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Seasonal Plasma Cortisol Level of Amur Common Carp among different Age Groups in Tarai Region of Uttarakhand

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ABSTRACT: Environmental and culture induced stressor like seasonal change in water parameters and handling of brooder are the major precursor for increased in cortisol level leading to detrimental effect on well-being especially growth and reproduction. The study was carried out to assess the cortisol levels of amur common carp in four seasons (spring, summer, autumn and winter)among three different age groups (0+, 1+ and 2+ years) using the RP-HPLC method with relation to its reproductive performance. The assumption on homogeneity of variances was tested and not satisfied based on Levene's *F* test, $F(19, 78) = 4.761$, $p < 0.05$. Age has a very significant effect on the plasma cortisol concentration among different group of the species, $F(4, 98) = 2846.238$, $p < 0.05$, $\eta^2 = 0.993$. Seasonal changes also showed significant effect in plasma cortisol concentration of the species, $F(3, 98) = 5157.560$, $p < 0.05$, $\eta^2 = 0.995$. The interaction between the different age groups and seasonal changes was also highly significant for Amur common carp, $F(12, 98) = 441.040$, $p < 0.05$, $\eta^2 = 0.985$. Since age and season has significant effect on the plasma cortisol concentration of the species, a Tukey's HSD post-hoc test found statistically significance between the means of all seasons. Between age groups, the means between male 1+ (37.87±8.46ng/ml) and female 1+ (37.65±2.51ng/ml) were not significant while the means between the remaining age groups were statistically significant ($p<0.05$). The plasma cortisol level was significantly correlated with gonado-somatic index ($r = 0.751$, $p < 0.01$) while non-correlation with hepato-somatic index ($r = -0.554$, $p < 0.01$). Plasma cortisol in all the age groups showed highest concentration in concomitant with breeding seasons. Highest level of cortisol was observed during spring season followed by summer, autumn and winter season in which male with older age group (2+ year old) dominated the plasma cortisol level followed by female 2+, male 1+, female 1+ and 0+ year old respectively. The above findings reveal that stress hormone - cortisol is instrumental in reproductive process however care must be taken not to augment secondary stressor from fish husbandry and handling processes.

Keywords: Amur common carp, cortisol, seasonal reproduction, Tarai region.

INTRODUCTION

Cortisol in fish body plays a governing role to maintained normal physiological functions and elevation of this hormone affects its bodily functions which lead to effects on traits that are imperative to aquacultures such as body growth, resistance to diseaseand reproduction. Thus, the impact of cortisol on growth may further affect the reproductive capability as fish reproduction is directly related to its size (Ellis *et al.,* 2012), gestation period (Kim *et al.,* 2018) habitat and seasons (Carbajal *et al.,* 2019). The inter-renal tissue embedded inside the anterior part of the kidney in known as the site for synthesized of corticosteroid in teleost fish. Milla *et al.* (2009) reported major corticosteroids *viz.*, cortisol, cortisone, 11-deoxycortisol and corticoster one that were isolated form the fish blood which are different based upon their the type of species, sex of the individual and state of reproduction. Many authors reported the variations in the levels of plasma corticosteroid throughout the reproductive cycle, although it remains as a basic indicator in reproductive behaviour of fish. Elevated level of plasma cortisol levels were observed during pre-spawning and spawning period in both male and female among some species (Wingfield and Grimm 1977; Pickering and Christie 1981; Kusakabe *et al.,* 2003; Noaksson *et al.,* 2005; Westring *et al.,* 2008) with higher concentrations in adult as compared to immature and young ones

indicating the importance of this hormone in reproduction (Milla *et al.,* 2009). In common carp, cortisol levels increased with increased in water temperature and it's found to be higher during the spawning season (Saha *et al.,* 2002). The role of cortisol level in male rainbow trout, with relation to its reproduction other than stresses have also been reported by Ya-Yi *et al.* (2001). Elevated level of serum cortisol level in the freshwater fish feather back (*Notopterus notopterus*) (Shankar and Kulkarni 2014) and in stinging catfish (Mishra and Chaube 2022) during breeding season for ovarian growth and vitellogenesis. The inhibitory effects of stress/cortisol on the secretion of plasma sex steroid have been earlier reported in fishes by Foo and Lam (1993).

MATERIALS AND METHODS

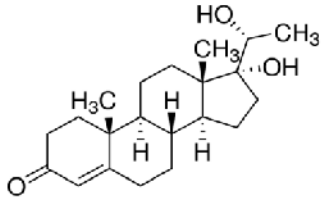
Maintenance of Fish. Amur common carp (*Cyprinus carpio haematopterus)* of three different age groups *i.e.* 0+, 1+ and 2+ year'swere colleceted from farm facility of the College of Fisheries, G. B. Pant University of Agriculture & Technology, Pantnagar (Uttarakhand) which were further maintained in earthen ponds for the study. Regular feeding @ 3% body weight/day in early morning with containing 25% protein supplementary pellet floating feeds.

Site and Agro-Climatic Conditions. The fish culturing earthen pond is located at Instruction fish farm inside the farm facility of College of Fisheries, GBPUAT, Pantnagar at the Lat. 29.01°N, Long. 79.3°E, 344 MSL at Tarai region of the Himalayas at the Shivalik range. It has a sub-tropical type of climatic.

Sampling Protocol. Samples were collected only from separate pond A and B for three age groups *i.e* 0+, 1+ and 2+ during four seasons *viz.,* summer, autumn, winter and spring. Clove oil (Velisek *et al.,* 2005) was used to anesthetize @ 30 mg/l prior regular handling the specimens for collection of blood for biochemical analysis. Samples were collected and handled normally within 5 to 10 mins. The blood drawn was collected into a heparin (lithium) coated normal serum tubes for hormone. Theheparinizedsample was centrifuged at 10,000rpm (11180x g) for about 12 mins at temperature 4°C and the supernatant was further collected in 2ml microcentrifuge tubes and analyzed or stored at below -20°C sealed with parafilm.

Water Quality Observations. Some important water quality parameters viz. water temperature, total dissolved solids (TDS), pH, dissolved oxygen (DO) and free carbon dioxide (CO_2) of the two earthened ponds were regularly monitored during sampling periods/seasons. Digital meter were used for water temperature, TDS (accuracy±2%) and pH (accuracy 0.01 pH) while titrimetric method (APHA, 1992) was used for dissolved oxygen (DO) and Carbon dioxide (CO_2).

Pure hormone samples. Pure hormones were purchased from Sigma India for standardization of the experimental protocol.



Hydrocortisone ≥98.0% (HPLC) H4001CAS no. 50-23-7

Molecular formula : $C_{21}H_{30}O_5$
Mol. Wt. : 362.46gm/mol
Melting Point. : 220°C
Colour Appearance : White to off-white
Form of Appearance : Powder
Solubility (Colour) : Colourless to faint yellow colour
Solubility (Turbidity) : Clear
Synonyms: Cortisol 11β, 17α, 21-Trihydroxypregn-4-ene-3, 20-dione, 17-Hydroxycorticosterone, 4-Pregnene-11β,17α, 21 – triol-3,20-dione, Kendall's compound F, Reichstein's substance M

Hormone Estimation

Reversed phased high-performance liquid chromatography (RP-HPLC) of Dionex Ultimate 3000, using by Chromele on software (version 6.8) was used to estimate cortisol hormone in blood samples under chromatographic condition that includes validation, quantitation, linearity of the assay, accuracy, stability, repeatability and precision (Soranganba and Singh 2018). SPE (Solid Phase Extraction) LiChrolut RP-18 (40-63 µm) 100 mg 1 ml standard PP-tubes [119855] was used as pre-treatment of each aliquot (Budzinski *et al.,* 2006; Chen-HaoZhai *et al.,* 2009) with slight modifications.

Statistical Study. Statistically analyzed of the observed data were tested using analysis of variance (ANOVA) and significance level at $p< 0.05$ were considered for differences. All the observed data were expressed in mean±SEM.

RESULT

A descriptive statistics of plasma cortisol observed across all age groups showed maximum mean plasma cortisol level of 54.17±3.8ng/ml during the spring season followed by summer (36.4±1.89ng/ml), autumn (32.79±2.49ng/ml) and winter (20.88±1.43ng/ml) season respectively. Among the age groups, male 2+ year's group has the highest mean plasma cortisol level of 45.55±3.83ng/dl followed by female 2+ (43.49±4.44ng/ml), male 1+ (37.87±8.46ng/ml), female 1+ (37.65±2.51ng/ml) and 0+ (16.54±1.21ng/ml) years old fishes. The mean seasonal plasma cortisol level of individual age and sex groups of Amur common carp groups is shown in Table1and diagrammatically represented in Fig. 1.

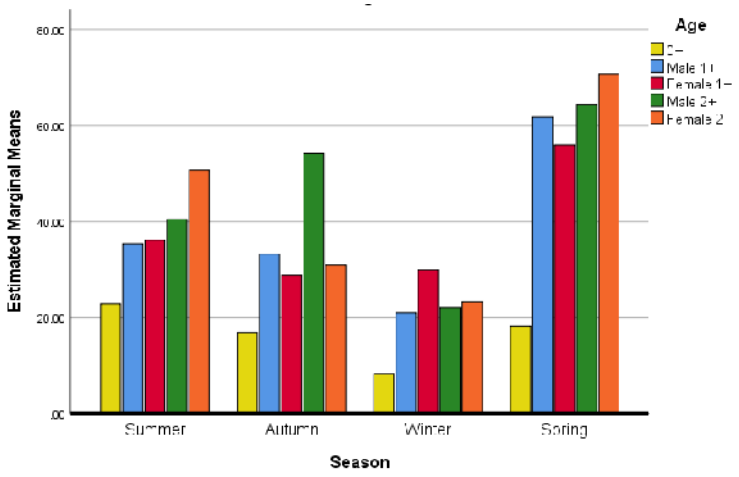


Fig. 1. Seasonal changes in plasma cortisol level of different age and sex groups of Amur common carp.

Seasonal differences might relate to the changes in the plasma cortisol level of the fish and such changes might differ between age groups and sexes. The assumption on homogeneity of variances was tested and not satisfied based on Levene's *F* test, F (19, 78) = 4.761, p< 0.05. ANOVA-two way tested the plasma cortisol of the fishes according to change in the different seasonal condition during summer, autumn, winter and spring among 0+ year old and male and female of 1+ and 2+ years old groups. Age has a very significant effect on the plasma cortisol concentration among different group of the species, F (4, 98) = 2846.238, p <0.05, η^2 = 0.993. Seasonal changes also showed significant effect in plasma cortisol concentration of the species, F (3, 98) = 5157.560, p <0.05, η^2 = 0.995. The interaction between the different age groups and seasonal changes was also highly significant for Amur common carp, F (12, 98) = 441.040, p <0.05, η^2 = 0.985. Since age and season has significant effect on the plasma cortisol concentration of the species, a post-hoc test (Tukey's HSD) was conducted for comparison of differences between the means. It was found that the mean plasma cortisol level were statistically significant between the means of all seasons. For age groups, the means between male 1+ (37.87±8.46ng/ml) and female 1+ (37.65±2.51ng/ml) were not significant while the means between the remaining age groups were statistically significant (p<0.05). Highly significant positive correlation was observed between plasma cortisol with gonadal T (r = 0.712, p<0.01) and GSI (r = 0.751, p< 0.01) while no correlation with HSI (r =-0.554, p<0.01).

Increased in metabolic hormone like cortisol during breeding season indicates the probable diversion of body energy for reproductive activities (Shankar and Kulkarni 2014). This steroidogenic hormone is produced from inter-renal gland in fishes and also reported as an important metabolic hormone with changes in seasonal activities corresponding to reproductive behaviour of the fish (Mishra and Chaube 2022). Significantly different seasonal plasma cortisol levels were observed with highest concentration during spring followed by summer, autumn and winter season respectively. Seasonal change in plasma cortisol has been reported in *Notopterus notopterus* (Shankar and Kulkarni 2014) and live bearer **(**Kim *et al.*, 2018). Many authors have reported the presence of this hormone in ovary, sperm and seminal fluid of fishes supporting gonadal cortico-steroidogenesis (Scott *et al.*, 2010). The levels of plasma cortisol differs greatly throughout the reproductive cycle in both male and female during the pre-and/or spawning period (Pickering and Christie 1981; Kusakabe *et al.*, 2003; Noaksson *et al.*, 2005; Westring *et al.*, 2008; Carbajal *et al.*, 2019) and have been reported in *Salmotrutta*, goldfish *Carassius auratus* and common carp *Cyprinus carpio* (Pickering and Christie 1981). Male in both the age groups have higher cortisol level with increased in age with 0+ year old having the least cortisol value. Since cortisol is a metabolic hormone and also regulates reproductive activities, higher cortisol level as compared to the younger ones has been justified. The level of cortisol elevates earlier in males than in females salmon but the magnitude was higher in females (Pickering and Christie 1981**;** O'Conno *et al.*, 2010). The difference in plasma cortisol level in opposite sex might be due to its species specificity (Milla *et al*., 2009). There was a positive correlation between plasma cortisol with GSI while negative correlation between HSI. Cortisol probably disrupt GnRH and function of gonadotropin actions while sex-steroid have reciprocal effect by regulating corticosteroid productions and probably plays a role in modulation of the reproductive endocrine control (Milla *et al*., 2009). Thus, it have some implications in the plasma and gonadal sex-steroid level in Amur common carp.

CONCLUSION

Plasma cortisol studied in all the age groups has highest level concomitant with other sex hormone and breeding seasons. Highest level was observed during spring

season followed by summer, autumn and winter season. Male 2+ year old dominated the plasma cortisol level followed by female 2+, male 1+, female 1+ and 0+ year old groups respectively. Cortisol being a stress hormone, it could vary according to stress induced while handling and also the active mating environment during peak season. It is observed that cortisol manifest the physiological activity having profound effect in the reproductive process of the fish besides secondary stressor.

FUTURE SCOPE

The study will help to ascertain the brood handling and husbandry practice for seed production industry and help reduce secondary induced stress for successful aquaculture.

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Conflict of Interest. None.

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