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Low density lipoproteins in reproductive physiology of Amur common carp

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Abstract

The present investigation was taken up to determine the seasonal change in low density lipoproteins (LDL) 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. Positive correlation of serum and gonadal LDL levels with GSI in 1+ and 2+ year's age groups were observed and might be indicative of its active role in gonadal development. Significant effect of serum and gonadal level with age and season F (4, 98) = 293.1, $p<0.05$, $\eta^2 = 0.938$; F (3, 98) = 353.252, $p<0.05$, $\eta^2 = 0.931$ and F (3, 69) = 224.371, $p<0.05$, $\eta^2 = 0.926$; F (3, 69) = 257.825, $p<0.05$, $\eta^2 = 0.935$ respectively. Based on the present investigation, it can be inferred that seasonal changes in LDL 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: low 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) [20]. Factors that affect the biochemical properties of fish includes the age of the fish (Svetina *et al*., 2022) [19], species, strain & traits (Langston *et al*., 2002) [12], environmental conditions like temperature (Magill and Sayer, 2014) [13], reproductive & gonadal periodicity (Bayir, 2015) and seasonal alterations (Sreevalli and Sudha, 2014; Soranganba and Singh 2018) [18, 17]. Several biochemical parameters in terms of species-specific, seasonal and diurnal changes have been observed in *Tinca tinca* (De Pedro *et al*., 2015) [5]. Bastami *et al.*, (2019) [2] 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) [9]. 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 made by the liver from triglycerides, cholesterol and apolipoproteins enable fats and cholesterol to move within the water-based solution of the bloodstream for various physiological functions in Nile tilapia *Oreochromis niloticus* (Singh *et al.*, 2012) [15]. 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. 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. The endogenously synthesized triacylglycerols is delivered to adipose tissue by VLDL and the residue is converted and transformed into LDL in the bloodstream, which is rich in cholesterol esters. Thus, the role of LDL is to transport cholesterol to peripheral tissues and regulate *de novo* cholesterol synthesis at such sites (Karatas *et al.*, 2014) [10]. 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) [6].

Kulkarni (2017) [11] reported higher level of LDL in male *Notopterus notopterus* than female thus indicating sex-specific higher nutrients demand and continuous utilization for the development and gonadal maturation.

Material and Methods

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] and Cequier-Sánchez *et al*., (2008) [4] method.

a. Mixed the tissue with 10 ml (20 times the tissue volume) of 2:1 ratio dichloromethane and methanol solution.
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.

Estimation

According to the formula derived from Friedewald *et al.*, (1972) [8], LDL concentrations were calculated as follows:

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

Results and Discussion

Seasonal change in the physiological conditions with significant correlation with GSI in Amur common carp in Tarai region of the Himayalan range was reported by Soranganba (2022) [16].

Table 1: Seasonal LDL (mg/dl) Level of 1+ and 2+ Year's old Amur Common Carp

Age Groups	Sample	Summer season		Autumn season		Winter season		Spring season	
		Male	Female	Male	Female	Male	Female	Male	Female
2+	Muscle	-	-	-	-	-	-	-	-
	Gonadal	4.86±0.15	6.19±0.12	1.01±0.13	1.12±0.17	1.81±0.20	1.96±0.16	2.37±0.17	3.09±0.16
	Hepatic	-	-	-	-	-	-	-	-
	Serum	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
1+	Muscle	-	-	-	-	-	-	-	-
	Gonadal	1.23±0.12	1.81±0.10	-	-	0.17±0.03	0.36±0.13	0.20±0.19	1.08±0.22
	Hepatic	-	-	1.66±0.40	1.09±0.12	0.32±0.05	-	-	-
	Serum	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

[Data are given as mean±SEM (n=5)]

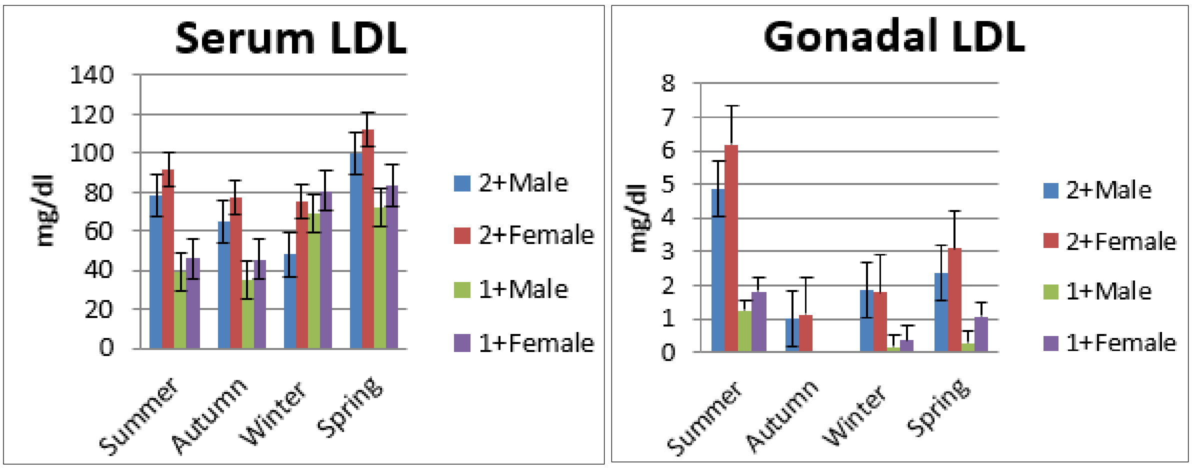

Fig 1: Observations on muscle, gonadal, hepatic and serum LDL levels in 1+ and 2+ year's age groups in different seasons are shown in Table.

A descriptive statistics observed across all age groups showed maximum mean serum LDL level of 86.87±3.51mg/dl during the spring season followed by winter (67.93±2.31mg/dl), summer (62.54±4.17mg/dl) and autumn (57.58±3.15mg/dl) season respectively. Among the age groups, female 2+ year's group has the highest mean serum LDL level of 83.76±3.68gm/dl followed by male 2+ (72.42±4.61gm/dl), female 1+ (64.02±4.18gm/dl) and male 1+ (53.69±3.94gm/dl) -year-old groups. Seasonal differences might relate to the changes in the serum LDL level of the fish and such changes might differ between age groups and sexes. The assumption on the homogeneity of variances was tested and not satisfy based on Levene's F-test, F (19, 78) = 1.877, p<0.05. A two-way analysis of variance tested the serum LDL of the fishes according to change in the different seasonal condition during summer, autumn, winter and spring among male and female of 1+ and 2+ -years-old groups and observed that age has a very significant effect on the serum LDL concentration among different group of the species, F (4, 98) = 293.1, p<0.05, η^2 = 0.938. Seasonal changes also showed significant effect in serum LDL concentration of the species, F (3, 98) = 353.252, p<0.05, η^2 = 0.931. The interaction between the different age groups and seasonal changes was also highly significant for Amur common carp, F (12, 98) = 88.715, p<0.05, η^2 = 0.932. Since age and season has a significant effect on the serum LDL concentration of the species, a Tukey's HSD *post-hoc* test was conducted to compare differences between the means. It was found that the mean serum LDL level was statistically significant between means of all seasons. For the age groups, the mean serum LDL level between male 2+ (72.42±4.61gm/dl) and 0+ (66.58±0.71gm/dl) were not statistically significant while the means between the remaining age groups were statistically significant (p<0.05).

Low-density lipoprotein was not detected from muscle tissue in both the age groups while hepatic LDL level was detected in autumn and winter season in male and in winter season in female of 1+ year's age group specimen. A descriptive statistics observed only in the 1+-year-old group for autumn and winter season showed mean hepatic LDL level of 1.49±0.29mg/dl and 0.32±0.5mg/dl respectively. Among the age groups, the female has higher mean hepatic LDL of 1.27±0.35mg/dl than a male with 1.09±0.11mg/dl. The mean seasonal hepatic LDL level is shown in Table. Seasonal differences might relate to the changes in the hepatic LDL level of the fish and such changes might differ between sexes. The assumption on the homogeneity of variances was tested and satisfy based on Levene's F test, F (2, 6) = 3.558, p = 0.096. A two-way analysis of variance tested the hepatic LDL of the fishes between autumn and winter among male 1+ old male and female. Age has a non-significant effect on the hepatic LDL concentration among different group of the species, F (1, 9) = 0.838, p = 0.385, η^2 = 0.123. Seasonal changes also showed non-significant effect in hepatic LDL concentration of the species, F (1, 9) = 4.659, p = 0.074, η^2 = 0.437.

A descriptive statistics observed across all age groups (except for 1+-year-old male during autumn) showed maximum mean gonadal LDL level of 3.35±0.47mg/dl during the summer season followed by spring (1.89±0.31mg/dl), winter (1.1±0.19mg/dl) and autumn (0.91±0.11mg/dl) season respectively. Among the age groups, female 2+ year's group has the highest mean gonadal LDL level of 3.04±0.45gm/dl followed by male 2+ (2.53±0.33gm/dl), female 1+ (1.03±0.17gm/dl) and male 1+ (0.65±0.15gm/dl) -year-old groups. The mean seasonal gonadal LDL level of individual age and sex groups of Amur common carp groups is shown in Table. Seasonal differences might relate to the changes in the gonadal LDL level of the fish and such changes might differ between age groups and sexes. The assumption on the homogeneity of variances was tested and satisfy based on Levene's F test, F (14, 54) = 1.055, p = 0.417. A two-way analysis of variance tested the gonadal LDL of the fishes according to change in the different seasonal condition during summer, autumn, winter and spring among male and female of 1+ and 2+ -years-old groups. Age has a very significant effect on the gonadal LDL concentration among different group of the species, F (3, 69) = 224.371, p<0.05, η^2 = 0.926. Seasonal changes also showed significant effect in gonadal LDL concentration of the species, F (3, 69) = 257.825, p<0.05, η^2 = 0.935. The interaction between the different age groups and seasonal changes was also highly significant for Amur common carp, F (8, 80) = 24.55, p<0.05, η^2 = 0.784. Since age and season has a significant effect on the gonadal LDL concentration of the species, a Tukey's HSD *post-hoc* test was conducted to compare differences between the means. It was found that the mean gonadal LDL level was not statistically significant between winter (1.1±0.19mg/dl) and autumn (0.91±0.11mg/dl) season while the means between the remaining seasons were statistically significant. For the age groups, the mean gonadal LDL was statistically significant (p<0.05).

Low-Density Lipoprotein (LDL) carries the lipid secreted in the liver to extra-hepatic tissue for cholesterol synthesis. Circannual variations in the LDL level in both the sexes could be controlled by an endogenous biological clock which may be coupled with the external rhythm and which enables sexual maturation and the moment of ovulation (Wallaert & Babin, 1994) [21]. In the present study, seasonally different LDL levels were observed the males and females of both the 1+ and 2+ years' old age groups. The serum LDL concentrations from the present study were similar with the findings by Azeez & Mohammed, (2017) [1] in *Cyprinus carpio* with relatively high LDL values from both cultured and wild groups. The gonadal LDL followed a similar trend with higher and significant level during the peak breeding season (spring and summer season). This was also evident from the positive correlation with GSI associated with gonadal CHO, PHO and HDL respectively. Variation in LDL concentrations could be related to feeding, environmental conditions and sexual status of the fish (Karatas *et al.*, 2014) [10].

Positive correlation of gonadal and 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. Higher LDL level in female comparable with higher level of GSI in female as compared to male is correlated with higher energy/lipids constituent requirements in ovarian development. Seasonal variations in LDL concentrations related to age and sexes had earlier been reported in *Salmo gaidneri* by Black and Skinner (1987) [3]. The variations in serum LDL concentration observed in the present study were similar with the findings of Azeez and Mohammed (2017) [1] in *Cyprinus carpio* with relatively high seasonal variations corresponding to gonadal maturation. Sheridan (1988) reported that variations in inter-conversion of LDL-cholesterol from liver toward gonadal development are not well defined in *Salmo gairdneri*. 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) [21]. Other factor including feeding, environmental conditions and sexual status could also play a cascading effect on the variations in LDL concentrations in the fish (Karatas *et al.*, 2014) [10].

The study have showed that major lipoprotein like LDL plays an important role in gonadal maturity and reproductive process of common carp and proper understanding of this lipid class could pave way towards dietary supplement and nutritional well-being of brood stock management and breeding programme.

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