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RESEARCH ARTICLE

A COMPARATIVE STUDY OF PROTEIN IN MUSCLES AND REPRODUCTIVE PHASES OF GONDAS OF CHANNA GACHUA (HAM)


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ABSTRACT

The comparative study of protein has been estimated from the muscles and gonads of Channa gachua. The changes of protein level in muscles appear to be accordance with the specific needs of different reproductive phases of the gonads.

INTRODUCTION

The phenomenon of reproduction is a complex process directed to growth, survive and continuity of the species. Development of gonads requires a specific quantum of protein and other biochemical content. During the reproductive season fish draw upon these content from the muscles, for the growth and development of the gonads.

Lots of works is on records on the biochemical composition of many teleostean fishes (Gerking, 1995; Brett et al; 1969; Niimi, 1972; Elliott, 1976; Eliaussen and Vahl, 1982; Luzzana, et al; 1996) but very few (Bailey et al; 1952; Keller and Britness, 1958; and Craig, 1977) have related it with reproductive phases. An attempt has been made to compare and correlate the variation of protein to the reproductive phases of gonads.

MATERIAL AND METHODS

Sexually mature fishes Channa gachua were collected regularly during the breeding season (March to September) of year 2004 from the river Kham near Aurangabad. (North longitude 190-200; East longitude 740-760). Gonads and muscles are processed for biochemical estimation of protein (Lowry et al; 1951) content.

RESULTS

Persual of Table 1 and Fig. 1 reveals that protein content of muscles observe constant values from preparatory (5.66) to early pre-spawning phase (5.38).

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At the same time gonads showed gradual increasing values from preparatory (testis 5.06, 12.00 ovary 6.49, 12.23) to pre-spawning (testis 23.68, 32.68 and ovary 24.06, 32.37). This decline of protein in muscles may probably be due to its active utilization by the gonads for the development.

During spawning phase the muscles protein has been observed to rise in their percentual values rising maximum (8.90). This shoot up in muscles protein may be the result of favourable feeding condition during this phase. (Silvertstein,1935). Gonads reached peak values (testis 38.86 and ovary 39.87) of their protein content may require for the spawning.

Muscles protein showed gradual increasing from August to September (10.02 and 10.82) and gonads showed decline in protein content. Decline in gonadal protein con is attributed to its transfer in to muscles to meet energy requirement of fish (testis 21.19., 15.43 and ovary 22.39., 1613).

DISSCUSSION

Variations in the protein contents in gonads during reproductive phases of fish, Channa gachua viz, preparatory, pre-spawning, spawning and post spawning phase took place in involving biochemical event. Protein has a significant role in growth and all metabolic process. According Wallace and Selman, (1985) in Fundulus heteroclitus, the major protein changed both qualitatively and quantitatively during development of gonads. It has been observed that in C. punctatus, H. fossilis and T. mossambica, (Verma et al; 1985, 1989), S. baccata (Fisca
Table 1. Comparative study of protein in gonads and muscles of *Channa gachua*

<table>
<thead>
<tr>
<th>REPRODUCTIVE PHASE</th>
<th>MONTHS</th>
<th>PROTEIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TESTIS</td>
</tr>
<tr>
<td>Preparatory Phase</td>
<td>May</td>
<td>5.96</td>
</tr>
<tr>
<td></td>
<td>April</td>
<td>12.00</td>
</tr>
<tr>
<td>Pre-spawning Phase</td>
<td>May</td>
<td>23.68</td>
</tr>
<tr>
<td></td>
<td>June</td>
<td>32.68</td>
</tr>
<tr>
<td>Spawning Phase</td>
<td>July</td>
<td>38.86</td>
</tr>
<tr>
<td>Post-spawning Phase</td>
<td>August</td>
<td>21.19</td>
</tr>
<tr>
<td></td>
<td>September</td>
<td>15.43</td>
</tr>
</tbody>
</table>

Fig. 1. Variation in protein, in gonads and muscles of *Channa gachua*

and Ravindra, 1999), *Schizothorax richardsoni* and *Glyptothorax pectinopterus* (Singh and Nautiyal, 1990), *C. orientalis* (Sharma and Saxena, 1991), and *Clarias batrachus* (Saksona and Agarwal, 1991) the level of protein was highest and lowest during spawning and preparatory phase respectively. In *Channa gachua* the high content of protein may be active utilization of gonads from muscles (Gupta et al: 1997) support for their may drawn from the observation of Love, (1970), who stated that the building of gonads is always accomplished at the expense of body protein. Dambergs (1964) who observed a decline in muscles protein of *Gadus morhua* during growth phase. John and Hameed, (1995) who also observed a gradual decline in muscles protein in *Nemipterus japonicus* In spawning phase, the muscles protein also showed increased values of protein, a similar increase in muscles protein with of developmental of gonads. Similar result had reported by Macay and Tunision, (1936), Jafri, (1968). According to them highest protein in muscles and gonads might be of active feeding during this phase.

Gonadal protein stared decline gradual from August (post spawning phase) muscles protein shows increasing through this phase. Scheepkin, (1972) working on *Trachurus mediterranea Ponticus* found increased muscles protein in post spawning phase. A similar trend seen in present study, it was compared in between muscles protein and gonads protein of *Channa gachua* during different reproductive phases.

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Ovaprim induced effect on testis of *Channa gachua*

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**Keywords**
- Testicular reorganization
- Spermatogenesis
- Sertoli cells and Leydig cells
- *Channa gachua*

**Abstract**
The testis of *Channa gachua*, after administration of Ovaprin, showed changes in the morphology of testis and in the serum androgen level within period of 72 hrs. Morphological changes included spermatogonial proliferation, activation of Leydig's and Sertoli cells, organization of seminiferous lobules and formation of lobular lumen in testis. Leydig cells were enlarged, exhibited characteristics of steroid producing cells. Sertoli cells became elongated, showed signs of high cellular activities and remained in close contact with spermatogonia. The lobular organization was achieved much earlier than the progression of spermatogenesis.

1. **Introduction**

In vertebrates various components of testis forms well defined cellular organisation. The association of the cells and sequences of their appearance in the seminiferous tubules are highly organised. However, in the fishes, each spermatogenetic cycle, followed by a resting stage for the release of spermatozoa during the process of late spermatogenesis by the rearrangement of germ cell cysts and somatic cells especially Sertoli cells.

Induction of spermatogenesis and maturation has been observed by the administration of Ovaprin doses for different duration. In present investigation, an attempt has been made to study the organization of testis of germ cell and observations after induction of spermatogenesis in fresh water fish, *Channa gachua* under the influence of Ovaprin.

2. **Material and Methods**

Live species of *Channa gachua* were collected from Kham river, near Aurangabad (M.S) India. Fish were collected during period of March 2007 to September 2008. They were brought to Laboratory, weighted (35.6 to 298 gm and length 8.5 to 20.5 cm) and kept in freshwater aquarium. Ovaprin was administered intraperitoneally to fish at a dose 0.25ml/kg (Syndel laboratory, Canada). A single dose is normally sufficient to induce maturation (0.25ml/kg).

3. **Results**
The testis of *Channa gachua* consists of germinal tissue and intermingles with connective tissue (Fig. 1A). The germinal tissue was disposed into chord-like testicular lobules containing spermatogonia and few Sertoli cells. The interlobular connective tissue also contains interstitial cells and blood capillaries. The spermatogonia were rounded in shape with rounded nuclei and prominent nucleoli. The cell boundaries, nuclear boundaries, and darkly stained granules were distinctly visible (Fig. 1B). The Sertoli cells were found amongst spermatogonia were irregular shaped with well defined nuclei.

In present study, activation of sertoli cells and Leydig cells after 12 hrs, spermatogonial division after 24 hrs and lobular organization after 72 hrs were achieved by injecting a single dose of Ovaprin (Fig. 1D). After 72 hrs when the primary spermatogonia and secondary spermatogonia along with sertoli cell organize themselves in such a manner that a quite distinct lobular structure with the lumen was formed as compared to controlled (Fig. 1C). The interlobular connective tissue was reduced.

Spermatogonia have a sheet of cytoplasm around large rounded (Fig 2B), homogeneously dense nuclei compared to control (Fig 2A). Single sometimes double nucleoli with dense granules were observed. Sertoli cells were found surrounded the spermatogonia, whether the cysts were in cluster of the seminiferous lobules. They posses irregular nuclei and contain round lipid globules. Rounded but sometimes elongated Leydig cells were disposed...
singly or in groups at the periphery of the testicular cysts separated by basal lamina along with fibroblast cells and other connective tissues. The testicular organization remains the same but the spermatagonia were increased (Fig. 2D). Compare to control (Fig. 2C). Sertoli cells were elongated with an irregular nucleus containing more electron dense material towards its periphery. Sertoli cells were received invaginations of spermatogonial cytoplasm indicating very close physiological association between them. Leydig cells were further activated.

Plate No. 1 T.S.of Testis. A- Controlled (Preparatory Phase). Note the connective Tissue (CT). Note the less No. of primary spermatogonia (PSG), B- Injected (Preparatory Phase) Note the primary and secondary spermatogonia (PSG and SSG) abundant in No. and large in size, Dark stained indicated the large secretion of gonadotropin, C- Controlled (Pre-spawning Phase) Less No. of Secondary spermatogonia (SSG), D- Injected (Pre-spawning Phase) Secondary spermatogonia (SSG) and primary spermatocyte (PSC) more in No. Sertoli cell (SC) and Leydig cell (LC) becoming active with better cytoplasm.

Plate No. 2 T.S. of Testis. A- Controlled (Spawning Phase) Less No. of primary and secondary spermatocyte (PSC and SSC). Note signs of secretary activity, B- Injected (Spawning Phase) Elongated sertoli cell (SC). Note the association between secondary spermatocyte and sertoli cell. Leydig cell with better cytoplasm. C- Controlled (Post-spawning Phase) Note the No. of residual sperm. Leydig cell with less cytoplasm. D- Injected (Post-spawning Phase) Note the No. primary secondary spermatocyte and residual sperm.
Seventy two hours after injection, spermatogonia were produced. These cells have dense nuclei with heterogenous distribution of electron dense granules. The cellular bridges between these cells were also observed. Sertoli cells were much elongated. The lipid globules were usually present in the sertoli cells. Leydig cells at this stage were large and found in the groups and assume characteristics of steroids synthesizing cells.

The testicular lobules contain spermatogonia and sertoli cells. Early spermatogonia took part in the cysts formation. The sertoli cells were found surrounded the germ cells and located in clusters filled up the spaces in chord like lobules. With increased spermatogonial division, and number and size of testicular cysts were increased and groups of sertoli cells becomes localized in the centre. Further, increased in the size of cysts took places due to division of spermatogonia. As the result, central sertoli cells were pushed apart leaving a gap in between, in the shape of tubular lumen. The testicular lobules thus formed consist of cysts containing spermatogonia surrounded by sertoli cells with a lumen in the centre and Leydig cells were lying just outside the cysts.

4. Discussion

The testis in most teleosts consists of compact paired structures lying in the abdominal cavity and composed of mass elongated, branched tubular structure with thin fibrous walls which lack a permanent lining, seminiferous epithelium and because of this reason, they are generally referred to as lobules, crystal or canals (Lofts,1968). On the basis of distribution of spermatogonia and spermatogenic pattern, two kinds of testicular structures namely, tubular and lobular types have been identified (Hoar 1969; Billard et al., 1982; Nagahama 1983; 1986; Redding and Patino, 1993).

The pituitary gonadotropic function seems to be responsible for suspended maturity in male Channa gachua in river. Successful attempts have been made to induced sexual maturity in Channa gachua by means of hormonal injections. Colombo et al., (1987) and Khan et al., (1987) induced spermatogenesis and production of spermatocytes in European eel by administering a single dose of hCG after 1month and 3 months, respectively. Sugimoto and Takahashi (1979) have shown that interstitial (leydig) cells were activated in the testis of Japanese eel during hCG induced maturation. Recently, Miura et al., (1991a) had induced sexual maturation in Japanese male eel with in 18 days by administering a single dose of hCG. The histarchitectue of testes after 6, 12, and 18 hours of treatment remained unchanged. After 24 hrs of hormonal injection, the dramatic changes took place and lobular organization after the 72 hrs. Thus the pituitary-gonadotropic function was restored by exogenous administration of Ovaprim resulting in the induction of spermatogenesis.

The structure and deposition of spermatogonia were similar to the description of earlier workers (Gresik et al., 1973; Sugimoto 1979; Griet 1975; Billard 1984; Colombo et al., 1987; Miura et al., 1991a). In Channa gachua primary spermatogonia were always surrounded by sertoli cells, while secondary spermatogonia were restricted to testicular cysts and continue to divide until spermatozoa formation. Each cysts was enclosed in a covering of sertoli cells and the cells extended in between and remains in close contact with spermatogonia. Clusters of sertoli cells were also occured inside the testicular lobules. The leydig cells occurring singly or in groups always lies the outside the interstitial cells separated by basement membrane.

The sertoli cells in the testes testis perform several functions including support and structural organization of the cysts, lobules and tubules help in the formation of spermatogonia and eventual conversion of metabolites and hormones towards the germ cells or central cavity phagocytosis of germ cells and in isolation of cysts compartments beyond the spermatocytic stage (Billard, 1986). These cells have also been implicated with steroid production in certain species (Weiβ 1969; Bars 1969; Van den Hurk et al., 1980). The sertoli cells remains in very close and direct association with germ cells which they support physically and nurture by modifying the chemical environment (Redding and Patino, 1934). The sertoli cells becomes active within 12 hrs of Ovaprim treatments as evidenced by increasing cell and nuclear size, organization and distribution of dense globule material. The peak activity was achieved after 72 hrs.

The Leydig cells were typically interspersed in the connective tissue surrounding germ cells--sertoli cells unit and their primary function is to produce steroids needs for gametogenesis. These are characteristically steroids-producing cells in Channa gachua testis which becomes elongated after Ovaprim treatments. The Leydig cells in the present study also became activated within 72 hrs synthetic hormones treatments. Same results were found by Sugimoto and Takahashi, 1979; Colombo et al., 1987; Miura et al., 1991b; Follinius and Porte, 1960.
It might be concluded that, Ovaprim first stimulated androgen; testosterone and mainly 11-
ketotestosterone in turn induce spermatogonial proliferation and organization of testicular lobules
with a wide lumen within a 72 hrs. The sertoli cells remain in close contact with the spermatogonia, and
maintained supply of metabolites and other substances later. They also took part in the lobular
organization and formation of its lumen. This further strengthened our earlier conflict regarding the
stimulatory effect of Ovaprim on the induction of spermatogenesis and sexual maturation.

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SEASONAL VARIATIONS OF PROTEIN IN THE OVARY OF FISH CHANNA GACHUA

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Abstract

The present study was carried out in the breeding seasons from 2004 to 2006. The ovary of fresh water fish Channa gachua was analyzed for its protein content during period of four different phases of reproductive cycle, in Channa gachua i.e. preparatory, pre-spawning, and post-spawning. Protein content found in preparatory phase was non-significant, and significantly increased from pre-spawning to spawning and declined in post-spawning (mg/g wet weight of ovary).

Key Words: Protein content, ovary, Channa gachua.

Introduction

Fish protein contains all essential amino acids which are easy to digest. The protein digested and assimilated is mostly incorporated in to muscles of the fish. Fats, on the other hand, have a high caloric value and stored in muscles, liver, intestine and gonads.

In the breeding season, the fish draws up from muscles protein and used and for the growth and development of the reproductive organs.

Knowledge of biochemical composition of fish is of great help in evaluating its nutritive value (Kingston and Venkatararnani 1954). Though lot of work on biochemical composition has been undertaken very few (Bailey et al; 1952; Idler and Blmers, 1958; Brown, 1957; Gupta and Raina Sujata, 1977) have correlated with reproductive cycle. The protein content was studied in number of teleosts such as Oreochromis mossambicus (Pathan and Baile 2005), Heteropneustes fossilis (Hunge and Baile 2003), Channa orientalis (Saksena & Sexena 1999), Claris batrachus (Bana, 1977). Garra mullaya (Khan & Mehrotra 1991), Schizothorax richardsoni & Glyptocephalus pentinopterus (Singh & Nauriyal, 1990). Reproduction in fishes depends upon co-ordinated actions of various hormones associated with brain-pituitary-gonadal axis (Evans, 1998). The hypothalamic-pituitary-gonadal level concerning the possible biochemical interaction in teleost was along this axis (Pathan and Baily, 2005). In the present study, ovary has been selected to establish the possible correlation of metabolites and reproductive cycle.

In India, the data available on the chemical composition of fish, especially the fresh water fish, related mainly to their nutritive value. The present study has been undertaken to correlate the variations in biochemical composition of ovary in fresh water fish, Channa gachua to its reproductive phases. This attempt has been made to find out whether the biochemical constituents i.e. protein of Channa gachua at different times, could be related to reproductive cycle of ovary.

Materials and Methods

Live species of Channa gachua, were collected from Kham river near Aurangabad. Fishes were collected during the period of early March to late September. They were brought to Laboratory, weighted, scarified after pitting, to take out their ovaries. The ovaries were observed in each case and reproductive cycle was noted. Protein was estimated by drying ovary for 24 to 36 hours in an ovan maintained at 68°C. This ovary was processed for their biochemical estimations of protein (Lowry et al, 1951).
Result and Discussion

The values of protein obtained in female *Channa gachua* There were two years data were given in table No.1. Protein content was found highest during spawning phase and attained peak values were (34.94±4.023, 38.0608±1.8304 and 36.0042±0.3426) and lowest in preparatory phase were (9.094±0.549, 8.5386±0.7058 and 9.8674±0.6704) mg/g.

Table No 1: Seasonal variation in the protein in ovary of *Channa gachua* (mg/g wet weight of ovary)

<table>
<thead>
<tr>
<th>Phases of Reproductive Cycle</th>
<th>Percentage of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>2005</td>
</tr>
<tr>
<td>Pre-spawning Phase</td>
<td>17.357 ± 0.956</td>
</tr>
<tr>
<td>Spawning Phase</td>
<td>34.944±4.023</td>
</tr>
<tr>
<td>Post-spawning Phase</td>
<td>16.835±0.733</td>
</tr>
</tbody>
</table>

In (early March to early June) preparatory and pre-spawning phases the low level of protein might be due to its active utilization by ovaries during the process of vitellogenesis. Observations in the present study correlates positively to the observations of Love, (1970) who stated that, the building up of gonad is always accomplished at the expense of body protein. Similar results have been reported by John and Hameed, (1995).

During spawning phase (i.e. late June to July) protein found to be increased and reaching maximum in spawning phase was attributed to low metabolic activity. Bano, (1977), Macay & Tunison, (1938), Jahir, (1968) also noted the increased protein content in muscle and they also attributed in increment with gonad maturity. Increase in protein content of muscle with maturation of gonads which was the result of active feeding in pre-spawning phase.

Shireni (1980) stated that the protein cycle in fishes can be synchronized with maturity of fishes than feeding. The efforts have been put forth by Damberg (1964) and noticed a decline in muscles protein of *Gadus morhua* during growth of gonads.

During the present study it was noticed that ovarian protein started declining in August to early September i.e. period of post spawning phase Jafri and Khawaja (1968) reported protein cycle in of *Ophicephalus punctatus* and showed correlation between feeding and spawning. Muscle protein started declining gradually during spawning and post-spawning phases. This decline of muscle protein can be attributed to its transfer in to ovaries to meet energy requirement of fish during spawning and post-spawning phases. Decline of protein has also been reported by Srikanth et. al. (1979) in *Clarias batrachus*; Somavanshi, (1983) in *Garra mulya*; and Luzzana et. al., (1996) in *Coregonid bondella*.

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