The Pharma Innovation Journal 2022; SP-11(11): 81-84

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(11): 81-84

www.thepharmajournal.com
Received: 01-08-2022
Accepted: 04-09-2022

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Assessment of seasonal change in cholesterol level of amur common carp associated with reproduction in different age groups

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Abstract
The present investigation was conducted to study the correlation of reproductive profile with biochemical parameters cholesterol (CHO) 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. Water quality parameters were recorded on seasonal basis. Sampling for 1+ and 2+ year's age groups were carried out in four different seasons – summer (July), autumn (October), winter (January) and spring (March). CHO level showed an increasing trend from the initial detection in summer until winter season. Comparison of physiological profiles in 1+ and 2+ year's age groups of Amur common carp revealed two major peak periods - spring season and summer season. There was inverse correlation of GSI and HSI in all seasons irrespective of age and sex. The biochemical changes showed significant positive correlation with GSI. The changed in CHO levels were observed to be of higher amplitude in 2+ year's age group specimens than 1+ year's age group except HSI. Based on the present study, it may be inferred that seasonal changes biochemical parameters (CHO) have a profound effect on the scale of reproductive success of Amur common carp, *Cyprinus carpio haematopterus,* during spring and summer seasons and 2+ year's age group showed better reproductive potential.

Keywords: Cholesterol level, amur common carp, age groups, biochemical parameters cholesterol

Introduction
Biochemical indices are one of the important tools for assaying physiological changes that are widely used by fish biologists (Gabriel *et al.,* 2011) [9]. Gonadal development is stimulated by steroidal hormones and lipids play an essential role in meeting energy requirements during gonadal development. Lipids and their constituent fatty acids along with proteins is the major organic constituent associated with growth and reproduction (Tocher, 2003) [23]. Like in other vertebrates, cholesterol is the precursor to all steroid hormones. Fish steroidogenic tissues acquire cholesterol from circulating lipoproteins, intracellular cholesterol esters, dietary intake or by *de novo* synthesis. Reproduction is an energy-consuming process requiring an adequate generation of ATP from cellular energy stores. It has been reported that lipids are generally mobilized from the liver to the developing gonads during recrudescence, compared with general lipogenic hepatic activity preceding gonadal development (Sharpe and MacLatchy, 2007) [16].
Lipids are stored energy providing components for membrane synthesis and precursors of steroid hormones in substantial quantities in various tissues including muscle, liver and mesenteric depots. Variation in lipid classes in gonads, muscles and liver of adult fish are directly associated with the sexual maturity and spawning of the fish (Huynh *et al.,* 2007) [10]. Other than 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 carangids, *Scomberoides lysan* (Sutharshiny and Shivashanthini, 2011) [21] and Nile tilapia *Oreochromis niloticus* (Singh *et al.,* 2012) [17]. Karataş *et al.,* (2014) [12] observed the differences in the serum total cholesterol between 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. Lipids are also an important source of female egg production and for male breeding activities such as courtship behaviour, competitions, parental care and nesting (Ebhrahimnezhadarabi *et al.,* 2011) [5].

Shankar and Kulkarni (2014) [14] observed a correlated increase in cholesterol content in the ovary and liver during the breeding phase in freshwater fish, *Notopterus notopterus* indicating active requirements for the ovarian development and vitellogenesis. Changes in cholesterol were highly correlated with changes in the reproduction cycle in male and female groups of *Capoeta trutta* (Eroğlu and Şen, 2017) [7]. Since reproduction is a high energy demanding process, limitation in energy supply in the form of starvation shows a declining trend for cholesterol with sex-specific significance in both male and female *Notothenia coriiceps* (Stepanowska *et al.*, 2006) [19]. Biochemical profiles changed in relation to sex and season in *Barilius bendelisis* were reported by Sharma *et al*., (2015) [15]. Kulkarni (2017) [13] reported higher level of cholesterol in male *Notopterus notopterus* than female thus indicating sex-specific higher nutrients demand and continuous utilization for the development and gonadal maturation.

Material & Methods

Biochemical analysis for cholesterol was carried out using analytical kits from Erba, Germany.

Sample extraction

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) [8] 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) [3].
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.

Cholesterol (CHO) estimation

a) Prepared the blank by mixing 20 µl of distilled water into 1 ml cholesterol reagent supplied with the kit.
b) Similarly, prepared the standard solution by mixing 20 µl of the cholesterol standard (200 mg/dl) into the reagent solvent.
c) Prepared the test samples by mixing 20 µl of the test into the reagent solvent.
d) Incubated all the solutions in a preheated oven at 37^0C for 10 mins and took absorbance of the test and the standard using UV spectrophotometer at 505 nm against the reagent blank.
e) Calculated cholesterol concentration using the formula below:

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{x Concentration of Standard (mg/dl)}$$

Precaution during biochemical analysis

a) Samples and reagents were thawed at room temperature before analysis
b) Proper incubation temperature and duration were maintained.

Blank and standard readings were checked intermittently

Results and Discussion

Observations on CHO level of 1+ and 2+ year's age groups for muscle, gonads, hepatic, serum samples in different seasons are shown in Table. Muscle, gonadal and serum CHO level in both the age groups showed similar pattern with highest level in spring season, which decreased slightly in summer and continued to decrease to the lowest level in autumn season followed by an increase in winter season. Hepatic CHO level in both the age groups showed highest level in autumn season, which decreased in winter and continued to decrease to the lowest level in spring followed by slight increase in summer season. Statistically significant differences ($p<0.05$) in CHO levels were observed in both the age groups in relation to age, seasons and interaction (age & seasons) for muscle, gonadal, hepatic and serum samples. Higher CHO levels were recorded in 2+ years than 1+ year's age groups with female having higher level than male in both age groups. Pearson's correlations ($p<0.01$) showed significant positive correlation between GSI with muscle CHO, gonadal CHO and serum CHO while negative correlation was observed between hepatic CHO and GSI.

Table 1: Cholesterol (mg/dl) Level of 1+ and 2+ Year's old Amur Common in Different Seasons

Age Groups	Samples	Summer season		Autumn season		Winter season		Spring season	
		Male	Female	Male	Female	Male	Female	Male	Female
2+	Muscle	2.14±0.06	2.04±0.13	1.78±0.07	1.65±0.03	1.98±0.06	1.83±0.11	2.81±0.21	2.63±0.14
	Gonadal	10.31±0.15	12.32±0.13	5.13±0.09	5.73±0.14	8.28±0.17	9.60±0.14	14.45±0.18	16.72±0.17
	Hepatic	6.28±0.09	6.96±0.06	12.85±0.17	13.59±0.10	7.28±0.14	8.84±0.15	5.13±0.18	5.68±0.25
	Serum	178.66±2.14	195.15±1.57	152.67±0.45	164.60±0.97	142.16±0.97	170.70±1.12	208.18±1.88	222.04±2.45
1+	Muscle	1.94±0.07	1.06±0.09	1.69±0.06	1.59±0.06	1.85±0.17	1.75±0.13	2.09±0.15	1.86±0.13
	Gonadal	6.93±0.09	7.39±0.09	3.51±0.08	3.93±0.12	5.29±0.06	5.83±0.06	8.59±0.19	8.95±0.19
	Hepatic	3.07±0.05	3.16±0.06	9.36±0.40	9.44±0.36	5.07±0.09	4.89±0.64	2.41±0.11	2.86±0.11
	Serum	123.76±0.71	136.54±0.55	104.50±0.56	118.63±0.97	144.84±0.82	161.52±0.82	167.95±1.21	185.22±0.80

[Data are given as mean±SEM (n=5)]

Muscle, gonadal and serum CHO levels in 1+ and 2+ year's age groups showed significant positive correlation with GSI level, having peak during two seasons- spring and summer. These positively correlated higher levels of muscle, gonadal and serum CHO might be due to the mobilisation of CHO towards developing gonads and increase in gonadal tissue is due to accumulation of CHO related to reproductive processes during peak spawning season. Inverse relationship between changes in hepatic CHO and GSI levels and positive correlation between hepatic CHO and HSI might indicate

about mobilisation of CHO from liver to gonads via blood circulation. Liver is considered site for *de novo* synthesis of CHO during the period coinciding with the developing gonads, and it forms the main substrate for biosynthesis of steroidal hormones. Higher CHO levels observed in 2+ year's age group might be indicative of higher requirement for CHO during its gonadal development and biosynthesis of steroidal hormones as corresponding to higher levels of GSI in 2+ year's age group. Higher CHO level in all tissues of female studied as compared to male could be related to higher requirement of CHO during ovarian development for vitellogenesis corresponding to higher reproductive potential as indicated by GSI levels. Increased in serum lipids during pre-spawning and spawning seasons related with utilization of lipids as an energy source and for synthesis of steroidal hormones for reproduction have been reported in *Tinca tinca* (Svoboda *et al.*, 2001) [22] and *Leuciscus cephalus* (Aras *et al.*, 2008) [1]. Reports with varied observations concerning changes in serum CHO levels with age differences. Ejrae *et al.*, (2015) [6] reported no significant changes in serum CHO level with age in *Ctenopharyngodon idella*, while an increase in serum CHO levels with age and size were reported in *Tenualosa ilisha* (Jawad *et al.*, 2004) [11] and *Dicentrarchus labrax* (Coz-Rakovac *et al.*, 2005) [4]. High cholesterol levels occurred as a prerequisite for gonadal steroidogenesis and production of basal steroidal hormones (Young *et al.*, 2005) [24]. Kavadias *et al.*, (2003) [25] observed higher plasma total cholesterol concentrations having positive correlation with gametogenesis in *Dicentrarchus labrax*. During sexual maturation in *Capoeta capoeta umbla*, CHO were to be mobilised from tissues in which previously CHO was stored towards gonads for gonadal development and to act as substrate for steroid production (Bayir, 2005) [2]. Shankar and Kulkarni (2014) [14] reported active requirements of CHO in the ovary during the breeding phase in freshwater fish *Notopterus notopterus* as indicated by the correlated increase in cholesterol content in it with ovarian development and vitellogenesis. Seasonal changes in CHO concentrations in somatic tissues with relation to gonadal development of *N. notopterus* were reported by Sudarshan and Kularni (2013) [20]. Decrease in CHO level in the ovary during the post-spawning and resting seasons was observed in *Mystus vittatus* (Sreevalli and Sudha, 2014) [18]. High levels of hepatic CHO during recrudescence period (autumn) and its gradual decrease during gonadal development reported in *Carassius auratus* (Sharpe and Maclatchy, 2007) [16] are similar to the present observations in Amur common carp.

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