Growth and body composition of catfish *Heteropneustes fossilis* fed testes 3X supplemented diet

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Abstract: Supplementation of the minced meat diet with a homoeopathic glandular preparation (Testes 3X) promoted growth of catfish *Heteropneustes fossilis* and increased the tissue concentrations of RNA, protein and glycogen. DNA content per unit weight of tissue declined. Presumably, cellular DNA content remained constant. Testes 3X ingredient caused lipolysis and hydration in the body tissue. RNA/DNA ratio emerged as an index of the living condition of fish. Specific growth rate, Food conversion efficiency and food quotient were enhanced after the supplementation of testes 3X.

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1. Introduction:

Recent years have witnessed that considerable interest in trials of cost-effective dietary additives involving cultivable fishes. Search continues for anabolic and growth – promoting ingredients. Several workers reported the performance of sex steroids in fish (Guerrero, 1976; McBride & Fagerlund, 1976; Fagerlund & McBride, 1977; Matty & Lone, 1979; Fagerlund *et al*., 1979, 1980; Lone & Matty, 1980, 1981). Some of the recent researchers using androgenic growth promoters in fish presented encouraging results (Ankley *et al.*, 2003; Crossin *et al.*, 2010; Khalil *et al.*, 2011). The study reported here was under taken with a view to determine the nutritional efficacy of a homoepathic glandular formulation (Testes 3X). In addition to body weight gains, tissue was also analyzed for their chemical quality. Some of the chemical constituents examined include protein, RNA, DNA, glycogen, fat and water. Mustafa & Zofair (1984) amply emphasized the need to evaluate biochemical composition of tissues and haematological parameters in assessing health and general condition of fish. A number of researcher have investigated the biochemical parameters especially RNA/DNA ratio to evaluate the growth of fish and other animals (Chicharo & Chicharo, 1995; Azuma *et al.*, 1998; Ali *et al.*, 2006; Koueta *et al*., 2006; Glemet & Rodriguez, 2007; Abdel-Tawwab *et al.*, 2013). Danabas & Altun (2005) have studied the effect of testosteroneundeconoate on growth of *Mugil aurata* and found a significant increase in growth rate. Addition of 11 –ketotestosterone to fish meal diet significantly augmented fish growth (Davis et al., 2010). In another study, Montajami (2012) has reported that supplementation of hormone methyltestosterone has promoted the growth rate to a significant level over the control. The RNA/DNA ratio is positively correlated with growth of some fishes (Bullow *et al.*, 1981; Tripathi *et al.*, 2002; Smith and Buckley, 2003; Mukherjee & Jana, 2007; Raksheskar, 2012). Different growth parameters like specific growth rate, food conversion efficiency and food quotient were also determined.

2.Materials and Methods

Live specimens of *Heteropneustes fossilis* (Total lenth 10.5-14 cm and body weight 13.7-14.9 g) were collected from freshwater bodies at Aligarh and transported to well aerated laboratory aquaria. After acclimatizing the specimens for a week during which the stock was maintained on minced meat diet, the entire lot was grouped into two batches (each batch consisting of 20 individuals) and reared in separate aquaria (75x25x30 cm). Fish were fed minced meat at 4% body weight. Food of one batch was supplemented on alternate days with a homoeopathic glandular preparation (Testes 3X) at the rate of 5 mg/g minced meat. Weight of this substance was adjusted by reducing equal weight of minced meat for constant ration size. Unused food was removed and weight at 24 hours interval when water was changed. The experiment was run for 60 days, after which the specimens were taken out, recorded for length and weight and decapitated. Liver and gonad were immediately dissected out and weighed. Muscle sample were also excised without loss of time from the trunk region below the dorsal fin and processed for biochemical assays.

A known amount of fresh tissue (3 g) was placed in preweighed petridish and transferred to a thermostat running at 100□C for 40 hours. The sample was then reweighed. Loss in weight gave the amount of water in the tissue sample which was expressed as percentage. Fat was estimated by the help of soxhlet extraction technique using petroleum ether (boiling point 40-60□C) as followed earlier by

Mustafa & Jafri (1981). Procedure of Ashman & seed (1973) was followed for the extraction of glycogen and was determined by the method of Montgomery (1957).

The protein, RNA and DNA were determined in fat free dry tissue sample. Technique of Webb and Levy (1955) was followed for the preparation of dry fat free samples. Method of Lowry *et al.* (1951) was followed for the estimation of protein. The method described by Schneider (1957) was employed for the extraction and quantification of RNA. This method is based on the reaction of orcinol with pentose sugar of RNA. Quantity of RNA was determined by the help of a calibration curve prepared using yeast RNA as the standard. DNA was extracted from the dry fat free sample by the method of Webb & Levy (1955) and was determined according to the technique of Ashwell (1957). This is a simple and easy method and gives reliable results.

3.Results and Discussion

Specimens of *Heteropneustes fossilis* supplied diet supplemented with testes 3X utilized more food and grew at faster rate. Data pertaining to food intake, food conversion efficiency and growth rate obtained in the laboratory have been embodied in Table 1.

Table 1. Food utilization, growth and condition of *Heteropneustes fissilis* fed Testes 3X supplemented diet

Parameters	Minced meat diet (Control)	Testes 3X supplemented mince meat diet (experimental)
Total gain in body weight (g)	9.0	11.0
% gain in body weight (g)	3.02	4.02
Specific growth rate[a] (%)	0.069	0.092
Total food consumed (g)	376.44	386.06
Gross food conversion efficiency[b] (%)	2.39	2.84
Food quotient[c]	41.82	35.09
Ponderal index[d]	0.613 ± 0.01	0.624 ±0.016
Liver-somatic index[e]	1.479 ± 0.06	1.531 ± 0.181
Gonado-somatic index[f]	0.094 ± 0.013	0.138 ± 0.008

a= 100 (Log_e final weight, g- Log_e initial weight, g) ÷ Days of feeding
b= Wet weight gain, g x100 ÷ quantity of food offered, g
c= Quantity of food offered, g ÷ wet weight gain, g
d= Intact body weight, g x 100 ÷ cube of total length, cm
e= Liver weight, g x100 ÷ intact body weight, g
f= Gonad weight, g x 100 ÷ intact body weight,g

The ingredient increased the appetite only marginally but improved the assimilation efficiency rather appreciably. Interesting biochemical changes in the body occurred and were related to biosynthesis of tissue building and storage substances. Results of biochemical analysis of the white muscle of the test and control specimens have been shown in Table 2.

Table 2. Concentrations of protein, nucleic acids, glycogen, fat and moisture in muscle of control and testes 3X fed specimens of *Heteropnuestes fossilis*. Values are mean ± standard error.

Parameters	Control	Testes 3x supplemented diet
Protein (mg/100 mg)	56.53 ± 1.86	64.70 ± 1.82*
RNA (µg/100 mg)	1218.03 ± 31.48	1406.25 ± 26.94*
DNA (µm/100 mg)	194.26 ± 14.17	159.16 ± 13.06*
RNA/DNA ratio	6.28 ± 0.13	8.84 ± 0.16*
Glycogen (mg/100 g)	137.08 ± 6.06	165. 97 ±6.70*
Fat (%)	1.627 ± 0.084	1.588 ± 0.061
Moisture (%)	77.92 ± 3.29	77.70± 4.26

*Indicate significant difference with control (P<0.05)

Fish fed testes 3X in the diet synthesized more RNA and protein and accumulated more glycogen. The concentration of DNA and fat were, however, lowered. RNA level in the body is influenced by dietary intake (Mustafa and Jafri (1977), Mustafa & Mittal, 1982). But in the absence of significant difference in the ration size of the two batches of fish, the increase in concentration of RNA can be credited largely to the homoeopathic ingredient (Ration quality). The RNA arising in larger quantity is instrumental in turning out greater quantity of protein. Such a quantitative relationship between RNA and protein biosynthesis has been documented earlier by Mustafa and Jafri (1977). Mustafa & Zofair (1983) have even elucidated this macromolecular progression through regression formula. Spurt in metabolic activity following administration of glandular extract containing androgens justifies rise in RNA level if the findings of Mustafa & Shams (1982) vis-à-vis correlation between RNA level and metabolic status of the tissues of catfish *Clarias batrachus* are given credence.

Testes 3X seemed to promote the synthesis of glycogen by way of enhancing the conversion of monosacharides (glycogenesis) or anabolic transformation of the products of catabolism of lipids (glyconeogenesis). Results left no doubt that a small proportion of body fat was broken down on feeding the homoeopathic additive to fish. In view of its protein – sparing action, fat mobilization is no surprise. Such a rescue role of fat for protein has been reported earlier by Takeda *et al.* (1975); Adron *et al.* (1976); Takeuchi *et al.* (1979); Bromley (1980). That the androgen treatment leads to decline in lipid reserve of the body is well known (McBride & Fagerlund, 1976; Fagerlund & McBride 1977; Fagerlund *et al.*, 1979, 1980; Higgs *et al.*, 1982). It is one of the reason why male farm animals are castrated for fattening.

It is clear from the present data that out of the non-protein energy substances, the fish prefers glycogen over fat when supplied exogenous androgen preparation. Rise in water content is consequential. Glycogen accumulation involves deposition of considerable quantity of water. Storage of each gram of glycogen is accompanied by at least three grams of water (Nielsen, 1979). The amount of fat utilized is replaced by water which accompanies glycogen accumulation. The reported negative fat-water relationship (Anon, 1966; Coppini, 1967; Jafri, 1973; Love, 1980) in fish tissue owes to this factor. Mustafa & Jafri (1981) correlated the dynamics of change in glycogen with condition factor of the catfish, *Heteropneustes fossilis*. They explicitly indicated glycogen as an index of robustness or general well being of fishes. High ponderal index and liver somatic index of fish synthesizing more protein and accumulating glycogen confirm these views.

In the light of proven metabolic stability of DNA even under serious conditions (Leslie, 1955; Mustafa & Mittal, 1982) the apparent decline in the amount of DNA/unit weight of tissue sample of specimens of *Heteropneustes fossilis* can not be considered as a result of negative interaction of this nucleic acid with the ingredient of Testes 3X. A survey of literature in the field of molecular biology

does not reveal any evidence of disruption of DNA synthesis by the male gonadal hormone. Stability of DNA is commensurate with its genetic role. Decrease in the concentration may be a consequence of the smaller number of cells/unit weight of tissue sample. Cells of fish fed Testes 3X are more robust, being rich in RNA, protein, glycogen, and must certainly be heavier and more voluminous that the cells of other batch of fish. As a matter of fact a smaller number of these cells form a unit weight of tissue processed for chemical assay compared to a larger number of the less healthier cells of smaller weight and volume. Thus it is no surprise that a given weight of muscle sample of control fish constituted of greater number of cells yields higher concentration of DNA. The influence of cell number on DNA concentration has been thoroughly worked out by Bulow (1981), Mustafa & Jafri (1977), Mustafa (1977a,b), Buckley (1980), Mustafa & Mittal (1982), Mustafa & Shams (1982), Mustafa & Zofair (1983). Dagg & Littlepage (1972) showed that increased growth is represented by increase in protein and a decline in the DNA content. With constant DNA and increased RNA, higher RNA/DNA ratio in Testes 3X fed fish expected. Inasmuch as RNA level is related to protein synthesis and growth as described earlier in the discussion, the RNA/ DNA ratio serve well to indicate recent growth rates and biological condition of the fish. Buckley (1984) has stated that the high ratios could indicate either healthy, well fed, rapid growing fish, and those under stress have low ratios. Thomas & Diwan (1990) has explained the high ratios as a good indicator of the better nutritional status of the animals. A higher gonadosomatic index of test fish signified that the homoeopathic additive supplies ingredients which promote development of gonads.

4.Conclusion

The present study revealed that the homoeopathic glandular formulation has an anabolic effects on fish which is evident from the better growth rate showed by fish fed testes 3X supplemented diet. Along with the increase in weight the biochemical parameters were also improved. The accumulation of macromolecule like protein, RNA and glycogen in the white muscle of the fish fed testes 3X added diet was enhanced. Hence, the testes 3X can be used as a growth promoter in fish.

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