Role of Dietary Administered Hormones on pattern of excretion of ammonia and orthophosphate in holding water in C. punctatus culture system

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Abstract— Fish were fed diet containing 17 Alpha methyltestosterone (MT) at doses 0 (control), 0.5, 1.0 and 2.0 mg/kg of diet and thyroxine (T_4) at doses at doses 0 (control) 20, 50 and 100 mg kg-1 of diet for 80 days . Fish administered with 17 Alpha methyltestosterone (MT) in feed exhibited increased growth (in terms of mean increase in length, live weight gain, growth percent gain in body of weight and specific growth rate) with increase in the dose of MT from 0.5 to 1.0 mg/kg body weight and thereafter further increase in the dose of MT no improvement in growth was observed indicating 1.0 mg of MT/kg of diet appear to be optimum for growth performance in C. punctatus. Significantly (P <0.05) low amounts of Ammonia and Phosphate in the holding water in comparison to the fish fed on low dose of MT (0.5 mg) or control. T4 administration in diet at lower doses (50 mg kg-1) significantly P<0.05) enhanced growth in C. punctatus. Similarly Significantly (P <0.05) low amounts of Ammonia and Phosphate in the holding water in comparison to the fish fed on low dose of T_4 (50 mg) or control. Use of hormones as food additives can reduce the post prandial excretion of Ammonia ($N\text{-}NH^{+}_4$) and orthophosphate ($O\text{-}PO^{-}_4$) in holding water which enhances the growth performance of slow growing fish species.

I. INTRODUCTION

The main purpose to study the exceretory pattern of Ammonia ($N\text{-}NH^{+}_4$) and orthophosphate ($O\text{-}PO^{-}_4$) in holding water depends on dietary intake and how much dietary protein incorporated in fish it also has ecological and environmental relevance. The amount of residue deposited into the rearing tanks has increased significantly Due to the great intensification of fish farming. Nitrogen (N) and phosphorus (P) are the main end-products of fish culture, and can affect not only the rearing water, but also the environment as a whole. The oral administration hormones 17 Alpha methyltestosterone (MT) and thyroxine (T4) in fish feeds is a good alternative which can help to reduce Ammonia (NH4-N) and orthophosphate ($O\text{-}PO^{-}_4$) waste. The balance diet should be standardized and the N and P excretion rates in several rearing systems (mainly the intensive farms) should be measured since a two- to three-fold decrease in the excretion of those pollutants in the fish culture

Ammonia production by fish is primarily dependent on the protein intake and metabolic efficiency of the fish, which is species specific and also affected by waterborne ammonia, levels (Dosdat, 2003). The rate of postprandial ammonia excretion in fish culture fish has been found to be dependent on dietary protein levels (Jayaram and Beamish, 1992) Most fish eat protein-rich diets and ammonia is, metabolically, the least expensive means of removing the N produced by deamination of amino acids (Carter and Brafield, 1992). It means the amount of nitrogen excreted is related to the different N content of the diets. Freshwater species tend to excrete more total ammonia nitrogen (TAN) than marine species (jobling, 1995). The postprandial excretion rate of ammonia in culture fish was dependent on dietary protein levels (Jayaram MG, Beamish FWH, 1992). Influence of dietary protein and lipid on nitrogen and energy losses in lake trout, Salvenalis namayeush,1992 . The effect of high temperature and diet protein level on metabolic utilization of diets by rainbow trout.observed by Olva-Teles Aand Rodrigues AMin 1995. Similar effect was observed by Medale et .al 1995 in rainbow trout.dietary protein/energy ratio, ration size, dietary energy source and water temperature on nitrogen excretion in rainbow trout was observed by Medale et .al 1995.Cowey, 1995 observed the net retention of dietary nitrogen by fish is quantitatively similar to that of omnivorous birds and mammals (40–50%) so that up to 60% of assimilated N is excreted in water in soluble form, and causes

eutrophication and it is difficult to reduced by conventional dietary The need to formulate diets which minimize fish P excretion and consequent eutrophication of the water requires the replacement of fish meal with low-P protein sources (LALL, 1991). The use of high protein ingredients that have a high percentage of digestible P may help to reduce the unavailable P concentration of the feed (CHO et al., 1994). Diurnal pattern of ammonia excretion in fed and starved rainbow trout (29 to 70 g at 14 °C) was studied by Rychly and Marina in 1977.

(Kalla, 2002) reported two peaks in the diurnal graph when ammonia was sampled every 2 h for the general pattern of ammonia remains fluctuating. Postprandial pattern of ammonia and phosphate excretion reported that their production in the holding water was greater in diets with animal origin protein in comparison to plant origin proteins (Garg.et.al., 2002). Most of the studies have revealed that the growth performance/rate is less when ammonia and phosphorus excretion is high in holding water (Kalla, 2002 and Rajaharia, 2008). (Rjaharia, 2008) has reported that even in plant protein based diets optimum level of calcium and magnesium are essential for decreasing nitrogen and phosphorus excretion in holding water, in case of culture of Cirrhinus mrigala. Daily periodicity of ammonia and orthophosphate excretion in the fish Catla catla with different levels of inclusion of probiotics Bacillus coagulans in processed soybean and Duck weed based diets (approx. 40 percent protein)studied by.(Raparia and Bhatnagar, 2016)An alternative technique to improve fish health, combat pollution in the holding water and to improve fish growth.

These results clearly indicate that protein source, its level of inclusion and dietary addition of hormones at different doses play a important role in the management of excretory levels of ammonia and phosphorous which in turn improving growth performance of fish. Therefore, aim of present studies is to determine diurnal excretory patterns of Ammonia (N-NH+4) and orthophosphate (O-PO-4) in holding which help to evaluate the relationship of potential waste with fish production. Decrease the potential waste load of farm effluents enhances the growth of slow growing fish species.

II. MATERIAL AND METHODS

Specimens of *C. punctatus* (BW 4.80-4.86) were obtained from fish dealers of Hisar. Fish were placed in transparent glass aquaria (60x30x30 cm) kept in laboratory where the temperature was maintained at 25^{0C}±1('C and the lighting schedule at 12h of light (08°°-20°°h) alternating with 12h of darkness (20°°- 08°°). The average intensity of light inside the laboratory was approximately 1000 lux. Fish were acclimated in the laboratory for a minimum of seven days prior to the initiation of experimental treatments and were fed ad *libitum* on a feed containing fishmeal as the protein source between 16°° and 19°°h. The water in the aquaria was renewed daily with water which had been previously equilibrated to the desired temperature (25°C).Experiments were conducted in an air-conditioned laboratory under a light regimen of LD 12:12 at 25°C.

Preparation of diets: Ingredients were ground to pass through 0.5 mm sieve prior to the addition of hormones and analysis for proximate composition. Soybean seeds were cleaned , autoclaved for 15 minutes at 121°C (15 Ibs) to remove ANFs and used as the protein source without adversely affecting the growth (Garg et al., 2002), dried in an oven maintained at 60°C. Other source of diet were mixed in certain ratios (Groundnut oilcake 375.5),(Processed soybean 112.5),(Rice bran 25.0),(Wheat flour, 25.0). Simultaneously proximate analysis was also conducted for moisture,crude protein, crude fat, crude fiber, ash NFE and phosphorus. Each kg contains: Copper 312 mg. Cobalt 45 mg, Magnesium, 2.114 g, Iron 979 mg, Zinc 2.130 g, Iodine 156 mg, DL-Methionine 1.920g, L-Lysine Mono Hydrochloride 4.4 g. Calcium 30%, Phosphorus 8.25% (Table 1). The pelleted diet supplemented with MT hormone at doses of 0.5, 1.0 and 2.0 mg/kg of diet and thyroxine at doses of 50.0, 100.0 and 150.0 mg/kg. Fish were fed at the rate of 3% per day body weight for 80 days. Control group was also fed with diet without hormone for 80 days. The ration size was adjusted at every 15 days intervals after bulk weighing of the fish. Individual weight and length of fish was recorded at the beginning and at the end of experiment for the determination of condition factor. Water samples were collected 6-8 hr post-feeding for the determination of Ammonia and Phosphate levels from the holding waters following APHA (1998). Duncan Multiple Range Test (Duncan, 1995) was used to evaluate the differences among treatment groups at the 0.05 level of significance. Group means were compared by Students 't' test (Snedecor and Cochran, 1982).

Individual weight of fish was recorded at the beginning and end of the experiment and also at the 15 day interval with the help of a top panelectronic balance (Make, AFCOSET FX-1200). In order to maintain the optimum water quality, temperature, pH and Dissolved oxygen (DO) were regularly monitored. Water samples were collected 6-8 h post-feeding to determine $N\text{-}NH^{+}_{4}$ and $O\text{-}PO^{-}_{4}$ levels from the holding water as follows (APHA, 1998):

Determination of Ammonia-nitrogen ($N\text{-}NH^{+}_{4}$)

The ammonium ion reacts with alkaline solution of Nessler's reagent (potassium mercury iodide) to form a yellow brown colored complex of ammonium mercury iodide. The light absorption is measured at wavelength of 425 nm. Rochelle salt solution: Dissolve 50g of potassium sodium tartrate tetra hydrate (KNaC4H4O6 .4 H2O) in 100 ml distilled water. Remove the ammonia by boiling off approximately 30 ml of the solution. After cooling make the volume to 100 ml by adding more distilled water.

Nessler's Reagent: Dissolve 1 00g HgI2 and 70g of KI in a little distilled water-A. Dissolve 160g of NaOH in little distilled water-B. Mix solution A and Band make the volume to IL by adding distilled water. Store it in a rubber stoppered amber colored bottle. Stability of this solution is I year. Standard stock ammonium solution: Dissolve 0.831g anhydrous NH4Cl, (dried at 100°C) in distilled water and make the volume to 1L by adding distilled water.

50 ml of filtered water sample was taken in erlenmeyer flask. Then, added 2 drops of Rochelle salt solution and mixed it well. Added 2 ml of Nessler's reagent and waited for 10-20 minutes for colour development and the light absorption was measured at wavelength of 425nm. The concentration of NH-4 N was deduced from the standard curve prepared by dissolving NH 4Cl (E.Merk) in distilled water to prepare standard ammonia solution of 1.0 mg L-1 (concentrations 0.2-1.0mgL-1).

Note : Use ammonia free water for preparation of all reagents. Total ammonia excretion was calculated by using the following formula:

$$\text{Total ammonia excretion} = \frac{\mathrm{NH_4{-}N(mg\,l^{-1})in\ aquarium\ water}}{\text{Fish body weight(kg)per L of water}}$$

Determination of orthophosphate (soluble reactive phosphorous)

The orthophosphate (O-PO-4) reacts with acidified ammonium molybdate solution and form molybdophosphoric acid, which is then reduced to a blue complex in the presence of stanuous chloride. This is measured spectrophotometrically at 690 nm.

Ammonium molybdate strong acid solution: Dissolve 5g of Ammonium Molybdate (NH4)6 Mo7 O24• 4H2O) in 3 5 ml of distilled water. Add 62 ml of cone. H2SO4 to 80ml of distilled water, cool and add the molybdate solution and make the volume to 200 ml by adding more distilled water.

Stannous chloride solution: Dissolve 0.5g SnCl2.2H2O in 2 ml conc. HCl. Make the volume to 20 ml with distilled water. Stock phosphate solution for standard curve: Dissolve 219.5mg anhydrous KH2PO4in 1000 ml distilled water-stock solution. 25 ml of the water sample was taken in a conical flask (100 ml). Also run distilled water blank simultaneously. Added 1 ml ammonium molybdate solution, 3 drops stannous chloride solution and shook the contents. Blue colour appeared, waited for 10 minutes and took the reading on spectrophotometer at 690 nm and deduced the value of O-PO4 mg l-1 with the help of a standard curve.

Reactive phosphate excretion was calculated by using the following formula:

$$\text{Reactive phosphate excretion} = \frac{\mathrm{O{-}PO_4(mg\,l^{-1})in\ aquarium\ water}}{\text{Fish body weight(kg)per L of water}}$$

IV. RESULT

Fish administered with 17 α methyltestosterone (MT) in feed exhibited increased growth (in terms of mean increase in length, live weight gain, growth percent gain in body weight and specific growth rate) with increase in the dose of MT from 0.5-1.0 mg per kg, and thereafter, with further increase in the dose of MT no improvement in growth performance was observed, indicating 1.0 mg of MT per kg of diet appear to be optimum for growth performance in *C. punctatus*. Significantly, ($P<0.05$) highest percent gain in body weight was observed in fish fed on a diet containing 1.0 mg of MT (108.64%), which was only marginally low (103.66%) in the fish fed on 2.0 mg of MT (Fig. A).

T4 administration in diet at lower doses (50 mg kg-1) significantly P<0.05) enhanced growth performance (Live weight gain, per cent gain in body weight and SGR) in C. punctatus. Feeding the fish on higher dose of T4 (100 and 150 mg kg-1) resulted in low growth, which was evident in lower gain in both weight and length values versus control group. (Fig. B).

Effect of fish fed on methyltestosterone administered diet on metabolite excretion

Effect on $N\text{-}NH^{+}_{4}$ excretion and $O\text{-}PO^{-}_{4}$ production: fish fed on MT @ 1.0 or 2.0 mg per kg of diet, excreted significantly (P<0.05) low amounts of $N\text{-}NH^{+}_{4}$ and $O\text{-}PO^{-}_{4}$ in the holding water in comparison to the fish fed on low dose of MT (0.5 mg) or controls fed on hormone free diet (Table 2).

Effect of fish fed on thyroxine administered diet on metabolite excretion

Fish given T_4 at lowest dose excreted significantly (P<0.05) low levels of $N\text{-}NH^{+}_{4}$ and $O\text{-}PO^{-}_{4}$ in the holding water in comparison to the fish fed on higher dose of T_4 (100 and 150 mg kg-1) or control fed on hormone free diet (Table 3).

V. DISCUSSION

The water quality deterioration is the major problem because of accumulation of metabolic wastes like ammonia and orthophosphate excretion in the holding water. Use of hormone reduces the quantity of ammonia and nitrite in water which is utilized in enhancing the muscle proteins. The administration of more than 1.0 mg/kg of MT was observed to decrease the excretion of ammonia and increase nitrogen retention in Juvenile O. masou (Santandreu and Diaz, 1994). Present results on C. punctatus have also revealed a decrease in the excretion of ammonia and O-PO-4 production as result of MT administration (0.5-1.0 mg kg-1 of diet).

The results of the present study also show that administration of MT and thyroxine significantly reduced the ammonia excretion and increased nitrogen retention and hence suggest a reduction in the degradation of body proteins. In mammals also similar responses have been observed in relation to the decrease in the excretion of nitrogenous compounds (Kochakian, 1935: 1950a,b; Lewis et al., 1981;). Further studies revealed that incorporation of probiotics in the diet further decrease the excretion of ammonia and phosphate in the holding water because of better nutrient utilization (Sumagaysay-Chavoso, 2003, Bhatnagar and Sushma, 2008 and Bhatnagar and Raparia, 2014).

T4 treatment also decreased nitrogen and phosphate excretion in fish fed on a diet containing @ 50-100 mg kg-1 of T4. At higher doses an increase in excretory level of N-NH4+ was observed. Smith and Thorpe (1977) have also observed that administration 0.25-5.0 mg kg-1 of T4 reduced nitrogen excretion in rainbow trout. This may again attribute to low feed utilization and when dietary utilization is low deamination of unutilized feed protein occurs and excretion of metabolites in the holding water increases. These result are justified by previous findings of Kallaand Garg 2004 and Jindle and Garg 2005 and Jindle, 2011.

In conclusion, the growth and digestibility parameters were found to be negatively correlated with (N-NH+4) and O-PO-4. A comparison of weight gain in fish administered with diet containing 17 alpha MT and thyroxine show low excretion of metabolites is related to maximum growth performance. (Table 2 and 3)(Fig A and B)

The use of 17 α-methyltestosterone and thyroxine incorporated diet in C. punctatus appears to be promising from the standpoint of improving growth and feed conversion under optimal and advantageous for fish culture and it also reduces the excretion of N-NH+4 and O-PO-4 in the holding water.

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Table 1 : Composition of experimental diet

[36] Ingredients	[37] g in food
[38] Groundnut oilcake	[39] 375.5
[40] Processed soybean	[41] 112.5
[42] Rice bran	[43] 25.0
[44] Wheat flour	[45] 25.0
[46] Mineral premix and amino acids (MPA)*	[47] 5.0
[48] Chromic oxide	[49] 5.0
[50] *Each kg contains: Copper 312 mg. Cobalt 45 mg, Magnesium, 2.114 g, Iron 979 mg, Zinc 2.130 g, Iodine 156 mg, DL-Methionine 1.920 g, L-Lysine Mono Hydrochloride 4.4 g, Calcium 30%, Phosphorous 8.25%	

Table 2: Effect of feeding 17 α methyltestosterone (MT) on nutrient retention and postparandial excretory levels of total ammonia (N-NH+4) and reactive phosphate production (O-PO-4) in C. punctatus -80 day treatment (LD 12:12 at 25°C)

[51] [52] Parameters	[53] Dietary level (mg per kg)			
	Control	**0.5**	**1.0**	**2.0**
$N\text{-}NH^{+}_{4}$ (mg KG^{-1} BW d^{-1})	$56.18^{A}\pm0.951$	$50.47^{B}\pm0.47$	$15.44^{C}\pm0.33$	$15.40^{C}\pm0.20$
$O\text{-}PO^{-}_{4}$ (mg KG^{-1} BW d^{-1})	$42.78^{A}\pm0.06$	$39.82^{B}\pm0.87$	$22.45^{C}\pm0.70$	$23.18^{C}\pm0.72$
Values are mean ±SE of mean of three observations. Means with the same letter/s in the same row are not significantly (P<0.05) different. Data were analysed by Duncan's multiple range test.				

Table3: Effect of feeding thyroxine (T_4) on post parandial excretory levels of total ammonia ($N\text{-}NH^{+}_{4}$) in *C. punctatus* -80 day treatment (LD 12:12 at 25°C)

Parameters	**Dietary level (mg kg^{-1})**			
	Control	**50.0**	**100.0**	**150.0**
$N\text{-}NH^{+}_{4}$ (mg KG^{-1} BW d^{-1})	$92.88^{B}\pm3.15$	$58.05^{D}\pm0.30$	$85.62^{C}\pm0.57$	$182.34^{A}\pm2.92$
$O\text{-}PO^{-}_{4}$ (mg KG^{-1} BW d^{-1})	$85.03^{C}\pm0.21$	$37.94^{D}\pm1.11$	$113.19^{B}\pm2.17$	$183.04^{A}\pm9.73$
Values are mean ±SE of mean of three observations. Means with the same letter/s in the same row are not significantly (P<0.05) different. Data were analysed by Duncan's multiple range test.				

Figure: A

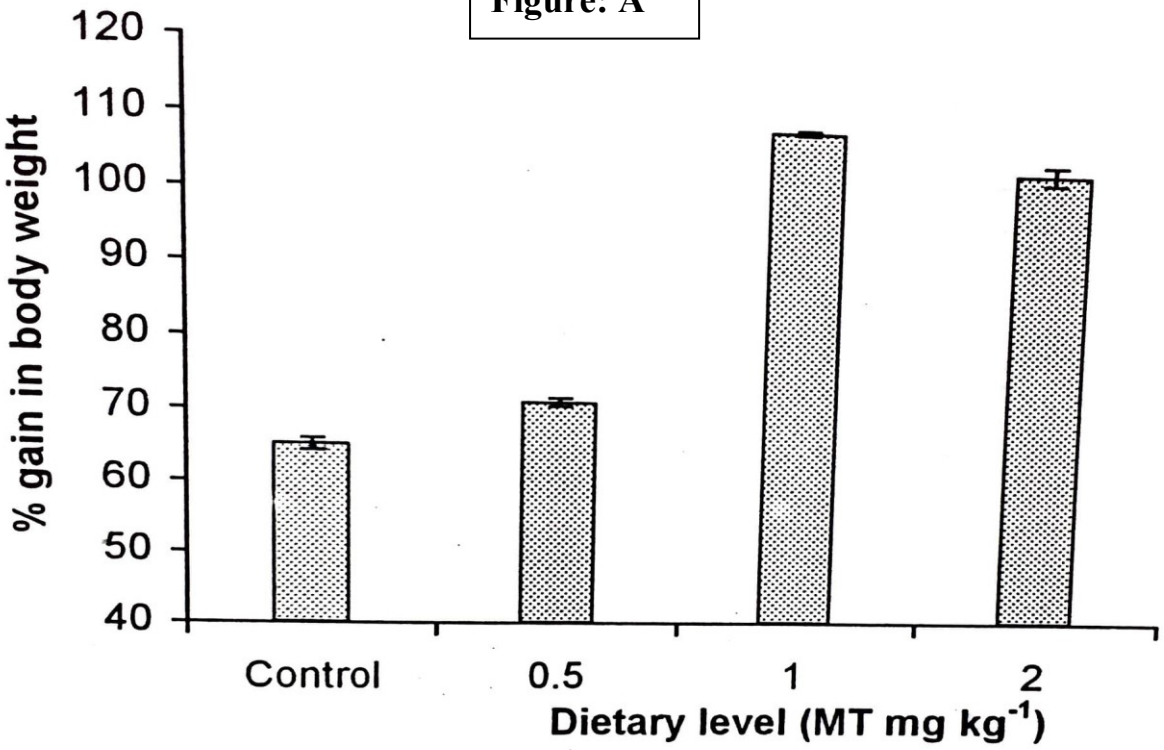

Figure A : Effect of oral administration of 17 alpha methyltestosterone on percent gain in body weight *C. punctatus*

Figure: B

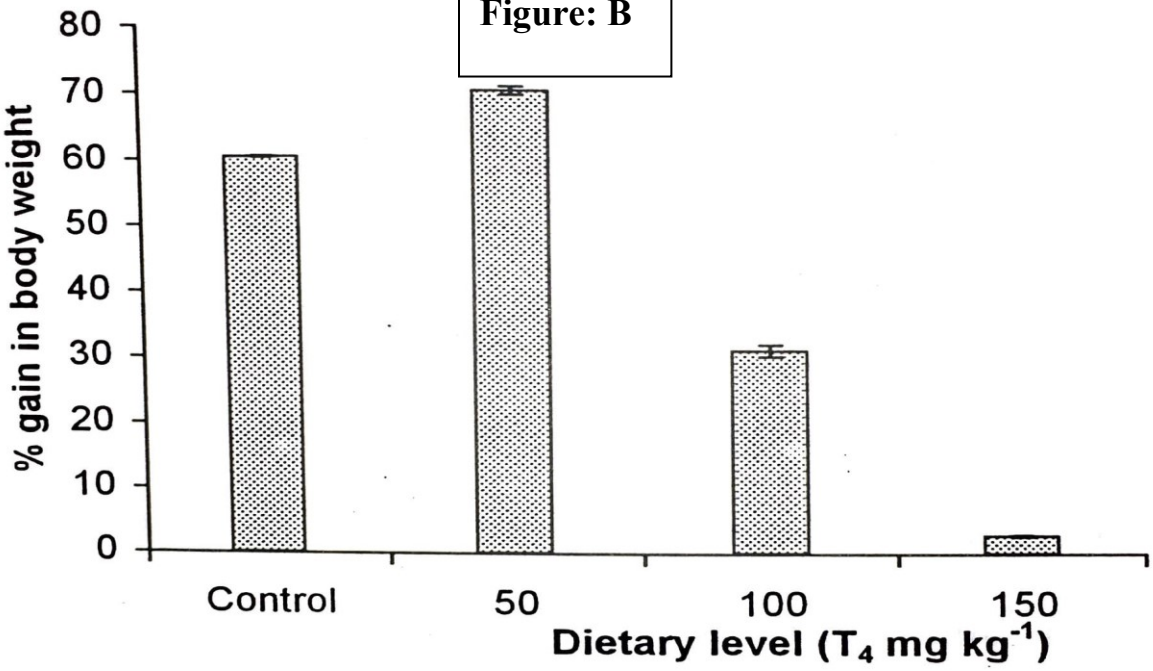

Figure B : Effect of oral administration of thyroxine (T_4) on percent gain in body weight in *C. punctatus*