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Research Paper

Effect of some hormones on the muscle tissue biochemical contents of the fresh water fish *Notopterus notopterus*

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Abstract

The uses of steroid hormones as dietary supplements for growth production are an accepted practice in cattle industry. In the present study an effort is made to understand the importance of the hormone such as testosterone and thyroxin on muscle tissue biochemical changes. In muscle the protein and glycogen content is increased in the testosterone treated fish in comparison to control during all the four reproductive phases. The lipid content of muscle in the testosterone treated fish is found to be increased in all the reproductive phases, where as the cholesterol content of muscle in testosterone treated fish is decreased during preparatory and pre spawning phase, while it is reached maximum during spawning and post spawning phase as compared to control fishes. The biochemical contents were also estimated in the fish implanted with thyroxin tablets during pre spawning phase. The result indicated that the muscle protein, glycogen, lipid and cholesterol contents were less in thyroxin treated fish as compared to control fishes.

Keywords: Thyroxine, testosterone, muscle tissue, *Notopterus notopterus*

Introduction

Biochemical composition of fish tissue is considerable interest for their specificity in relation to food values of fish and evaluating their physiological needs at different periods of life. A number of workers have observed depletive effects of malnutrition and spawning in the chemical composition of fish[1,2,3]. Protein acts both as a nutrient and as an energy source. Consequently addition of energy to a diet not only increases energy intake but also lowers the protein energy; total energy ratio. Both factors are likely to influence nitrogen balance and their effects, although related may be separate. Lack of attention to this point has led to lose statement "increasing the protein level with constant energy always resulted in improved efficiency[4]. Aquatic and terrestrial macrophytes have been tried as unconventional source of plant proteins to evolve suitable fish feeds[5,6,7,8]. Lipids are the essential nutrients that provides concentrated energy. They are the major energy stores, and are extremely important in maintaining structural and physiological integrity of cellular and subcellular membranes. The role of lipids in the transport of substrates via the circulatory system in both vertebrates and invertebrates is a vital one [9]

The liver cells, can store up to 5 to 8 percent of their weight as glycogen, muscle cells can store upto one percent glycogen. The molecular weight of glycogen being 50, 00,000 or greater, most of it precipitate in the form of solid granules. This conversion of the monosaccharides into the high molecular weight precipitated compound makes it possible to store large quantities of carbohydrates, without significantly altering the osmatic pressure of intracellular fluids [10]. In fish large amount of yolk nutrient including carbohydrate is transferred from body to the gonad for its subsequent growth and maturation during prespawning season. However, stored nutrient including carbohydrate in liver and muscle of the body are transferred to the gonad to ensure its growth and maturation leading to subsequent spawning[11]. The changes in carbohydrate reserve in fish seems to reflect the requirements of developing gonads specially females.

Cholesterol is the main precursor for the synthesis of steroid hormones which is in turn influence the maturation of gonads [12]. The nature of cholesterol molecules, presumably allows it to fit more or less within any of the proposed protein / phospholipids matrices[13] in male rats, estrogen lower plasma cholesterol but increase liver cholesterol, with increased cholesterol turnover[[14] . The cholesterol contents in steriodogenic tissues has been shown to be associated with synthesis of female hormone [15]. The use of methyl testosterone for promoting weight increase in juvenile pacific Salmon while Simpson and his colleagues in 1974 were the first to show that *Rainbow trout* and *Atlantic Salmon* parr when fed with a diet supplemented with either ethyloestranol or methyl testosterone exhibited a significant increase in growth and an improvement of food conversion efficiency[16] .Estradiol-17β is the major ovarian steroid of most teleosts[17] and are involved in the synthesis of vitellogenin in the liver. This protein (Vg) synthesized in the liver is transported through the blood to the oocyte where it is absorbed and deposited as yolk.

Materials and Methods

About 40 fish *Notopterus notopterus* were collected during each period of January-March, April-July, August-October, and November-December. With a total of 160 fish for all the four phases in cortisol and testosterone treatments. These months fall under preparatory prespawning, spawning and postspawning phases respectively. For thyroxine treatment only 20 fish were collected ruing prespawning phase (April-July). The live fishes were brought to the laboratory and were kept in large plastic pool tanks having size of 90 cms diameter and 60 cms in height. Each tank accommodated 10-15 fishes about 8-10 days were needed for the fishes to aclimatise. During acclimatization antibiotic tablets (chloromphenol 80 mg in one gallon of water) has been given to prevent from infections. Fishes of both control and experimental groups were fed with live earthworms; boiled eggs, small fishes (Gambusia) and tadpole larvae.

Experimental work

Hormones such as testosterone and thyroxine used for the experimental study to know the tissue biochemical changes.

Hormonal treatment

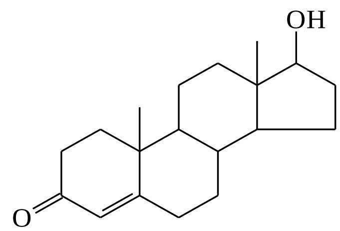
a**) Testosterone**

The hormone testosterone (SISTANON 250 inj) which is commercially available, the 1 Ampule of 1 ml contains; testosterone propionate I.P. 30 mg, testosterone phynylpropionate 60 mg, testosterone isocaproate B-P-60 mg and testosterone decanoate B.P. 100 mg.The hormone testosterone was dissolved in 100 ml olive oil. The fishes control and experimental group were maintained separately in a aquarium, the group I (control) 10 fishes received only 0.1 ml olive oil/fish whereas the group II fishes received 0.1 ml of testosterone hormone containing 250 μg intraperitonially per fish for a period of 10 days.

This experiment was carried out in all the four different reproductive phases separately. After injecting the hormone regularly for a period of 10 days, all the fishes were sacrified by decapitation and the tissue liver and muscle were used for biochemical estimation in the respective phase.

Details of Testosterone

Trivial Name: Testosterone (T)
Systematic Name: 17β-Hydroxyandrost-4-en-3-one
Structural formula



Solubility: Oil soluble
Company: Central drug house (P) Ltd., P. Box No. 1495, Delhi-6.

b**) Thyroxine**

The thyroxine harmone tablets of 10mg were implanted in the muscle by making a small incision in the dorsal region. The trade name for thyroxine is Eltroxn, a synthetic thyroid hormone. After the implantation the incised area was covered with vasoline and left for 10 days. The fishes were divided in to two groups of 10 each. Group I served as control which was not received thyroxine hormone; Group II served as experimental one which received 10mg of the thyroxine hormone tablet implantation. This experiment was carried out during the prespawning phase. After 10 days treatment all the experimental, and control fishes were scarified by decapitation and tissues are analysed for biochemical contents**.**

Biochemical determination

The biochemical content such as protein, lipid, glycogen and cholesterol were determined in muscle of the fish *N. notopterus*. These biochemical parameters were determined by using the following methods.
Estimation of protein by – Lowry's method (1951).
Estimation of lipid by – Barnes and Black stock (1973).
Estimation of cholesterol by – Liberman and Burchard method (1952).
Estimation of glycogen by – Carrol *et al.,* (1956).

Results and Discussion

It is known that seasonal increase in steroid hormones in fish for gearing up of metabolism to provide energy for protein and lipid synthesis. In blenny fish (*Parablennius Sangvinolentus parvicornis*) that the liver played an important role in the storage of energy before the reproductive season and reserves were utilized during the breeding season. This could be due to females investing energy for oocyte development [18,19.]. The analysis of plasmatic steroids by RIA during the annual reproductive cycle of the Indian major carps, *Labeo rohita, Cirrhanna mrigala,* murrel *Channa punctatus* and cat fish *Heteropneustes fossils* and reported that exceptionally high circulating levels of in the post-vitellogenic fishes and in some instances testosterone levels in the female are higher than male fish of the same species[20].

Biochemical contents of muscle

The biochemical contents such as protein, glycogen, lipid and cholesterol has been studied in the muscle tissue in response to the hormones testosterone and thyroxine during the different phases of the reproductive cycle of the freshwater fish *N. notopterus*.

Proteins

Protein plays a central role in cell function and cell structure proteins are high molecular weight polypeptides. They are not only responsible for comprising the structure of cell but are involved in all the aspects of cell physiology such as catalysis, tissue repair, building, defense reactions etc., through hormones, they regulate the metabolic aspects of the animal.

There are also reports regarding the effect of androgen on protein metabolism in culture practice. An improvement in the quality of flesh in terms of higher protein after the inclusion of 17α-methyl testosterone for 30 days a dosage of 300 mg/kg diet of *Cyprinus carpio.* In case of muscle, the protein content is increased in the testosterone treated fish in comparison to control during all the four reproductive phases [21].

Glycogen

The muscle glycogen is found to be increased in the testosterone treated fish during all the reproductive phase in comparison to control. The effect of testosterone propionate and methyltestosterone (0.5 μg/g body weight) on different chemical constituent of liver and ovary in the Indian catfish *Clarias batrachus* during preparatory phase. Although it is reported that testosterone has no effect on vitellogenin production in the Indian cat fish *H. fossilis* [22].

Lipid

Lipids are of immense nutritional importance from the stand point of both quality and quantity. They provide maximum energy, besides providing vitamins like A, D, E and K. Lipids are also source of some essential fatty acids. In the present study the muscle lipid content in the testosterone treated fish is found to be increased in all the reproductive phases in comparison to control.

Cholesterol

The muscle cholesterol in the testosterone treated fish was found to be less during preparatory, pre spawning phase and is found to be maximum during spawning post spawning phase when compared to control. The biochemical contents were also estimated in the fish implanted with thyroxine tablets during prespawning phase. The result indicates that all the hepatic and muscle protein, glycogen, lipid and cholesterol contents were less in thyroxine treated fish as compared to control.

Conclusion

Hence from the above study it is clear that, the metabolic hormones such as testosterone and thyroxine hormone play an important role in enhancing the tissue biochemical contents for nutritional purpose.

Table 1: Showing muscle biochemical contents of the freshwater fish *N. notopterus* during different reproductive phases after testosterone hormone treatment

	Preparatory	Prespawning	Spawning	Post spawning
Control				
Protein	4.50 ± 1.33	0.76 ± 0.05	3.32 ± 0.01	1.10 ± 0.09
Glycogen	0.90 ± 0.12	0.56 ± 0.03	0.63 ± 0.01	0.73 ± 0.09
Lipid	4.6 ± 0.38	1.56 ± 0.05	1.9 ± 0.19	1.3 ± 0.07
Cholesterol	1.16 ± 0.07	3.4 ± 0.13	4.33 ± 0.12	1.8 ± 0.05
Treated				
Protein	5.46 ± 0.433	2.01 ± 0.25**	4.16 ± 0.26	3.29 ± 0.02*
Glycogen	1.34 ± 0.29*	0.81± 0.14	1.71 ± 0.16*	2.74 ± 0.03**
Lipid	6.05 ± 0.34*	1.93 ± 0.31	2.13 ± 0.28*	2.90 ± 0.03*
Cholesterol	0.77 ± 0.14*	1.49 ± 0.21**	2.70 ± 0.22**	10.81 ± 0.01***

Tissue expressed as mg/gm.

Each value is expressed as mean ± SE, N = 6.

NS = Not significant * = significant $P < 0.05$; ** = significant $P < 0.01$; *** = significant $P < 0.001$; when compared with control.

Table 2: Showing hepatic and muscle biochemical contents of the fresh water fish *N. notopterus* during prespawning phase after thyroxine treatment

Biochemical contents	Hepatic biochemical contents		Muscle biochemical contents	
	Control	Treated	Control	Treated
Protein	0.93 ± 0.10	0.72 ± 0.06	0.76 ± 0.05	0.67 ± 0.03
Glycogen	0.62 ± 0.02	0.53 ± 0.02	0.56 ± 0.03	0.50 ± 0.03
Lipid	2.76 ± 0.38	0.97 ± 8.36**	1.56 ± 0.05	0.95 ± 0.01*
Cholesterol	1.33 ± 0.04	0.95 ± 0.01	3.4 ± 0.13	1.91 ± 0.16**

Tissue expressed as mg/gm.
Each value is expressed as mean ± SE, N = 6.
NS = Not significant * = significant P < 0.05; ** = significant P < 0.01; when compared with control.

Fig. 6.4. Showing muscle biochemical contents of the freshwater fish *N. notopterus* during different reproductive phases after testosterone treatment

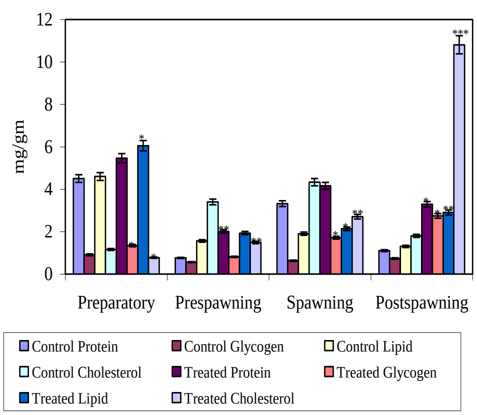
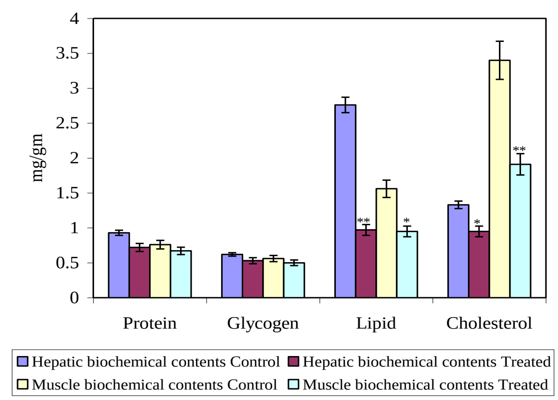


Fig. 6.5. Showing hepatic and muscle biochemical contents of the fresh water fish *N. notopterus* during prespawning phase after thyroxine treatment



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