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Assessment of seasonal change in biochemical parameters of Amur common carp associated with reproduction in different age groups

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Abstract
The present study was taken up to assess the role of some biochemical indices (TP, CHO, TG, HDL, LDL, VLDL, PHO) from blood serum of 1+ and 2+ year's age groups Amur common carp, *Cyprinus carpio haematopterus* in relation to physiological indices (like GSI, HSI) and reproductive traits seasonally. The study showed that 2+ year's age group have higher TP than 1+ year's age group correlating with GSI and indicating better higher reproductive potential and protein requirement. Similarly, CHO, TG, HDL, LDL, VLDL and PHO were observed with higher concentration in 2+ than 1+ age groups. Higher levels of GSI in 2+ year's age group corresponding to CHO indicates its mobilisation for gonadal development and other biosynthesis. High TG recorded in male can be related to low level energy mobilisation in male gonadal development as compared to female counterparts. Elevated major lipid class HDL observed in 2+ year's age group indicates its role in reproductive physiology and vice versa for LDL showing positive correlation with GSI. High concentration of VLDL indicates the transport of endogenous lipids towards gonadal development coinciding with peak spawning season and higher level in female correlates with female vittelogenesis. PHO plays an important role in cellular integrity and as energy reserves which in evident from the higher level observed during the study. The above biochemical obervations corresponding with season and spawning period indicates the imortace of these biomolecules and lipid class in gonadal development of Amur common carp.

Keywords: Biomolecules, lipids class, Amur common carp, biochemical parameters

Introduction
Common carp mostly cultured in India are having the problem of poor body growth due to early gonadal development, early maturation and multiple spawning behaviour, which results in stunted growth and fewer carcasses to body weight ratio. This leads to the negative impression as good candidate species for culture aspect and on consumer preferences. Biochemical indices are one of the important tools for assaying physiological changes that are widely used by fish biologists (Gabriel *et al.,* 2011) [13]. Lipids and their constituent fatty acids along with proteins is the major organic constituent associated with growth and reproduction (Tocher, 2003) [34]. There is limited study related to plasma protein level with reproductive cycle and gametogenesis of fish (Popovski *et al.,* 2017) [24]. Lipids are certainly an important source of energy in fish but proteins also play a vital role (Chellappa *et al.,* 1995) [5]. 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. Fatty acid provides a potent source of such energy upon demand, which is stored intra-cellularly as triglycerides within lipid droplets (Dunning *et al.,* 2014) [10]. In teleost species, acquired energy is primarily stored in the form of triglycerides and is hydrolyzed from liver and muscle stores during lipid mobilization to yield free fatty acids (FFA) and glycerol. 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) [28]. Similarly, other lipoproteins like the high density lipoprotein.

Material and Methods
The experimental site is situated at IFF inside the Experimental farm facility of College of Fisheries, G.B.

Pant University of Agriculture & Technology, Pantnagar (Uttarakhand) located at the latitude of 29.01°N, longitude 79.3°E, 344 metres above mean sea level (MSL) at Tarai region, the Shivalik range of the Himalayas. Specimens of 1+ and 2+ year's age group weighing approximately 170-200 gm and 590-700gm were held in earthen ponds A and B respectively. Random samples of 5 (five) male and female for 1+ and 2+ year's age groups were collected during all four seasons – summer, autumn, winter and spring from pond A and B respectively. Samples for 0+ year's age group were collected only from pond B during summer, autumn and winter seasons. Fish were anesthetized using clove oil for collection of blood and later sacrificed for tissues/organs collection for biochemical assay. The gonads and liver of 1+ and 2+ year's age groups fish specimens were dissected out and weighed for calculation of GSI and HSI. About 5-10 gm muscle, gonadal and hepatic tissues were collected from 1+ and 2+ year's age groups and blood samples incase of 0+ year's age group for biochemical analysis and stored at below -20^{0}C sealed with parafilm till analysis. Biochemical analysis for protein, cholesterol, triglycerides and HDL were carried out using analytical kits from Erba, Germany. According to the formula derived from Friedewald *et al.*, (1972) [12], VLDL and LDL were calculated as follows:

$$\mathrm{VLDL} = \frac{\mathrm{Triglycerides}}{5}$$

$$\mathrm{LDL} = \mathrm{Cholesterol} - \frac{\mathrm{Triglycerides}}{5} - \mathrm{HDL}$$

Phospholipids were calculated by the formula given by Covaci *et al.*, (2006) [7] as:

$$\mathrm{Phospholipid} = \mathrm{Cholesterol} \times 0.73 + 90$$

The unit of LDL, VLDL and phospholipids was derived from the unit of the respective compound like triglycerides and cholesterol i.e. mg/dl.

Results and Discussion

Serum total protein (TP) (Table 1), Cholesterol (CHO), Triglyceride (TG), High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and Phospholipids (PHO) levels of 1+ and 2+ year's age groups are given in Tables 2. Total protein level of both the age groups recorded level in spring season, which declined slightly in summer season and continued to decline in autumn until the lowest level in winter season. It showed statistically significant differences ($p<0.05$) in both the age groups in relation to seasons and their interaction (age & seasons). 2+ years showed higher level than 1+ year's age group with female dominating the male group. Pearson's correlations ($p<0.01$) showed significant correlation with GSI and serum TP. Changes in serum TP level seems to be correlated with seasonal changes in reproductive status of the fish, which might be indicative of the importance of this biomolecule in fish reproductive processes. Higher serum TP level in 2+ year's age group than 1+ year's age group could be correlated with higher GSI level indicative of higher reproductive potential and requirement of TP during reproductive.

Table 1: Serum total protein (gm/dl) Level of 1+ and 2+ Year's old Amur common carp in different seasons

Age Groups	Samples	Summer season		Autumn season		Winter season		Spring season	
		Male	Female	Male	Female	Male	Female	Male	Female
2+	Serum	3.69±0.01	3.81±0.07	3.30±0.03	3.52±0.18	3.15±0.02	3.22±0.01	3.48±0.06	3.65±0.16
1+		3.10±0.02	3.24±0.02	3.10±0.03	3.14±0.04	2.96±0.05	3.01±0.03	3.31±0.02	3.44±0.04

[Data are given as mean± SEM (n=5)]

Table 2: Serum cholesterol, triglyceride, HDL, LDL, VLDL, phospholipids (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+	CHO	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
	TG	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
	HDL	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
	LDL	78.19±1.90	91.53±2.36	65.09±1.94	77.04±1.48	48.04±0.90	74.92±1.05	99.49±2.14	112.10±2.23
	VLDL	26.36±0.14	27.72±0.12	19.72±0.09	17.20±0.13	20.92±0.17	18.90±0.16	32.95±0.24	30.67±0.27
	Phospholipids	220.42±1.56	232.46±1.15	201.44±0.33	210.16±0.71	193.78±0.72	214.61±0.82	241.97±1.37	252.09±1.79
1+	CHO	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
	TG	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
	HDL	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
	LDL	38.89±1.08	46.10±1.60	34.72±1.18	45.81±0.70	68.59±5.23	80.76±1.21	72.56±1.60	83.39±0.75
	VLDL	19.16±0.08	18.65±0.10	15.49±0.21	13.53±0.11	16.41±0.12	15.62±0.17	25.40±0.52	27.39±0.18
	Phospholipids	180.35±0.52	189.67±0.40	166.28±0.41	176.61±0.71	195.73±0.59	207.91±0.61	212.61±0.88	225.21±0.58

[Data are given as mean± SEM (n=5)

processes. Female had higher serum TP than male group, which might be due to their higher requirements in vitellogenesis and/or formation of chorion or vitelline membrane during gonadal development. Suresh *et al.*, (2008) [32] reported cyclic changes in plasma protein levels correlating with reproductive phases in both sexes of Indian major carp, *Labeo rohita*. Seasonal changes in protein levels of *Capoeta capoeta umbla* with increase in temperature were reported by Bayir *et al.*, (2007) [4]. There were reports on mobilization of protein during breeding periods in addition to uses of lipid in *Plruronecrus plutessu* (Dawson and Grimm, 1980) [9] and *Gusterosteus uculeutus* (Chellappa *et al.*, 1989) [6]. Svobodova *et al.*, (2001) [33] reported increase in serum TP level with age and sex in common carp (*Cyprinus carpio*).

Similar differences in serum TP level with change in age and sex have also been reported in hybrid striped bass (Hrubec *et al.*, 2001) [16], Indian shad, *Tenualosa ilisha* (Jawad *et al.*, 2004) [17] and sea bass (*Dicentrarchus labrax*) (Coz-Rakovac *et al.*, 2005) [8]. Proteins are directly related to gonadal development since it is required for the formation of the vitelline membrane or chorion (Hamazaki *et al.*, 1989; Oppen-Bemsten *et al.*, 1991) [15, 22].

Serum CHO level in both the age groups also showed 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. 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 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 and CHO. These positively correlated higher serums 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 for gonadal development and biosynthesis of steroidal hormones as corresponding to higher levels of GSI in 2+ year's age group. 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) [33]. Reports with varied observations concerning changes in serum CHO levels with age differences. Ejrae *et al.*, (2015) [11] 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) [17]. High cholesterol levels occurred as a prerequisite for gonadal steroidogenesis and production of basal steroidal hormones (Young *et al.*, 2004) [37]. Kavadias *et al.*, (2003) [20] 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) [3]. Shankar and Kulkarni (2014) [31] 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).

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 serum samples. For age group, TG levels were higher in 2+ years as compared to 1+ year's age group. Pearson's correlations ($p<0.01$) in both the age groups showed significant positive correlation between GSI with serum TG. Significant positive correlation of increased in serum TG levels in 1+ and 2+ year's age groups with GSI might be an indication of mobilisation 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. Higher serum TG level in male might be related to low level energy mobilisation in male for gonadal development in comparison to female specimens. Seasonal variations in TG levels related to reproduction were reported in *Carassius auratus* (Sharpe and MacLatchy, 2007) [28]. Age and sex-related differences in overall TG pool have been described in striped bass (Lund *et al.*, 2000) [21]. 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) [14]. Singh and Lal (2008) [29] 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.

Observations on HDL level showed 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. 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 serum samples. For age group, 2+ years had higher HDL level than 1+ year's age group. Higher level of serum HDL was observed in male as compared to female. Pearson's correlations ($p<0.01$) showed any significant correlation between GSI and serum HDL. 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 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. Jerez *et al.*, (2006) [18] observed lipogenic 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) [30]. Considerable increase in HDL was observed during sexual maturation in both male and female *Leuciscus cephalus* reaching substantial levels during spermiation and ovulation (Aras *et al*., 2008) [1].

Serum LDL level in both the age groups showed highest level in spring season but decreased in summer reaching to the lowest level in autumn followed by slight increase in the winter season. Statistically significant differences ($p<0.05$) in LDL levels in serum samples were observed in both the age groups in relation to age, seasons and interaction (age & seasons). Higher level of serum LDL was observed in female as compared to male in both age groups. Pearson's correlations ($p<0.01$) showed significant positive correlation between GSI with serum LDL. The variations in serum LDL concentration observed in the present study were similar with the findings of Azeez and Mohammed (2017) [2] in *Cyprinus carpio* with relatively high seasonal variations corresponding to gonadal maturation. Higher LDL level in female than male of Amur common carp observed in the present study might be due to the number of LDL receptors in the hepatic tissues that could have changed during the metabolic reorganization of liver following vitellogenesis due to the synthesis of vitellogenin in trout as reported by Wallaert and Babin (1994). Other factor including feeding, environmental conditions and sexual status could also play a cascading effect on the variations in LDL concentrations in the fish (Karataş *et al.*, 2014) [19].

Serum VLDL level in both the age groups showed similar pattern with highest levels in spring season which decreased 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 VLDL levels were observed in both the age groups in relation to age, seasons and interaction (age & seasons) for serum samples. Higher VLDL level was recorded in 2+ year's age group than 1+ year's age group. Higher level of serum VLDL levels were observed in male as compared to female. Pearson's correlations ($p<0.01$) showed significant positive correlation between GSI with serum VLDL**.** Positive correlation between VLDL levels in serum samples with changes in GSI levels seems to be indicative of the transport of nutrients towards developing gonads through blood circulation. Higher VLDL levels in 2+ year's age groups might be indicative of VLDL being a major constituent in gonadal maturation by transporting the endogenous lipids towards gonadal development, which coincided with peak spawning season during spring season. Higher VLDL levels in female than male might be more correlated with the higher energy requirements for production of vitellogenin in female, which are associated with gonadal development and maturation. Seasonal variations in plasma VLDL level have been reported in male and female trout (Wallaert and Babin, 1994), chub. Variation of VLDL levels with age, nutrition and sexual cycle could be observed in most of the teleost which occurred during feeding where excess dietary fatty acids were exported from the liver in the form of lipoproteins (VLDL) and accumulated and stored in the form of TG in specific lipid storage sites (Tocher, 2003) [34]. Oil droplets that will contribute to the lipid content of the oocytes are probably derived from VLDL transported to the ovaries through the bloodstream and taken up by the follicular complex during primary oocyte growth phase in three spined stickleback, *Gastersteus aculatus* (Wotton *et al.*, 1978) [36].

Serum PHO level in both the age groups showed similar pattern with highest level in spring season, which declined in summer and continued to decline to the lowest level in autumn followed by slight increase in autumn season. Statistically significant differences ($p<0.05$) in PHO levels were observed in both the age groups in relation to age, seasons and interaction (age & seasons) for serum samples. For age group, 2+ year's age group specimens had higher PHO level than 1+ year's age group. Level of PHO in serum was higher in female as compared to male. Pearson's correlations ($p<0.01$) showed significant positive correlation between GSI with serum PHO. Significant positive correlation of serum PHO levels with high GSI level during spring season followed by summer season might be indicative of PHO as a major constituent in lipid bilayer formation during gonadal development. In the present study, PHO levels were higher in 2+ years than 1+ year's age group, which matched with higher GSI level. It indicated involvement of higher PHO levels in gonadal development and its active part in reproductive processes of Amur common carp, as it forms an integral part in cellular integrity and energy reserves in fish. Higher level of serum PHO in males also seem to be higher correlated with testicular development. Its role in gonadal maturity based on low hepatic PHO levels during spawning, post-spawning and resting phases and sharp increase during the preparatory phase were reported in common carp (Rinchard and Kestemont, 2003) [25]. Positive correlation of PHO with GSI in Amur common carp are also supported by the similar reports of Aras *et al*., (2008) [1] in chub (*Leuciscus cephalus*) as gonadal tissues require ample supplies of phospholipids constituents for membrane formation. Increase in PHO levels with increased GSI levels have been reported in *Salmo gairdneri* (Weigand and Idler, 1982) [35].

Positive correlation of serum LDL levels with GSI in 1+ and 2+ year's age groups might be indicative of its active role in gonadal development. LDL like other lipids constituents - TG, CHO, PHO carried by this lipoprotein particle are required for gonadal development as a medium for meeting energy requirements and serving as substrate for various steroidal hormone biosynthesis, which generally attain peak during spawning (spring) season. Higher LDL level in 2+ year's age group corresponding to higher GSI level is indicative of higher demand related to development of gonads. The observations in biochemical parameters in different age groups during the study period corresponded with the seasonal changes in spawning period and GSI levels, which might be indicative of the role played by these biomolecules and lipid classes in gonadal development in terms of supply of energy and/or biosynthesis of steroidal hormones in Amur common carp.

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