Table : Seasonal fluctuations in liver glycogen, liver protein, liver moisture, HSI, ovarian glycogen, ovarian protein, ovarian moisture and GSI contents of *H. fossilis*.

Months	Liver glycogen (mg/g) wet wt. M±SE	Liver protein (mg/g) wet wt. M±SE	Liver moisture (%) wet wt. M±SE	HSI M±SE	Ovarian glycogen (mg/g) wet wt. M±SE	Ovarian protein (mg/g) wet wt. M±SE	Ovarian moisture (%) wet wt. M±SE	GSI M±SE
JAN.	28.05	61.67	73.65	1.62	1.76	115.68	67.25	0.72
	±1.12	±2.25	±1.33	±0.06	±0.10	±4.48	±1.22	±0.02
FEB	26.57	67.55	73.12	1.60	1.93	122.10	66.80	0.88
	±1.28	±2.20	±1.45	±0.02	±0.09	±3.23	±1.25	±0.03
MAR	26.73	68.20	73.25	1.30	1.12	130.25	66.60	1.77
	±1.25	±2.78	±1.55	±0.03	±0.11	±2.48	±1.54	±0.04
APR	25.28	82.32	74.00	0.95	1.61	166.22	66.40	4.40
	±0.48	±2.90	±1.25	±0.02	±0.08	±8.25	±2.10	±0.16
MAY	19.32	104.48	74 .12	0.83	1.94	210.50	64.40	10.20
	±0.95	±4.12	±1.62	±0.05	±0.12	±5,75	±1.34	±0.25
JUN	15.08	126.15	74 .55	0.°0	2.25	278.12	62.20	11.92
	±1.12	±3 35	±1.53	±0.06	±0.15	±6.66	±1.12	±0.58
JUL	15.62	225.50	75.50	0.85	3.38	302.24	60.05	15.20
	±1.15	±4.64	±1.12	±0.06	±0.20	±7.47	±0.95	±0.92
AUG	18.07	134.28	74.15	1.02	2.09	208.15	60.70	13.67
	±1.05	±2.32	±1.35	±0.04	±0.08	±7.34	±1.13	±0.75
SEP	22.54	105.18	74.20	1.58	2.02	191.12	63.92	7.55
	±1 26	±3 45	±1.74	±0.03	±.010	±2.10	±1.44	±0 23
OCT	22.86	92.55	74.25	1.63	1.77	165.00	65 20	0.82
	±1.30	±3 15	±1.80	±0.05	±0.11	±2.35	±1.72	±0.08
NOV	23:38	78.24	73.93	1.65	0.65	140 65	65.78	0.78
	±0.80	±2.44	±1.75	±0.09	±0.07	±4 25	±1 45	±0.05
DEC	29.42	60.65	73.78	1.72	0.45	110.32	67.12	0.70
	±0.95	±2.66	±1 45	±0.08	±0.05	±6.12	±1.62	±0.04

DISCUSSION

Large amount of energy is required by the fish during gonadal maturation cycle[16,17].

Hepatic glycogen probably represents the energy source to meet out the metabolic needs encountered in the ovarian growth.[11] The decrease in liver glycogen contents in spawning phase has been suggested to be due to steroid hormones released during this period.[18] Thereafter, the gradual diminition of the glycogen content during gonadal maturation in *H. fossilis* may be due to its breakdown into glucose through glycogenolysis for the production of energy and/or less synthesis. Decrease in HSI value as reported here in pre-spawning phase may be a reflection of this fact. Increase in ovarian glycogen during spawning phase suggests increased synthesis of glycogen in ovary and/or transfer of glycogen to ovary. The obser.ed increase in ovarian glycogen at a time when liver glycogen was found to decrease probably suggests that there is transfer of glycogen reserves from liver to ovary during spawning period.

In most of the oviparous vertebrates yolk proteins and its precursors are synthesised into the liver at the time of gonadal development which is transported to ovary through blood[9,19–23]. Yolk precursor like vitellogenin (female specific serum protein) were identified in number of teleosts and non-teleosts[5,24,25]. In this investigation protein contents of liver and ovary of *H. fossilis* were found to be maximum during spawning period. Similar observations have been reported in other fishes [3–6,26]. Increased amount of hepatic protein also coincided with increased contents of ovarian protein in *H. fossilis* during spawning phase. This seems to indicate that probably liver is the source for increased demand of nitrogenous protein in the body. The increased protein contents during spawning period may be due to increased synthesis or decreased degradation of protein by liver and ovary and/or increased amount of enzymes required for vitellogenesis. Increased level of GSI during spawning period may be an indication of this fact. During the present study lipid estimation could not be done and therefore, above suggestion is based on the analogy with other reports. Gradual decline in protein content of ovary during post-spawning period of this species may be due to the spent ovary and/or less demand of nitrogenous product. This change possibly suggests that during this period liver acts as storehouse of energy reserves.

It is significant to record here that in *H. fossilis* ovarian moisture percentage were maximum during the months of post-spawning when both glycogen and protein were at minimum. Presumably depleted glycogen and protein contents were replaced by water during this phase. In liver too moisture value was found to increase during gonadal maturity period and maximum moisture percentage coincided with spawning phase. Decreased percentage of water in ovary during spawning phase may seem to indicate excess deposition of dry matter. Similar findings have been recorded by Jafari and

Khawaja[3] in *Ophiocephalus punctatus*. Increased level of water content in liver during breeding season may be due to diversion of the dry nutrients towards gonadal development and maturity.

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ANNUAL VARIATIONS IN THE ACTIVITY OF THYROID GLAND IN RELATION TO OVARY AND LIVER OF A FRESHWATER TELEOST, *HETEROPNEUSTES JOSSILIS* (BLOCH).

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Seasonal variations in the activity of thyroid gland in relation to ovary and liver of a freshwater teleost, *Heteropneustes fossilis* were studied for one complete year. Thyroid activity index, ovadiameter measurements and gonosomatic index were found to be maximum during July–August and minimum during December-January. Hepatosomatic index revealed an opposite trend in being maximum during December and minimum in July. It is suggested that thyroid hormones are involved in ovarian maturation in this species.

Although various attempts have been made to correlate thyroid function with reproduction in cold blooded vertebrates, yet no definite function has been attributed to it, despite some excellent reviews[1,2] on this subject. Swift[3] mentioned two types of cyclical activity of thyroid in fish; one due to changes caused by external environment and the other inherent. Such cyclical changes in the activity of thyroid gland have been mainly associated with factors such as temperature, photoperiod and reproduction[4,5]. Much of these data are derived from the studies on temperate fishes and the report on tropical fishes, particularly of thyroid, gonad and liver axis in Indian freshwater teleosts are scanty. The present investigation was carried out with a view to find out probable correlation between thyroid and reproduction, if any, and the role of liver in mediating these processes,

MATERIALS AND METHODS

Ten female *H. fossilis* (body weight 38–40 g; total length, 17–18 cm) were collected around 15th of every month throughout the year from the ponds in and around Jaunpur City, India. They were brought alive to laboratory, decapitated and their lower jaw containing thyroid region and ovary were fixed in Bouin's fluid for the measurement of thyroid activity using the method of Fortune[6] and ova-diameter

with help of oculometer and stage micrometer respectively. Decalcification of jaw was achieved by changing the fixative twice a week for three weeks. Paraffin sections of both lower jaw and ovary were cut at 5 μm thickness. Thyroid was stained with PAS-Hematoxylin and ovary with Hematoxylin and Eosin. Camera lucida drawing of outlines of 50 thyroid follicles (45–165 μm) were drawn at 150 times magnification on a standard paper. These were then cut out and weighed. Epithelia outlines of the weighed paper were also cut out and weighed. Finally, the ratio between the total epithelial weight and follicular weight of the paper cutting was taken as thyroid activity index (TAI). Likewise, average of 100 largest ova (82–740 μm) from each fish every month were recorded for ova-diameter measurements. The weight of ovary and liver taken respectively for gonosomatic (GSI) and hepatosomatic indices (HSI) Stages of reproductive cycle were followed as Singh and Singh[7]. The degree of relationship between two variables was measured by correlation coefficient (r) and significance of 'r' values was checked by student t-test.

RESULTS :

TAI, ova-diameter measurements, GSI and HSI values for one complete year are presented in table 1. TAI values started increasing from February reaching its peak in July (0.61±0.02). Thereafter, it gradually declined and reached to its

Table 1 : Seasonal variations in thyroid activity index, ova-diameter, GSI and HSI of *Heteropneustes fossilis*.

Months	Thyroid Activity Index M±SE	Ova-diameter (μm) M±SE	GSI M±SE	HSI M±SE
January	0.18±0.03	87.58±2.09	0.72±0 02	1.62±0.06
February	0.20±0.01	156.00±4.88	0.88±0.03	1.60±0.02
March	0.25±0.04	162.17±4.96	1.77±0.04	1.30±0.03
April	0.28±0.02	255.87±4.69	4.40±0.16	0.95±0 02
May	0.36±0.03	650.00±9.68	10.20±0.25	0.83±3.05
June	0.57±0.01	654.13±10.10	11.92±0.58	0.82±0.06
July	0.61±0.02	725.65±17.78	15.20±0.92	0.80±0.06
August	0.60±0 02	723.27±14.37	13.67±9.75	1.02±0.04
September	0.47±0.03	606.95±8.23	7.55±0.23	1-58±0.03
October	0.32±0.05	445.25±9.50	0.82±0.08	1.63±0 05
November	0.22±0 02	178.52±5.25	0.78±0.05	1.65±0.09
December	0.18±0.03	100.34±1.81	0.70±0.04	1.72±0.08

minimal level (0.18±0.03) in December-January. Ova diameter measurements were recorded to be minimum during January (87.58±2.09) followed by a gradual increase reaching its peak in July (725.65±17.78). GSI variations paralleled with ova-diameter measurements ($r=0.9158$, $P < 0.001$) and the values were minimum during December (0.70±0.0) and maximum during July (15.20±0.92). When compared with ova-diameter measurements ($r=-0.6829$, $P < 0.01$), GSI ($r=-0.8272$, $P < 0.001$) or TAI ($r=-0.7868$, $P < 0.001$), changes in HSI revealed an opposite trend, and the values were found to be minimum during June (0.80±0.06) and maximum during December (1.72±0.08).

DISCUSSION :

Majority of the evidences showing cyclical activity of the fish thyroid are derived from histological and cytochemical studies conducted during different seasons or during metamorphosis. Earlier qualitative studies using histological methods in eleostean thyroid were based on size and number of thyroid follicles, height of epithelial cells and amount of colloid in it[8] [9]. Highest level of epithelial cell height[9] [10] and hyroid hormone production [10] [11] has been recorded during spawning phase and lowest n post-spawing phase. Recently, Dubey and Choubey[12] recorded positive correlation etween epithelial cell height, ovarian weight and ova-diameter in *Channa punctatus*. n this study highest TAI has been taken as reflection of the high activity of the land. It is evident from the result that in *H. fossilis* thyroid gland attains maximum ctivity during July-August (spawning phase). Once the spawning is initiated hyroid activity starts declining reaching its minimum level in January. It is remarkable o note that TAI is positively correlated with ova-diameter measurements ($r=0.8798$, < 0.001) and GSI ($r=0.8846$, $P < 0.001$) throughout the year. Two peaks of T_4 and $_3$ during autumn and spring have been found to be concerned with temperature ompensating mechanism and reproduction[13]. It tends to indicate that, in *H. fossilis* ariation in the thyroid activity are related with reproduction.

In *H. fossilis* HSI values were minimum during spawning phase, which can be scribed to augmented glycogenolysis to meet high demand of energy required for onadal maturation at this time. It is likely, therefore, that the observed association f thyroid in reproduction might be through this pathway, alteast for the energy urpose.

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Seasonal Variations in Glycogen, Protein and Moisture Contents in Liver and Ovary of a freshwater teleost, *Heteropneustes fossilis* (Bloch)

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Seasonal changes in glycogen, protein and moisture contents in liver and ovary of *Heteropneustes fossilis* were studied. Glycogen content in liver was reduced in pre-spawning and spawning phases which was associated with decrease in hepatosomatic index (HSI). However, ovarian glycogen increased during spawning phase. There was gradual increase in protein content of liver and ovary from preparatory to spawning phase which declined in post-spawning phase reaching almost to the initial preparatory condition. Increased level of protein in ovary during spawning phase coincided with enhanced gonosomatic index (GSI). Water percentage in liver slightly increased in spawning phase but remained constant in other phases. Ovarian moisture content gradually decreased from pre-spawning to spawning phase but increased in post-spawning phase.

Physiological factors such as maturation, spawning and feeding change the biological composition of fish during breeding season[1,2]. Higher amount of proteins are observed in gonads at the time of tip maturity [3-5]. In oviparous vertebrates complex form of olk protein is synthesised by the extraovarian tissues (heterosynthetic) such as liver and ransported to ovary through blood where it is taken up into oocytes[6-9]. In addition to eterosynthetic process, an intraovarian origin of yolk protein (autosynthetic) has also een reported[10]. Hepatic glycogen is used as the source of energy at the time of gonaal development and spawning[11]. The present study sets out the seasonal variation in lycogen, protein and moisture contents in liver and ovary of *Heteropneustes fossilis*

IATERIALS AND METHODS

Ten live adult specimens of female *H. fossilis* (weight range 38g-40g and length

range 17cm—19cm) were collected around 15th of each month from ponds in the vicinity of Jaunpur city, India. They were rapidly sacrificed and their liver and ovary were taken out, quickly washed with 0 6% saline, blotted to remove excess saline, weighed and used for the determination of glycogen, protein and moisture contents. Glycogen was estimated following the method of Kemp and Adrienne[12] as modified by Srinivasan and Krishnaswamy[13] and protein was determined according to Lowry *et al.* [14]. The moisture contents were measured by drying pre-weighed liver and ovary samples at 100°C until constant weight were obtained. The total weight of liver and ovary were recorded for hepatosomatic (HSI) and gonosomatic indices (GSI) using the established formulae. The studies were followed using the methods of Singh and Singh[15].

RESULTS

Results are presented in table. Liver glycogen content was minimum during pre-spawning and spawning phases reaching maximum during post-spawning period. However, ovarian glycogen was recorded maximum in spawning period which then declined and reached minimum during post-spawning phase. Protein values in both the tissues were more or less similar in all the reproductive phases. Hepatic as well as ovarian protein contents were found to be maximum during July which is the spawning phase. These values then decreased abruptly and reached minimum during post-spawning phase.

Hepatic moisture content increased slightly from preparatory phase reaching maximum during spawning phase. Later it was followed by a gradual decline till it reached to minimum during post-spawning phase. Ovarian moisture content started declining from preparatory phase and recorded minimum during spawning phase. Thereafter, it gradually increased upto post-spawning phase reaching its peak value in December. HSI value declined from preparatory phase and reached to minimum during spawning phase. Thereafter, it was followed by an increase upto post-spawning phase reaching max.mum in December. GSI value was recorded to be at its lowest level during post-spawning period. Subsequently there was rapid and tremendous increase in GSI value from preparatory to spawning phase when it reached to its peak.

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EFFECTS OF METHYL TESTOSTERONE AND TESTOSTERONE PROPIONATE ON PROTEIN, GLYCOGEN AND MOISTURE CONTENTS IN LIVER AND GONAD OF A FRESHWATER TELEOST, *HETEROPNEUSTES FOSSILIS* (BLOCH).

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Adult male and female *Heteropneustes fossilis* were injected with different doses (1.25, 2.50 and 5.00 μg/g body wt.) of methyl testosterone (MT) and testosterone propionate (TP) on alternate days till each fish received a total of four injections. Protein, glycogen and moisture contents were determined in liver and gonad. Protein and glycogen values of liver and gonad were significantly increased in both sexes in response to MT and TP. These changes were dose dependent. Moisture percentage did not change significantly in both the tissues following the steroids treatment. Careful statistical comparison of MT and TP showed that MT was more effective in inducing such changes in both the tissues at all dose levels tested.

The involvement of sex steroids in liver and gonad metabolism of fish has ttracted the attention of many investigators. It is evident from earlier studies that ıost of the biochemical constituents of liver and gonad of fish are subjected to ıarked seasonal changes which have been influenced by variations in external emperature, photoperiod, salinity and rainfall etc.) and internal (sex steroids) ɔctors[1]. In teleosts, exogenous administration of androgen increases vitellogenic otentiality in liver[2,3], plasma protein level[4,5] and liver glycogen[6]. The inadequate ıformations regarding the responsiveness of liver and gonad to sex steroids need

further investigation. The intention of this study is to determine the effect of methyl testosterone and testosterone propionate on some biochemical constituents of liver and gonad of *Heteropneustes fossilis*.

MATERIALS AND METHODS

Male and female specimens of *H. fossilis* (weight range 38g—40g and length range 17 cm—19 cm) were procured during December from ponds in the vicinity of Jaunpur city, India. They were acclimatised to laboratory conditions for three weeks before starting the experiments. Fishes were divided into seven groups of 10 males and 10 females each. They are first lightly anaesthesised with MS 222 and then groups 1, 2 and 3 were intramuscularly injected with 1.25, 2.50 and 5.00 μg MT/g body wt. and groups 4, 5 and 6 with 1.25, 2.50 and 5.00 μg TP/g body wt. respectively on alternate days till each fish received a total of four injections. Last group received comparable quantity of carrier vehicle only and served as control. The fishes were fed with a compound ground mixture of goat liver, dry prawn and wheat flour ad libitum at every alternate days throughout the experiments. Aquaria water temperature was not artificially controlled but ranged in between 16—18° C.

Twenty four hours after fourth injection fishes of all groups were decapitated and their liver and gonad were taken out, washed with 0.6% saline, blotted to remove excess saline, weighed and used for the determination of protein, glycogen and moisture contents. Protein was determined using the method of Lowry *et at.*[7] and glycogen by Kemp and Adrienne[8] as modified by Srinivasan and Krishnaswamy[9]. The water content was determined by drying pre-weighed tissues at 100°C in hot air oven until constant weight were obtained. Hepatosomatic (HSI) as well as gonosomatic (GSI) indices were also calculated using the established formulae.

All the data were analysed statistically with the help of Student's t-test.

RESULTS

Results were summarised in tables I and 2. Both hepatic as well as gonadal protein values were significantly increased ($P < 0.05, 0.01, 0.001$) in both sexes follo-

Table 1—Effects of methyl testosterone and testosterone propionate on liver glycogen, liver protein, liver moisture, HSI, testicular glycogen, testicular protein, testicular moisture and GSI of *H. fossilis*.

Hormone	Dose µg/g body wt.	Liver glycogen mg/g wet wt. M±SE	Liver protein mg/g wet wt. M±SE	Liver moisture % wet wt. M±SE	HSI M±SE	Testicular glycogen mg/g wet wt. M±SE	Testicular protein mg/g wet wt. M±SE	Testicular moisture % wet wt. M±SE	GSI M±SE
MT	1.25	28.16** ±1.53	75.04** ±3.07	71.43 ±2.14	1.80 ±0.07	3.14*** ±0.08	111.61 ±10.58	72.72 ±2.32	0.034 ±0.002
MT	2.50	27.17** ±1.57	82.88*** ±4.00	72.65 ±2.35	1.81 ±0.08	2.46** ±0.01	128.50** ±10.50	71.28 ±2.01	0.035 ±0.004
MT	5.00	27.11** ±2.11	95.94*** ±4.23	73.98 ±1.80	1.82 ±0.11	2.44** ±0.02	143.20*** ±11.55	71.81 ±1.85	0.036 ±0.002
TP	1.25	2 .27 ±1.17	58.58 ±4.05	72.97 ±2.24	1.76 ±0.10	1.90* ±0.08	104.40 ±8.91	72.20 ±2.21	0.032 ±0.002
TP	2.50	26.87* ±1.06	70.33* ±3.39	73.95 ±2.33	1.83 ±0.10	2.46** ±0.01	120.72* ±8.45	1.80 ±1.90	0.032 ±0.002
TP	5.00	26.26* ±1.03	89.14*** ±5.40	73.98 ±2.33	1.82 ±0.08	2.33** ±0.03	132.86** ±10.35	73.98 ±2.35	0.034 ±0.002
Control	—	20.02 ±1.78	53.94 ±2.40	73.74 ±2.45	1.75 ±0.10	1.56 ±0.04	94.42 ±3.98	72.50 ±2.30	0.032 ±0.001

Significance of difference from control *P <0.05, **P <0.01, ***P <0.001.

Table 2 –Effects of methyl testosterone and testosterone propionate on liver glycogen, liver protein, liver moisture, HSI, ovarian glycogen, ovarian protein, ovarian moisture and GSI of *H. fossilis*.

Hormone	Dose µg/g body wt.	Liver glycogen mg/g wet wt. M±SE	Liver protein mg/g wet wt. M±SE	Liver moisture % wet wt. M±SE	HSI M±SE	Ovarian glycogen mg/g wet wt. M±SE	Ovarian protein mg/g wet wt. M±SE	Ovarian moisture % wet wt. M±SE	GSI M±SE
MT	1.25	32.91* ±1.45	75.54* ±3.66	72.53 ±2.30	1.70 ±0.04	2.24*** ±0.08	118.54* ±10 71	71.25 ±2.55	0.72 ±0.03
MT	2.50	30.51* ±1.91	85 10** ±3.95	72.22 ±2.24	1.71 ±0.07	1.84*** ±0.04	132.79** ±14.77	70.53 ±2 40	0.74 ±0.01
MT	5.00	30.02* ±1.54	112.11*** ±4.50	72.84 ±2.32	1.72 ±0.08	1·80*** ±0.03	150.16*** ±14.38	70.54 ±2.50	0.75 ±0.02
TP	1.25	27.70 ±2.63	66.13 ±3.98	73.84 ±2.50	1.64 ±0.12	1.65*** ±0.06	96.79 ±5.18	73.29 ±2.50	0.70 ±0.01
TP	2.50	32.30* ±1.16	77.96* ±3.85	73.62 ±2.20	1.71 ±0.10	1.80*** ±0.08	103.44* ±6.36	71.93 ±2.24	0.72 ±0.01
TP	5.00	31.50* ±1.15	94.16** ±3,84	73.25 ±1.90	1.72 ±0.05	1.74*** ±0.08	129.50** ±4.02	71.04 ±2.22	0.73 ±0.02
Control	—	24.20 ±1.34	64.60 ±1.73	73.89 ±2.23	1.68 ±0.10	0.80 ±0.05	96.19 ±2.10	73.54 ±2.30	0.69 ±0.02

Significance of difference from control *P <0.05, **P <0.01, ***P <0.001.

ving MT and TP treatments when compared with controls. These changes were dose lependent.

Both the steroids tested significantly increased glycogen content of liver and ionad ($P < 0.05$, 0.01, 0 001) of both sexes in respect to controls. The optimum dose evel for MT and TP to increase glycogen value in liver and gonad were found to be .25 and 2.50 μg/g body wt. respectively.

All the doses of MT and TP did not significantly alter the moisture percentage f liver and gonad, HSI and GSI values in both sexes.

)ISCUSSION

In most of the non-mammalian vertebrates yolk proteins and its precursors are f extragonadal origin. Although hepatic synthesis of yolk protein, vitellogenin is nfluenced by estrogen[10–12] as well as androgens[2–5 13 14]. The serum concentration of ndrogens were extremely high throughout the course of vitellogenesis, gonadal deve-)pment and spawning migration in the fish and at that time plasma protein level is ncreased[15–19]. In the present findings both MT and TP enhanced the hepatic as well as onadal protein synthesis. Medda *et al.*[4] have reported that testosterone failed to ause any significant change in protein content of liver and gonad where as estradiol ncreased protein content of liver. However, androgen like testosterone may be erving as precursor for estrogen synthesis[20–22]. Therefore, it is likely that these ormones acted via estrogen as reported by LeMenn *et al*[3]. Increased amount of rotein in gonad may be due to the deposition of extragonadal or intragonadal riginated proteins.

In the present study both MT and TP increased the hepatic as well as gonadal lycogen content in both sexes suggesting that an anabolic role of these hormones n *H. fossilis*. Similarly Dasmahapatra and Medda[6] have reported anabolic effect of estosterone in liver and Power and Florini[23] in muscle of fishes. The role of male teroids on carbohydrate metabolism has also been demonstrated[24,25]. During itellogenesis, gonadal growth and spawning, high energy is required for muscular

contraction[1, 26-28]. This necessiates the storage of glycogen in the liver and gonad. It is evident that MT induced changes in protein and glycogen contents of liver and gonad at different dose levels tested were of higher magnitude than the TP. Hence, it is likely that MT is more potant than TP in inducing such changes.

Dasmahapatra and Medda[6] reported that testosterone cause no significant change in moisture percentage in liver and gonad of *H. fossilis*. Our findings support above view, although we have used anologs of testosterone, a slight change in moisture was observed. This could be due to onset of anabolic activitles prior to spawning.

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