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Assessment of seasonal triglycerides levels in different age groups of Amur common carp associated with reproduction

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Abstract
The present study was conducted to determine the seasonal correlation of triglyceride (TG) with physiological indices 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 of the trial ponds were recorded seasonally. 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). TG 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 TG data showed significant positive correlation with GSI and the changed were seen with higher magnitude in 2+ year's age group specimens than 1+ year's age group except for HSI. Based on the present study, it may be inferred that seasonal changes in TG level certain important role and sufficient available TG in dietary supplements can play a major role in reproductive success of Amur common carp, *Cyprinus carpio haematopterus*.

Keywords: Triglycerides, Amur common carp, seasonal, reproduction

Introduction
Biochemical profiling can also be used to assess the state of internal milieu of broodstock and sexes during the reproduction period (Svoboda *et al*., 2001) [25]. Several factors affect the biochemical parameters of fish blood including age (Svetina *et al*., 2002) [24], species and strain (Langston *et al*., 2002) [12], temperature (Magill and Sayer, 2004) [15], reproductive and gonadal cycles (Bayir, 2005) [2] and seasonal changes (Sreevalli and Sudha, 2014; Soranganba and Singh 2018) [22, 20]. Species-specific seasonal and diurnal variations in different biochemical parameters have been observed in *Tinca tinca* (De Pedro *et al*., 2005) [4]. Bastami *et al.*, (2009) [1] investigated (male and female) wild common carp and observed that there were significant differences between sexes and concluded that only hematological characteristics cannot provide the physiological condition of the fish in totality. Triglycerides or the triacylglycerol molecules are carried throughout the blood in lipoprotein particles routinely characterized by size, density and their chemical composition as chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high density (HDL) lipoprotein. Very-low-density lipoprotein (VLDL) is one of the major groups of lipoproteins made by the liver from triglycerides, cholesterol and apolipoproteins that enable fats and cholesterol to move within the water-based solution of the bloodstream. Studies have shown about energetic lipids (e.g. triglycerides) mobilization from tissues preferentially to structural lipids (e.g. phospholipids) during starvation (Henderson and Tocher, 1987) [9]. 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 striped bass, *Morone saxatilis* (MacFarlane *et al.*, 1990) [14], carangids, *Scomberoides lysan* (Sutharshiny and Shivashanthini, 2011) [23] and Nile tilapia *Oreochromis niloticus* (Singh *et al.*, 2012) [18]. Karataş *et al.*, (2014) [10] 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. Lipids like triglycerides 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].

Material & Methods

Biochemical analysis for Triglycerides (TG) 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) [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.

Triglycerides (TG) estimation

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

$$\text{Triglycerides}\left(\text{mg}/_{\text{dl}}\right) = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ x Concentration of Standard (mg/dl)}$$

Results & Discussion

Observations on TG level of 1+ and 2+ year's age groups for muscle, gonadal, hepatic, serum samples in different seasons are shown in Table. Muscle and hepatic TG level in both the age groups showed similar pattern with highest level in autumn season, which decreased slightly in winter and continued to decrease to the lowest level in spring followed by some recovery in summer season. Gonadal and serum TG levels 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 followed by an increase in winter season. Statistically significant differences ($p< 0.05$) in TG 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, TG levels were higher in 2+ years as compared to 1+ year's age group. Levels of TG for muscle and hepatic tissues were higher in female than male while it was higher in male in gonad and serum TG in both the age groups. Pearson's correlations ($p< 0.01$) in both the age groups showed significant positive correlation between GSI with gonadal TG and serum TG while negative correlation between muscle TG and GSI. No significant correlation was observed between hepatic TG and GSI.

Table: Triglyceride (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	31.43±0.23	33.53±0.10	67.77±0.21	59.16±0.40	52.95±0.46	48.20±0.22	28.19±0.23	24.83±0.52
	Gonadal	21.99±0.11	24.86±0.17	19.97±0.16	22.41±0.20	34.74±0.44	42.27±0.26	73.71±0.24	83.20±0.30
	Hepatic	27.72±0.37	37.13±0.20	80.61±0.25	98.15±0.27	68.21±0.34	74.30±0.18	52.49±0.27	56.03±0.28
	Serum	131.81±0.72	138.63±0.59	98.60±0.49	86.01±0.69	104.61±0.87	94.52±0.65	164.76±1.24	153.33±1.31
1+	Muscle	25.10±0.27	27.69±0.35	57.12±0.34	48.11±0.47	30.56±0.19	28.10±0.22	20.68±0.18	18.74±0.49
	Gonadal	25.73±0.14	24.72±0.08	22.51±0.09	19.02±0.18	28.27±0.24	30.22±0.09	52.49±0.26	47.88±0.23
	Hepatic	16.39±0.20	21.53±0.15	30.32±0.16	35.49±0.21	29.94±0.19	30.70±0.17	22.05±0.26	26.43±0.16
	Serum	95.80±0.44	93.28±0.52	77.48±1.09	67.69±0.55	82.05±0.60	78.11±0.87	127.04±2.60	136.95±0.92

[Data are given as mean±SEM (n=5)]

Significant positive correlation of increased in gonadal and serum TG levels in 1+ and 2+ year's age groups with GSI and negative correlation with muscle TG level might be an indication of mobilisation of TG from the muscle tissue towards gonadal development via blood circulations which signifies the active role of TG in reproductive process of the fish. Higher levels of TG in 2+ year's age group in most of the seasons seems to clearly indicative of the higher energy requirement for gonadal development in this age group for having higher GSI than the 1+ year's age group. Seasonal change in the physiological conditions of common carp has been reported by Soranganba (2022) [21]. Higher level of muscle and serum TG level in male might be related to low level energy mobilisation in male for gonadal development in comparison to female specimens. Higher gonadal and hepatic TG levels, matching with higher GSI in females might be due to more active mobilisation from liver followed by accumulation of the same in ovary. Morphological difference in male and female accounts to physiological difference in carps has been reported by Soranganba and Saxena (2017) [19]. Seasonal variations in TG levels related to reproduction were reported in *Pleuronectes platessa* (White *et al.*, 1986) [26], *Clarias batrachus* (Lal and Singh, 1987) [11] and *Carassius auratus* (Sharpe and MacLatchy, 2007) [16]. Age and sex-related differences in overall TG pool have been described in striped bass (Lund *et al.*, 2000) [13]. Increased in TG level due to age and gonadal maturation in relation to with the transformation of nutrition from certain organs to gonads via the blood and reaching maximum level during spawning season have been observed in *Huso huso* (Gharaei *et al.*,

2013) [8]. Singh and Lal (2008) [17] observed tremendous increase in ovarian lipids content during ovarian recrudescence in Asian catfish, *Clarias batrachus* because of lipid import from liver and adipose tissues to ovary. This lipid mobilization to the ovary is the characteristic feature of the ovarian growth and development (Lal and Singh, 1987) [11]. Weigand (1982) [28] reported that TG was preferentially synthesized in the ovarian tissue of trout with larger GSI indicating a positive correlation between gonadal size and TG synthesis in ovary.

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