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Seasonal change in very low density lipoproteins (VLDL) profile with relation to amur common carp reproductive physiology

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Abstract
Very-low-density lipoprotein (VLDL) is one of the major groups of lipoproteins made by the liver from triglycerides, cholesterol and apolipoproteins that enable them to move within the water-based solution of the bloodstream and help deliver endogenously synthesized triacylglycerols to adipose tissue thus converting the residue to low-density lipoprotein (LDL) and vice-versa for *de novo* cholesterol synthesis at peripheral tissues. The study was carried out to assess the seasonal profile of VLDL in amur common carp during four seasons (spring, summer, autumn and winter) among different age groups and sexes. The assumption on the homogeneity of variances was tested for serum, muscle, gonadal and hepatic VLDL and found not satisfy based on Levene's *F* test. ANOVA (2-way) for serum, muscle, gonadal and hepatic VLDL level were analysed for different age groups and sexes and observed significant effect among different age and seasons. Similar observations can also be seen between age and seasonal interactions. Similar observation could be seen between all seasons and target samples while conducting Tukey's HSD *post-hoc* test for checking the means differences. The above findings revealed age and seasons have profound effect on the general well-being and reproductive physiology of amur common carp as indicated by the gonadal cycle and changes which undertakes the importance of this lipoprotein in fish gross welfare regime.

Keywords: Amur common carp, very low-density lipoprotein (VLDL), seasonal reproduction

Introduction
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 (Mourente *et al.*, 2002; Huynh *et al.*, 2017) [12, 8]. Beamish *et al.*, (1996) [2] proposed about the assessment of reproductive age in females using body size and accumulation of lipids that provided the energy for reproduction. Biochemical profiles changed in relation to sex and season in *Barilius bendelisis* were reported by Sharma *et al*., (2018) [14] and in common carp by Soranganba and Singh (2018) [14]. Petenuci *et al.*, (2016) [13] showed significant variation in different class lipid based on the season with decreased of saturated fatty acid contents during flooded periods whereas significant increase in polyunsaturated fatty acid contents during the same period in from the Amazon Basin. 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.*, 1996) [11], Atlantic bonito *Sarda sarda* (Zohar *et al.*, 2010) [22], carangids, *Scomberoides lysan* (Sutharshiny and Shivashanthini, 2011) [18] and Nile tilapia *Oreochromis niloticus* (Singh *et al.*, 2012) [15]. 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. Karataş *et al.*, (2014) [9] observed the differences in the serum VLDL 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 (Ebrahimnezhadarabi *et al.*, 2011) [5].

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) [6] and Cequier-Sánchez *et al.*, (2008) [4] method. Mixed the tissue with 10 ml (20 times the tissue volume) of 2:1 ratio dichloromethane and methanol solution and agitated the homogenate for 20 mins using modified digital rocker. Centrifuged the homogenate at 2000 rpm for 10 mins and collected the liquid phase in centrifuged tubes. 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. After vortex, centrifuged the mixture at low speed of 2000 rpm and separated the two phases. 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) [7], VLDL is calculated as follows:

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

Statistical Analysis

ANOVA two factors and Correlation analysis - Pearson's correlations using SPSS.

Result 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) [16-17]. A descriptive statistic for muscle VLDL observed across all age groups showed maximum level of 11.61±0.32mg/dl during autumn season followed by winter (7.99±0.49mg/dl), summer (5.88±0.15mg/dl) and spring (4.62±0.17mg/dl) season respectively. Among the age groups, male 2+ year's group has the highest level of 9.01±0.74mg/dl followed by female 2+ (8.28±0.61mg/dl), male 1+ (6.67±0.65mg/dl) and female 1+ (6.135±0.49mg/dl) -year-old groups. The mean seasonal muscle VLDL level of individual age and sex groups of Amur common carp groups is shown in Table and diagrammatically represented in Figure.

Table 1: Seasonal VLDL (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	6.28±0.04	6.70±0.02	13.55±0.04	11.83±0.09	10.59±0.09	9.64±0.04	5.63±0.04	4.96±0.09
	Gonadal	4.39±0.02	4.97±0.03	3.99±0.03	4.48±0.04	6.94±0.08	8.45±0.05	14.74±0.04	16.64±0.06
	Hepatic	5.54±0.06	7.42±0.04	16.12±0.05	19.63±0.05	13.61±0.06	14.86±0.03	10.49±0.05	11.21±0.05
	Serum	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
1+	Muscle	5.01±0.05	5.53±0.71	11.42±0.07	9.62±0.09	6.11±0.03	5.62±0.04	4.13±0.03	3.74±0.09
	Gonadal	5.14±0.03	4.94±0.01	4.50±0.01	3.80±0.03	5.65±0.04	6.04±0.02	10.49±0.05	9.57±0.04
	Hepatic	3.27±0.04	4.30±0.03	6.06±0.03	7.09±0.04	5.98±0.03	6.14±0.03	4.41±0.05	5.28±0.03
	Serum	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

[Data are given as mean±SEM (n=5)]

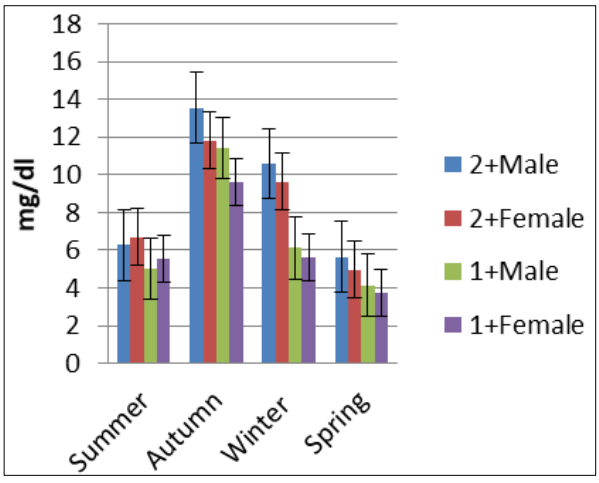

Fig 1: Muscle VLDL

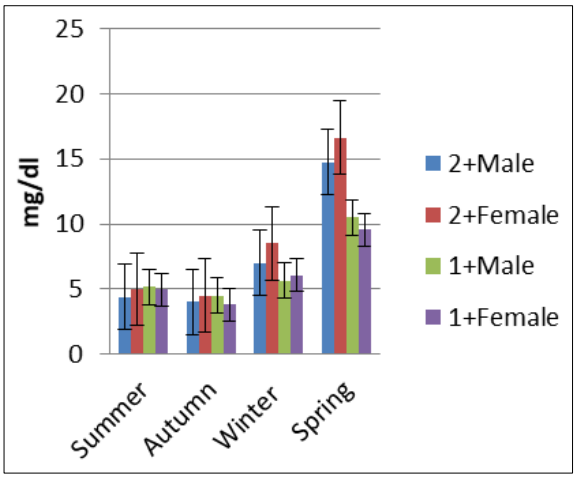

Fig 2: Gonadal VLDL

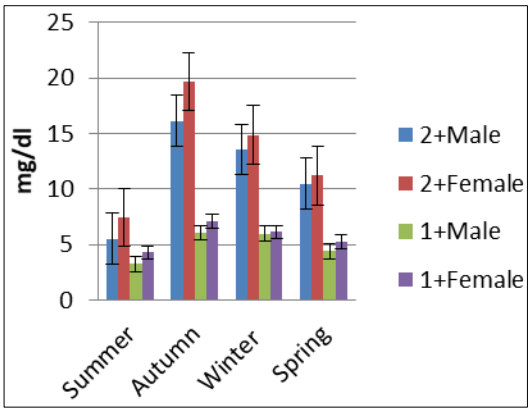

Fig 3: Hepatic VLDL

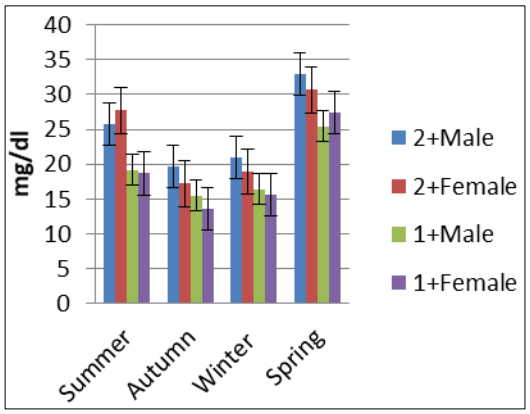

Fig 4: Serum VLDL

Seasonal differences might relate to the changes in the muscle VLDL level which 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 (15, 64) = 1.59, $p<0.05$. ANOVA (2-way) showed age and seasonal changes having very significant effect on the muscle VLDL concentration among different group of the species, F (3, 80) = 1736.482, $p<0.05$, $\eta^2 = 0.988$ and F (3, 80) = 8879.845, $p<0.05$, $\eta^2 = 0.988$ respectively. The interaction between the different age groups and seasonal changes was also highly significant, F (9, 80) = 222.380, $p<0.05$, $\eta^2 = 0.969$. Since age and season has a significant effect on the muscle VLDL concentration of the species, a Tukey's HSD *post-hoc* test was conducted to compare differences between the means and found statistically significant for different age groups and sexes across all seasons ($p<0.05$).

A descriptive gonadal VLDL statistics observed across all age groups showed maximum mean level of 12.86±0.67mg/dl during the spring followed by winter (6.8±0.25mg/dl), summer (4.86±0.06mg/dl) and autumn (4.19±0.07mg/dl) season respectively. Among the age groups, female 2+ year's group has the highest mean of 8.65±1.12mg/dl followed by male 2+ (7.53±1.0mg/dl), male 1+ (6.45±0.55mg/dl) and female 1+ (6.09±0.49mg/dl) -year-old groups. Details showed in Table and diagrammatically represented in Figure. Seasonal differences might relate to the changes in the gonadal VLDL level with such changes differing between age groups and sexes. The assumption on the homogeneity of variances was tested and not satisfy based on Levene's *F* test, F (15, 64) = 2.893, $p<0.05$. ANOVA (two-way) showed age and seasonal changes having a very significant effect among different group of the species, F (3, 80) = 2866.558, $p<0.05$, $\eta^2 = 0.993$ and F (3, 80) = 33473.397, $p<0.05$, $\eta^2 = 0.999$ respectively. The interaction between the different age groups and seasonal changes was also highly significant, F (9, 80) = 1415.906, $p<0.05$, $\eta^2 = 0.995$. Since age and season has a significant effect on the gonadal VLDL level, a Tukey's HSD *post-hoc* test was conducted to compare differences between the means and found that the mean gonadal VLDL level was statistically significant for different age groups and sexes across all seasons ($p<0.05$). There was a highly significant positive correlation between gonadal VLDL with GSI (r = 0.761).

A descriptive hepatic VLDL statistics observed across all age groups showed maximum mean level of 12.23±1.33mg/dl during the autumn followed by winter (10.15±0.94mg/dl), spring (7.85±0.69mg/dl) and summer (5.14±0.35mg/dl) season with female 2+ year's group having highest mean hepatic VLDL level of 13.28±1.03mg/dl followed by male 2+ (11.44±0.91mg/dl), female 1+ (5.71±0.24mg/dl) and male 1+ (4.94±0.27mg/dl) -year-old groups respectively. Details showed in Table and diagrammatically represented in Figure. Seasonal differences might relate to the changes in the hepatic VLDL level 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 (15, 64) = 0.928, p = 0.539. ANOVA (two-way) showed age and seasonal changes having very significant effect among different group of the species, F (3, 80) = 30422.251, $p<0.05$, $\eta^2 = 0.999$ and F (3, 80) = 16450.059, $p<0.05$, $\eta^2 = 0.999$ respectively. The interaction between the different age groups and seasonal changes was also highly significant for Amur common carp, F (9, 80) = 2056.057, $p<0.05$, $\eta^2 = 0.997$ with Tukey's HSD *post-hoc* test showing statistically significance for different age groups and sexes across all seasons ($p<0.05$).

A descriptive statistics observed across all age groups showed maximum mean serum VLDL level of 25.55±1.55mg/dl during the spring season followed by summer (18.65±1.74mg/dl), winter (16.07±3.21mg/dl) and autumn (14.65±0.86mg/dl) season respectively. Among the age groups, male 2+ year's group has the highest mean serum VLDL level of 24.92±1.27mg/dl followed by female 2+ (23.41±1.36mg/dl), male 1+ The assumption on the homogeneity of variances was tested and not satisfy based on Levene's *F* test, (19.12±0.89mg/dl), female 1+ (18.8±1.21mg/dl) and 0+ (7.96±0.55mg/dl) -year-old groups. The mean seasonal serum VLDL level of individual age and sex groups is shown in Table and diagrammatically represented in Figures. Seasonal differences might relate to the changes in the serum VLDL level and such changes might differ between age and sexes. F (19, 78) = 1.779, $p<0.05$. ANOVA (2-way) observed age and seasonal change have very significant effect among different group of the species, F (4, 98) = 4541.223, $p<0.05$, $\eta^2 = 0.996$ and F (3, 98) = 3039.501, $p<0.05$, $\eta^2 = 0.992$ respectively. The interaction between the different age groups and seasonal changes was found to be highly significant, F (12, 98) = 168.551, $p< 0.05$, $\eta^2 = 0.963$. Since age and season has a significant effect on the serum VLDL, a Tukey's HSD *post-hoc* test was conducted to compare differences between the means and found significant means between all seasons.

VLDL is assembled in the liver from mostly triglycerides, cholesterol and as the TG content decreases by uptake into cells it is converted into LDL in the bloodstream. It transports

these endogenous lipids and functions as the body's internal transport mechanism for lipids (Karatas *et al.*, 2014) [9]. LDL and HDL, which contain mostly cholesterol, can be taken up by the liver and re-assembled into VLDL. Since the VLDL in the present investigation were derivatives of triglyceride (TG) using the formula of Friedewald *et al.*, (1972) [7], a similar trend in the VLDL level i.e. one-fifth of TG levels were conclusive. Positive correlation between VLDL levels in gonadal and serum samples with changes in GSI levels and negative correlation with values in muscle and hepatic tissue seems to be indicative of the transport of nutrients from liver and peripheral muscle tissues 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. Serum VLDL level was high during the spring and summer coinciding with a reproductive period, temperature and suitable feeding period. Similar observations about the seasonal change in VLDL were reported in chub (*Leuciscus cephalus)* by Aras *et al.*, (2018) [1]. During feeding, excess dietary fatty acids are exported from the liver in the form of lipoproteins (VLDL) and are accumulated and stored in the form of TG in specific lipid storage sites (Tocher, 2013). Male have higher VLDL level in both the 1+ and 2+ -years-old group while 0+-year-old groups have the least. The lower level of VLDL in the female might be due to the excessive utilization of VLDL during the process of vitellogenesis as VLDL and vitellogenin are the two major components for oocytes formation. Oil droplets that will contribute to the lipid content of the oocytes are probably derived from VLDL transported to the ovaries in the bloodstream and taken up by the follicular complex during primary growth phase. The seasonal variations in plasma VLDL level has been reported in male and female trout (Wallaert & Babin, 1994) [20] and Amazonian pirarucu (Bezerra, 2013) [3]. The relative amount of VLDL varies with age, nutrition and sexual cycle (Tocher, 2013). In the present study, muscle VLDL does not follow with increased in the seasonal temperature and reproductive period with a maximum level during autumn followed by winter season. Age-wise, male were on the higher side than the females. After active feeding during warm temperature, much of the TG surge was evident and simultaneously the VLDL level. Gonadal VLDL follows the irregular pattern of maximum level during spring followed by winter and summer. Among the groups, female 2+ has the highest VLDL level followed by 2+ and 1+-year-old male. With significant differences between the seasons across all age groups, a highly positive correlation with GSI was observed indicating their role in gonadal maturity especially in females. The hepatic VLDL shows a similar pattern with muscle VLDL in seasonal and age groups with significantly different observations. Fatty acids involved in hepatic VLDL production may originate from different sources including intracellular TG stores, plasma FFA, *de novo* lipogenesis and incoming lipoproteins. (Kusnetsov *et al*, 2011) [10].

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