution by mixing 50 µl of the HDL standard (25 mg/dl) into the reagent solvent.
f) Prepared test samples by mixing 50 µl of the test into the cholesterol reagent solvent.
a) Incubated all the solutions in a preheated oven at 37 ºC for 10 mines and took absorbance of the test and the standard using UV spectrophotometer at 505 nm against the reagent blank.
b) Calculated HDL-cholesterol concentration using the following formula:

$$\text{HDL Cholesterol (mg/dl)} = \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \text{x Conc. of Standard (mg/dl)x dilution factor}$$

$$= \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \text{x } 25 \text{ x } 3$$

$$= \frac{\text{Abs. of Test}}{\text{Abs. of Standard}} \text{x } 75$$

Results and Discussion

Observations on HDL level of 1+ and 2+ year's age groups for muscle, gonadal, hepatic, serum samples in different seasons are given in Table. Muscle, gonadal and serum HDL levels in both age groups showed similar pattern being at highest level in spring season, which decreased in summer and continued to decrease to the lowest level in autumn followed by an increase in the winter season. Hepatic HDL in both the age groups showed highest level in autumn season, decreased in winter and continued to the lowest level in spring followed by slight increase in summer season. Statistically significant differences ($p < 0.05$) in HDL levels were observed in both the age groups in relation to age, seasons and interaction (age & seasons) for muscle, gonadal, hepatic and serum samples. For age group, 2+ years had higher HDL level than 1+ year's age group. Higher level of muscle, gonadal and serum HDL were observed in male as compared to female whereas female had higher hepatic HDL level than the male group in both age groups. Pearson's correlations ($p < 0.01$) showed significant positive correlation between GSI with gonadal HDL while no significant correlation was observed between GSI with muscle, hepatic and serum HDL. Seasonal change in the physiological conditions with significant correlation with GSI of male and female and age in common carp has been reported by Soranganba (2022) [16].

Table 1: HDL (mg/dl) Level of 1+ and 2+ Year's old amur common carp in different seasons

Age Groups	Sample	Summer season		Autumn season		Winter season		Spring season	
		Male	Female	Male	Female	Male	Female	Male	Female
2+	Muscle	0.12±0.02	0.11±0.02	0.15±0.02	0.08±0.01	0.13±0.01	0.12±0.02	0.18±0.01	0.16±0.01
	Gonadal	1.05±0.02	1.16±0.02	0.12±0.03	0.13±0.03	0.69±0.02	0.75±0.02	1.25±0.03	1.41±0.03
	Hepatic	1.23±0.06	1.31±0.04	2.49±0.04	2.78±0.03	2.20±0.05	2.41±0.03	1.21±0.04	1.32±0.03
	Serum	74.10±1.15	75.89±1.26	67.85±1.59	70.35±0.91	77.10±0.71	80.30±0.34	84.48±0.32	87.42±0.46
1+	Muscle	0.11±0.01	0.08±0.01	-	-	0.09±0.02	0.11±0.01	0.14±0.02	0.17±0.02
	Gonadal	0.55±0.02	0.63±0.02	0.27±0.03	0.36±0.01	0.49±0.03	0.52±0.02	0.67±0.04	0.74±0.04
	Hepatic	0.67±0.05	0.89±0.04	1.63±0.04	2.18±0.02	1.27±0.03	1.31±0.04	0.70±0.04	0.84±0.06
	Serum	66.07±1.31	71.42±1.31	54.28±0.91	59.28±0.66	62.81±0.29	67.97±0.32	76.73±0.47	81.71±0.12

Positive correlation of gonadal HDL level in 1+ and 2+ year's age groups with GSI might be indicative of active involvement of HDL during gonadal development in Amur common carp. Higher HDL level in 2+ year's age group commensurate with higher GSI level indicated about their role as major lipid class of energy source in reproductive processes. Higher level of gonadal, hepatic and serum HDL in female than male might be correlated with higher energy requirements for attaining higher GSI level, a prerequisite for reproductive success and proper gonadal development. Wallaert and Babin (1994) [23] observed considerable increase in HDL-cholesterol during sexual maturation in both males and females of *Oncorhynchus mykiss* reaching considerable level during permeation and ovulation. Jerez *et al.*, (2006) [10]

observed lysogenic capacity, considered as the mobilization of lipids from muscle and liver towards the gonad for the development of oocytes, in broodstock females of *Sparus aurata.* HDL showed an increasing trend in both male and female of *Capoeta trutta* correlated to change in the reproductive cycle (Stepanowska *et al.,* 2006 [19]). Considerable increase in HDL was observed during sexual maturation in both male and female *Leuciscus cephalus* reaching substantial levels during permeation and ovulation (Aras *et al*., 2008 [1]). The study have showed that major lipoprotein like HDL plays an important role in gonadal maturity and reproductive process of common carp and proper culture regime in brood stock management may focus on biosynthesis of this lipid class through feed supplements.

References

1. Aras M, Bayir A, Sirkecioglu AN, Polat H, Bayir M. Seasonal variations in serum lipids, lipoproteins and some haematological parameters of chub (Leuciscus cephalus). Italian Journal of Animal Science. 2008 Jan 1;7(4):439-448.
2. Atamanalp M, Solak K. Üç Farklı çiftlikte yetiştirilen gökkuşağı alabaliklari Oncorhynchus Mykiss, Walbaum, 1792 nın toplam kolesterol ve kolesterol tiplerinin karşilaştirilmasi. Gazi Üniversitesi Gazi Eğitim Fakültesi Dergisi. 2004;24:1.
3. Bastami KD, Moradlou AH, Zaragabadi AM, Mir SS, Shakiba MM. Measurement of some haematological characteristics of the wild carp. Comparative Clinical Pathology. 2009 Aug;18(3):321-323.
4. Bayir A. The investigation of seasonal changes in antioxidant enzyme activities, serum lipids, lipoproteins and hematological parameters of siraz fish Capoeta capoeta umbla living in Hinis Stream (Murat Basin). Degree dissertation. Ataturk University, Turkey Bethesda, MD: American Fisheries Society; c2005.
5. Cequier-Sánchez E, Rodriguez, Covadonga, Ravelo AG, Zarate, Rafael. Dichloromethane as a solvent for lipid extraction and assessment of lipid classes and fatty acids from samples of different natures. Journal of Agricultural and Food Chemistry. 2008 Jun 25;56(12):4297-4303.
6. De Pedro N, Guijarro AI, Lopez-Patino MA, Martınez-AŁlvarez R, Delgado MJ. Daily and seasonal variations in haematological and blood biochemical parameters in the tench, Tinca tinca Linnaeus, 1758. Aquac Res. 2005 Sep;36(12):1185-1196.
7. Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem. 1957 May 1;226(1):497-509.
8. Henderson RJ, Tocher DR. The lipid composition and biochemistry of freshwater fish. Progress in Lipid Research. 1987 Jun 1;26(4):281-347.
9. Hughes J, Kwong WY, Li D, Salter AM, Lea RG, Sinclair KD. Effects of omega -3 and -6 polyunsaturated fatty acids on ovine follicular cell steroid genesis, embryo development and molecular markers of fatty acid metabolism. Reproduction. 2011 Jan 1;141(1):105-118.
10. Jerez S, Rodriguez C, Cejas JR, Bolanos A, Lorenzo A. Lipid dynamics and plasma level changes of 17β-estradiol and testosterone during the spawning season of gilthead seabream (Sparus aurata) females of different ages. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 2006 Feb 1;143(2):180-189.
11. Karataş T, Kocaman EM, Atamanalp M. The comparison of total cholesterol and cholesterol types of cultured rainbow (Oncorhynchus mykiss, Walbaum, 1972) and brook trouts (Salvelinus fontinalis, Mitchill, 1815) cultivated under the same water conditions. International Journal of Fisheries and Aquaculture. 2014 Feb 4;6(2):16-19.
12. Langston AL, Hoare R, Stefansson M, Fitzgerald R, Wergeland H, Mulcahy M. The effect of temperature on non-species defence parameters of three strains of juvenile Atlantic halibut Hippoglossus hippoglossus L. Fish Shell Immunol. 2002 Jan 1;12(1):61-76.
13. MacFarlane RB, Harvey HR, Bowers MJ, Patton JS. Serum lipoproteins in striped bass (Morone saxatilis): effects of starvation. Canadian Journal of Fisheries and Aquatic Sciences. 1990 Apr 1;47(4):739-745
14. Magill AH, Sayer MDJ. The effect of reduced temperature and salinity on the blood physiology of juvenile Atlantic cod. Journal of fish biology. 2004 May;64(5):1193-1205.
15. Singh R, Singh AK, Tripathi M. Melatonin induced changes in specific growth rate, gonadal maturity, lipid and protein production in Nile tilapia Oreochromis niloticus (Linnaeus 1758). Asian - Australasian Journal of Animal Sciences. 2012 Jan;25(1):37.
16. Soranganba N, Singh IJ. Seasonal assessment of some water quality parameters in experimental fish ponds located at Tarai region of Uttarakhand. IJCS. 2018;6(2):428-430.
17. Soranganba N. Comparative study on seasonal change in physiological conditions in two different age groups of male Amur common carp (Cyprinus carpio haematopterus). The Pharma Innovation Journal. 2022;SP-11(10):1368-1372
18. Sreevalli N, Sudha HR. Total protein, glycogen and cholesterol content in the ovary and liver during post spawning and resting season of Mystus vittatus (Bloch). Current Biotica. 2014;7(4):321-325.
19. Stepanowska K, Nedzarek A, Rakusa-Suszczewski S. Effects of starvation on the biochemical composition of blood and body tissue in the Antarctic fish Notothenia coriiceps (Richardson, 1844) and excreted metabolic products. Polar Bioscience. 2006;20:46-54.
20. Sutharshiny S, Sivashanthini K. Lipid reserves of Scomberoides lysan (Pisces: Carangidae) from the Sri Lankan waters. International Journal of Biological Chemistry. 2011;5(3):170-83..
21. Svetina A, Matasin Z, Tofant A, Vucemilo M, Fijan N. Haematology and some blood chemical parameters of young carp till the age of three years. Acta Vet Hung. 2002;50:459-467.
22. Svoboda M, Kouril J, Hamackova J, Kalab P, Savina L, Svobodova Z, Vykusova B. Biochemical profile of blood plasma of tench (Tinca tinca L.) during pre-and post-spawning period. Acta Vet Brno. 2001;70(3):259-268.
23. Wallaert C, Babin PJ. Age-related, sex-related, and seasonal changes of plasma lipoprotein concentrations in trout. Journal of Lipid Research. 1994 Sep 1;35(9):1619-1633.