Annals of R.S.C.B., ISSN: 1583-6258, Vol. 27, Issue 1, 2023, Pages. 265 - 274
Received 15 June 2023; Accepted 31 July 2023.

Somatotropin Effects on Tissue Proteins and Transaminases of Fish, *Catla Catla* (Hamilton, 1822)

J. Swamy[1*] and G. Sunitha Devi[2]

[1, 2] Department of Zoology, University College of Science, Osmania University, Hyderabad 500007,Telangana State, India.

*jswamyzoology@gmail.com

ABSTRACT

Aquaculture is one of the main food production sectors to deal with the high demand for food due to the human population explosion. The demand for fish has increased in recent years due to population growth and the constant search for a healthy diet. The present study was conducted to assess the impact of Somatotropin hormone on Biochemical alterations such as Proteins, AST and ALT in certain tissues like Muscle, Liver and Gills of fish, *Catla catla*. The three different dosages of Somatotropin hormone such as 3mg, 6mg and 9mg per kg of diet was fed orally to the three experimental fish groups. The fourth group of fish were fed with diet without hormone. The biochemical alterations were recorded after 15days, 30 days and 45 days in Muscle, Liver and Gills of fish. The highest increment in tissue Protein content was observed under 9mg Somatotropin/kg diet followed by 6 mg Somatotropin/kg diet and 3 mg Somatotropin/kg diet in skeletal muscle, liver and gills of fish. Hence, it was clear that the anabolic hormones such as Somatotropin play an important role in enhancing the tissue protein content for nutritional purpose of man. Not much change was observed in AST levels of muscle, liver and gills of fish Catla catla. Significant decrease found in ALT levels of liver where as not much difference in muscle and gills.

Keywords: Somatotropin; Proteins; AST; ALT and *Catla catla*.

Introduction

Aquaculture, probably the fastest growing food-producing sector, now accounts for more than 50 percent of the world's food fish. Blue Transformation can meet the twin challenges of food security and environmental sustainability (FAO 2022)[5]. One of the major sources of animal protein for human consumption is fisheries resource. Therefore, considerable attention has been given to the production and growth of freshwater fish in aquaculture (Juin SK et al., 2017)[13]. Aquaculture plays a leading role in the fight against food insecurity, malnutrition and poverty globally (Munguti J M.Ogello EO., 2016 and Myers SS et al., 2017)[18,19]. Primary fish culture on a large scale for a commercial purpose is to obtain faster tissue growth on a low budget. The development of Aquatic Biotechnology has supported the application of experimental techniques to manipulate fish growth, as diets enriched in specific protein nutrients and administrating hormones like Prolactin, Insulin and Growth hormone (GH) (Pullin RS and Mc Conell RH.,1982)[21].

Somatotropin, also known as human growth hormone, is a peptide hormone that stimulates growth, cell reproduction and cell regeneration in humans and other animals. Somatotropin is a single chain polypeptide composed of 191 amino acids, the molecular weight is approximately 22 kDa, produced by the somatotropic cells of pituitary gland and with pleiotropic functions among vertebrates. It mainly regulates body growth, being also involved in reproduction, immunity and osmosis regulation in teleost fish and in metabolic regulation through its lipolytic activity and protein anabolism in vertebrates (Rousseau K and Dufour S., 2007)[24]. Somatotropin has also been recognized as relevant for the aquatic industry due to its role on growth and as immune stimulator(Farman farmaian A and Sun LZ., 1999 & JehHS, KimCH, LeeHK, HanK.,1998)[6,11].

In teleost fish, the Somatotropin secretion from the pituitary is under stimulatory and inhibitory regulation by the hypothalamus, with somatostatin being the major inhibitor of Somatotropin secretion seems to be present(Agustsson, T.2001 & Peng,C. and Peter, R.E., 1997)[1,20]. Somatotropin in fish elicits a variety of biological effects in several different tissues and cell types. Several authors stated that Somatotropin participated in nearly all main physiologic processes such as ionic and osmotic regulation; protein, lipid and carbohydrate

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 27, Issue 1, 2023, Pages. 265 - 274
Received 15 June 2023; Accepted 31 July 2023.

metabolisms as well as reproduction and immune system(Birzniece V, Sata A, Ho KKY. 2009; Hattori N. 2009 & Reinecke M et al., 2005)[2,8,23].

The present study was undertaken to know the impact of the Somatotropin hormone on some biochemical parameters such as Proteins, AST and ALT in certain tissues like Muscle, Liver and Gills of fish, *Catla catla*.

Material and methods

The fish, *Catla catla* used in the present study were procured from State fisheries culture tanks acclimatized to laboratory conditions for 10 days. Fish of uniform size of about 12±1 g were selected for each set of experiments. Fish were divided into four groups and kept in circular plastic troughs having a capacity of 20 liters. The fish were fed with commercial feed once a day at a rate of 2% of body weight both before and during the experimental period. The temperature was maintained at 27 ± 1°C and water in the containers renewed every 24 hours. During experimental period, the fish were fed with control diet (without Somatotropin hormone) for control group and experimental diets (Somatotropin hormone containing diets) for experimental groups.

Preparation of control and Growth Hormone containing diets:

The feed ingredients (commercially available Fish Meal) will be grinded and sieved to pass through 0.5 mm size sieve before incorporation of somatotropin (GH) with experimental diets. A control diet was prepared without somatotropin. Then, Somatotropin is mixed in 3 different groups of grinded fish meal at the level of 3mg, 6mg and 9mg per kg diet to prepare experimental diets. Required amount of water was added to mix diet ingredients to form a dough and pellets will be prepared using hand pelletizer, dried using air blower, sealed in vacuum packaged bags and stored until use to avoid bacterial or fungal contamination.

The diets containing Somatotropin (GH) were characterized as follows:

Diet [1]: Control diet [Without Somatotropin]

Diet [2]: 3mg of Somatotropin/kg of control diet

Diet [3]: 6mg of Somatotropin/kg of control diet

Diet [4]: 9mg of Somatotropin/kg of control diet

Impact of Somatotropin Hormone on Biochemical parameters such as Proteins, AST and ALT of fish, *Catla catla* were studied in three experimental fish groups along with control group.

Tissue Biochemical Analysis:

The biochemical parameters such as Proteins, AST and ALT were estimated in Muscle, Liver, and Gills of the fish, *Catla catla*. These biochemical parameters were estimated by using the following methods.

- **Total Proteins -** estimated by Bradford method (1976)
- **AST** (Aspartate Amino Transferase) – Bergmeyer method(1965)
- **ALT** (Alanine Amino Transferase) - Bergmeyer method(1965)

Statistical Analysis

All the data were presented as Mean±SE. For each experimental treatment, three replicates of 10 individuals were taken. For each replicate, under each treatment Mean was calculated. The three Mean values thus obtained were used to calculate experimental treatment Mean and Standard Errors of Mean. The student's 't'-test was applied between control values and each of the experimental treatment values. p values were determined using the t-statistics and degrees of freedom (df) and denoted as NS- Not Significant; * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$. The experimental data were analysed statistically by adopting valid statistical methods (Pillai and Sinha, 1968), the standard deviation and probability test i.e. 't' test were calculated. The Student's t-test was carried out to know the levels of significance.

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Received 15 June 2023; Accepted 31 July 2023.

Results and discussion

In the present study, the impact of Somatotropin (Oral mode) on tissue Proteins, AST and ALT of fish, *Catla catla* was studied. The different dosages of Somatotropin (3mg, 6mg and 9mg of somatotropin per kg of diet) were given to fish and studied the biochemical parameters after 15days, 30days and 45days of exposure.

Biochemical Analysis:

The biochemical contents such as total proteins, AST and ALT levels were estimated in response to the different dosages of Somatotropin for 15days, 30days and 45days. (Tables-1 to 9)

Protein levels in different tissues:

Proteins are large, complex bio-molecules or macromolecules consisting of one or more long chains of amino acid residues. Proteins perform massive functions within organisms, including catalyzing metabolic reactions, DNA replication, responding to stimuli and transporting molecules from one place to another. Many proteins are enzymes that catalyze biochemical reactions and are vital to metabolism. Proteins also have structural or mechanical functions, such as actin and myosin in muscle and the proteins in the cytoskeleton, which form a system for scaffolding that maintains cell shape. Other proteins are important in cell signaling immune responses, cell adhesion, and the cell cycle (Yoshihiro and Hideo, 2020) [28]. In animals, proteins are needed in the diet to provide the essential amino acids that cannot be synthesized.

At the end of experimental durations, the protein levels in different tissues were significantly increased. The increase of protein levels was more at higher dosage of somatotropin (9mg/kg) and longer duration (45 days). The order of increase was observed at higher dosage (9mg/kg) and longer duration (45 days) as Muscle (60.70%; P < 0.001) > Liver (52.10%; P <0.001) > Gills (45.54%; P <0.001) of fish. Silverstein et al., (2000)[26] found that recombinant bGH (Bovine Growth Hormone) injection induced protein and lipid content in USDA-103 strain and Norris strain of channel cat fish. In rainbow trout, GH increases white muscle and whole body protein along with a stimulatory effect of GH on muscle growth (Chen J.-Y et al., 2000; Fauconneau B et al., 1996)[4,7]. According to Liu et al. (1999)[15], crude protein and crude fat content of muscle in flounder were increased by feeding recombinant yeast containing salmon GH[15].

TABLE 1: Protein content in **Muscle** of fish, ***Catla catla*** fed with diet containing different levels of Somatotropin (mg/kg diet).

Somatotropin/Kg Diet		**Days of feeding Somatotropin along with Diet**		
		15 days	**30 days**	**45 days**
Control	**Mean**	64.080	68.900	72.740
	SE	± 0.932	± 0.796	± 0.655
3 mg/kg	**Mean**	69.450 ^{NS}	75.920 *	81.050 *
	SE	± 0.687	± 0.753	± 0.825
	%V	8.38	10.19	11.42
6 mg/kg	**Mean**	76.070 *	86.400**	96.500**
	SE	± 0.459	± 0.547	± 0.464
	%V	18.71	25.40	32.66
9 mg/kg	**Mean**	88.860***	102.350***	116.890***
	SE	± 0.823	± 0.617	± 0.854
	%V	38.67	48.55	60.70

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Each value is the Mean ± SE of six individual observations.
Values are expressed as mg of protein per gram weight of tissue.
%V - Percent Variation, SE - Standard Error.
NS - Not Significant, * P< 0.05, ** P< 0.01, *** P< 0.001

TABLE 2: Protein content in **Liver** of fish ***Catla catla*** fed with diet containing different levels of Somatotropin (mg/kg diet).

Somatotropin/Kg Diet		Days of feeding Somatotropin along with Diet		
		15 days	**30 days**	**45 days**
Control	**Mean**	51.310	54.730	57.26
	SE	± 0.561	± 0.715	± 0.834
3 mg/kg	**Mean**	55.870 NS	62.480*	67.530*
	SE	± 0.807	± 0.706	± 0.625
	%V	8.89	14.16	17.94
6 mg/kg	**Mean**	60.640*	69.960**	78.860***
	SE	± 0.621	± 0.773	± 0.751
	%V	18.18	27.83	37.72
9 mg/kg	**Mean**	70.880***	79.050***	87.090***
	SE	± 0.638	± 0.764	± 0.872
	%V	38.14	44.44	52.10

Each value is the Mean ± SE of six individual observations.
Values are expressed as mg of protein per gram weight of tissue.
%V - Percent Variation, SE - Standard Error.
NS - Not Significant, * P< 0.05, ** P< 0.01, *** P< 0.001

TABLE 3: Protein content in **Gills** of fish, ***Catla catla*** fed with diet containing different levels of Somatotropin (mg/kg diet).

Somatotropin/Kg Diet		Days of feeding Somatotropin along with Diet		
		15 days	**30 days**	**45 days**
Control	**Mean**	34.810	36.660	38.870
	SE	± 0.886	± 0.789	± 0.989
3 mg/kg	**Mean**	36.580 NS	41.320 *	46.120 *
	SE	± 0.833	± 0.890	± 0.884
	%V	5.08	12.71	18.65
6 mg/kg	**Mean**	41.410*	45.270**	51.640***
	SE	± 0.679	± 0.825	± 0.909
	%V	18.96	23.49	32.85
9 mg/kg	**Mean**	47.170***	52.050***	56.570***
	SE	± 0.882	± 0.781	± 0.721
	%V	35.51	41.98	45.54

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Values are expressed as mg of protein per gram weight of tissue.
%V - Percent Variation, SE - Standard Error.
NS - Not Significant, * P< 0.05, ** P< 0.01, *** P< 0.001

AST (Asparate amino transferase) levels in different tissues

Asparate transminase (AST) or Asparate amino transferase, also known as serum glutamic oxalo acetic transminase (SGOT), is a pryidoxal phosphate dependent transminase enzyme. AST catalyzes the reversible transfer of an α-amino group between aspartate and glutamate and, as such is an important enzyme in amino acid metabolism[Mohammad et al 2020][17]. Determination of transaminases has proven useful in the diagnosis of liver damage in fish [Maita M et al 1984, Sandens K et al. 1988][16,25]. Cell injury of certain organs leads to the release of tissue specific enzymes into blood stream [Heath AG 1995, Burtis CA 1996][9,3].The amino transaminases are considered as good sensitive tools for the detection of any variation in the physiological process of living organisms[Tolba MR et al 1997][27]. In fact, when the AST is higher than ALT a muscle source of these enzymes should be considered [Ranjan A et al., 2018][22].

In the present study, AST levels in different tissues like Muscle, Liver and Gills were not much changed when fed with different dosages of somatotropin (3mg/kg diet, 6mg/kg diet and 9mg/kg diet) compared to control (Diet without somatotropin). Josefina Blasco et al., (2021)[12] have revealed that there was not much difference in the levels of AST in Recombinant Bovine Growth hormone (rBGH) treated Gilt head Sea Bream (Sparus aurata) juveniles compared to Control.

TABLE 4: Aspartate aminotransferase (AST) levels in the **Muscle** of fish, ***Catla catla*** fed with the diet containing different levels of Somatotropin (mg/kg diet).

Somatotropin/Kg Diet		Days of feeding Somatotropin along with Diet		
		15 days	30 days	45 days
Control	Mean SE	43.260 ± 0.602	43.640 ± 0.734	44.120 ± 0.771
3 mg/kg	Mean SE %V	43.960 NS ± 0.733 1.62	44.420 NS ± 0.840 1.79	45.110 NS ± 0.942 2.24
6 mg/kg	Mean SE %V	44.270 NS ± 0.689 2.33	44.820 NS ± 0.872 2.70	45.740 NS ± 0.789 3.67
9 mg/kg	Mean SE %V	45.370 NS ± 0.855 4.88	45.870 NS ± 0.737 5.11	48.160 * ± 0.630 9.16

Each value is the Mean ± SE of six individual observations.
Values are expressed as μ moles of pyruvate / mg / hour.
%V - Percent Variation. SE - Standard Error. NS
- Not Significant, * P< 0.05

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TABLE 5: Aspartate aminotransferase (AST) levels in the **Liver** of fish, ***Catla catla*** fed with the diet containing different levels of Somatotropin (mg/kg diet).

Somatotropin/Kg Diet		Days of feeding Somatotropin along with Diet		
		15 days	**30 days**	**45 days**
Control	**Mean**	63.400	63.580	63.610
	SE	± 0.499	± 0.577	± 0.420
3 mg/kg	**Mean**	63.620 ^{NS}	64.020 ^{NS}	64.350 ^{NS}
	SE	± 0.515	± 0.583	± 0.707
	%V	0.35	0.69	1.16
6 mg/kg	**Mean**	64.260 ^{NS}	64.640 ^{NS}	65.640^{NS}
	SE	± 0.753	± 0.629	± 0.741
	%V	1.36	1.67	3.19
9 mg/kg	**Mean**	65.150 ^{NS}	66.550^{NS}	68.560*
	SE	± 0.510	± 0.639	± 0.530
	%V	2.76	4.67	7.78

Each value is the Mean ± SE of six individual observations.
Values are expressed as µ moles of pyruvate / mg / hour.
%V - Percent Variation. SE - Standard Error.
NS - Not Significant, * P< 0.05

TABLE 6: Aspartate aminotransferase (AST) levels in the **Gills** of fish, ***Catla catla*** fed with the diet containing different levels of Somatotropin (mg/kg diet).

Somatotropin/Kg Diet		Days of feeding Somatotropin along with Diet		
		15 days	**30 days**	**45 days**
Control	**Mean**	37.220	37.280	37.330
	SE	± 0.696	± 0.699	± 0.726
3 mg/kg	**Mean**	37.590 ^{NS}	37.670 ^{NS}	37.730 ^{NS}
	SE	± 0.663	± 0.562	± 0.620
	%V	0.99	1.05	1.07
6 mg/kg	**Mean**	37.960 ^{NS}	38.080 ^{NS}	39.850 ^{NS}
	SE	± 0.634	± 0.631	± 0.901
	%V	1.99	2.15	6.75
9 mg/kg	**Mean**	38.520 ^{NS}	39.370 ^{NS}	40.220 *
	SE	± 0.437	± 0.926	± 0.583
	%V	3.49	5.61	7.74

Each value is the Mean ± SE of six individual observations.
Values are expressed as µ moles of pyruvate / mg / hour.
%V - Percent Variation. SE - Standard Error.

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S - Not Significant, * P< 0.05

ALT (alanine aminotransferase) **levels in different tissues**

Alanine transaminase (ALT) is a transminase enzyme also called alanine aminotransferase (ALAT) and was formerly called serum glutamate pyruvate transminase (SGPT). ALT is found in plasma and in various body tissues but is most common in the liver (Liu W et al., 2010) [14]. It catalyzes the two parts of the alanione cycle. Serum ALT level, serum AST level and their ratios (AST /ALT ratio) are commonly measured clinically as biomarkers for liver health. Slightly altered ALT levels do not automatically mean that medical problems exist (Hejazi,2011) [10].

In the present study, ALT levels in different tissues like Muscle and Gills were not shown much difference but significant decrease was observed in Liver at higher dosage (9 mg/kg diet) and longer duration (45 days). Josefina Blasco et al., (2021)[12] a significant decrease was observed in the levels of ALT of Liver in Recombinant growth hormone (rBGH) treated Gilt head Sea Bream (Sparus aurata) juveniles compared to control.

TABLE 7: Alanine Aminotransferase (ALT) in the **Muscle** of fish, ***Catla catla*** fed with the diet containing different levels of Somatotropin (mg/kg diet).

Somatotropin/Kg Diet		**Days of feeding Somatotropin along with Diet**		
		15 days	**30 days**	**45 days**
Control	**Mean**	33.510	34.130	35.410
	SE	± 0.917	± 0.925	± 0.974
3 mg/kg	**Mean**	32.390 ^{NS}	32.920 ^{NS}	34.040 ^{NS}
	SE	± 0.794	± 0.812	± 0.654
	%V	-3.34	-3.55	-3.87
6 mg/kg	**Mean**	31.800 ^{NS}	32.120 ^{NS}	32.250 *
	SE	± 0.906	± 0.759	± 0.836
	%V	-5.10	-5.89	-8.92
9 mg/kg	**Mean**	31.210 ^{NS}	31.320 *	31.540**
	SE	± 0.680	± 0.842	± 0.870
	%V	-6.86	-8.23	-10.93

Each value is the Mean ± SE of six individual observations.
Values are expressed as µ moles of pyruvate / mg / hour.
%V - Percent Variation, SE - Standard Error .
NS - Not Significant, * P< 0.05, ** P< 0.01

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TABLE 8: Alanine Aminotransferase (ALT) in the **Liver** of fish, ***Catla catla*** fed with the diet containing different levels of Somatotropin (mg/kg diet).

Somatotropin/Kg Diet		Days of feeding Somatotropin along with Diet		
		15 days	**30 days**	**45 days**
Control	**Mean**	54.470	54.730	54.840
	SE	± 0.876	± 0.904	± 0.947
3 mg/kg	**Mean**	52.360 ^{NS}	51.620 ^{NS}	50.560 ^{NS}
	SE	± 0.987	± 0.755	± 0.613
	%V	-3.87	-5.68	-7.80
6 mg/kg	**Mean**	48.750*	46.430*	43.790**
	SE	± 0.726	± 0.577	± 0.431
	%V	-10.50	-15.17	-20.15
9 mg/kg	**Mean**	44.210*	41.300**	38.380***
	SE	± 0.473	± 0.700	± 0.656
	%V	-18.84	-24.54	-30.01

Each value is the Mean ± SE of six individual observations.
Values are expressed as μ moles of pyruvate / mg / hour.
%V - Percent Variation, SE - Standard Error.
NS - Not Significant, * P< 0.05, ** P< 0.01, *** P< 0.001

TABLE 9: Alanine Aminotransferase (ALT) in the **Gills** of fish, ***Catla catla*** fed with the diet containing different levels of Somatotropin (mg/kg diet).

Somatotropin/Kg Diet		Days of feeding Somatotropin along with Diet		
		15 days	**30 days**	**45 days**
Control	**Mean**	26.100	26.620	27.120
	SE	± 0.535	± 0.748	± 0.688
3 mg/kg	**Mean**	25.310 ^{NS}	25.760 ^{NS}	26.180 ^{NS}
	SE	± 0.657	± 0.701	± 0.786
	%V	-3.03	-3.23	-3.47
6 mg/kg	**Mean**	24.610 ^{NS}	25.010 ^{NS}	25.450 ^{NS}
	SE	± 0.808	± 0.641	± 0.707
	%V	-5.71	-6.05	-6.16
9 mg/kg	**Mean**	23.810 ^{NS}	24.270 ^{NS}	24.430*
	SE	± 0.809	± 0.779	± 0.881
	%V	-8.77	-8.83	-9.92

Each value is the Mean ± SE of six individual observations.
Values are expressed as μ moles of pyruvate / mg / hour.
%V -

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Percent Variation, SE - Standard Error. NS - Not Significant, * P< 0.05

Conclusion

Based on the results obtained from the present investigation, it was concluded that Somatotropin elevated the Proteins in Muscle, Liver and Gills. But not much difference in AST levels in different tissues of *Catla catla*. Significant decrease was observed in ALT levels of Liver where as not much difference was found in ALT of muscle and gills. Hence, it is clear that the anabolic hormones such as Somatotropin hormone play an important role in enhancing the tissue protein content in fish for the nutritional purpose of Man.

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