TECHNICAL REPORT
OF
CONTRACT RESEARCH PROJECT

Title of the Project

Transgenic Bt-cotton seed (JKC 738 Bt., Event 1, containing *Bacillus thuringiensis* Cry 1Ac [truncated] gene) feeding studies in Indian major carp, *Cirrhinus mrigala*

11th June, 2004 to 31st March, 2005

Name of the Principal Investigator

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Name of the Co-Principal Investigator(s)

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Performing Laboratory

Aquafeed Laboratory of Fish Nutrition and Biochemistry Division
Central Institute of Fisheries Education
(Deemed University),
Versova, Mumbai-400 061 (MS), India

Laboratory Project ID

Study : ALNUBI-CR-2004-05
Experiment : MOU-CR/CIFE-JKAGEL/ALNUBI/2004/1

Abbreviation

ALNUBI	:	Aquafeed Lab. of Nutrition & Biochemistry Division
Bt.	:	*Bacillus thuringiensis*
$CaHPO_4$	:	Calcium Hydrogen Phosphate
CCC	:	Laboratory Control Cotton Cake
CIFE	:	Central Institute of Fisheries Education
CR	:	Contract Research
DO	:	Dissolved Oxygen
FCR	:	Food conversion ratio
FER	:	Feed Efficiency ratio
g	:	Gram
GH	:	Gram Hull
ID	:	Identification
IU	:	International Unit
JKAGEL	:	JK Agri Genetics Limited
mg	:	Milligram
MM	:	Mineral Mixture
MOU	:	Memorandum of Understanding
MP	:	Maize Powder
ND	:	Not detected
NTGC	:	Non-transgenic Cotton Seed
PCC	:	Parental Control Cotton Seed
PER	:	Protein Efficiency Ratio
RB	:	Rice Bran
RBD	:	Random Block Designing
SBM	:	Soybean meal
SE	:	Standard Error
SPSS	:	Statistical Package
TGC	:	Transgenic Cotton Seed
VM	:	Vitamin Mixture
WB	:	Wheat Bran
µg	:	Microgram

1.	Title of the Project	:	**Transgenic Bt-cotton seed (JKC 738 Bt., Event 1, containing *Bacillus thuringiensis* Cry 1Ac [truncated] gene) feeding studies in Indian major carp, *Cirrhinus mrigala***
2.	Name of the Investigator(s)	:	1. **Dr. S. C. Mukherjee, PI** 2. **Dr. P. P. Srivastava, Co-PI** 3. **Dr. K. K. Jain, Co-PI**
3.	Research Scholar (for 3 months each)	:	1. **Mr. B. Ravi Kanth** (Period 05.08.2004 to 05.11.2004) 2. **Mr. Thulasida, G.** (Period 18.11.2004 to 17.02.2005)
4.	Implementing Institution	:	Central Institute of Fisheries Education* (Deemed University), Versova, Mumbai-400 061 (MS), India
5.	Laboratory/Division involved in experimentation	:	Aquafeed Laboratory of Fish Nutrition and Biochemistry Division
6.	Funding Agency	:	M/s. J. K. Agri Genetics Ltd., 1-10-177,4th Floor, Varun Towers Begumpet, Hyderabad – 600 016 (India)
7.	Date of Contract Research undertaken	:	11th June, 2004
8.	Tenure of Contract Research	:	From June 11, 2004 to March 31, 2005
9.	Date of completion of Contract Research	:	31st March, 2005
10.	Submission of draft report	:	17th March, 2005
11.	Submission of final report	:	31st March, 2005

NOTE: ***Record retention** : All study specific raw data; protocols, and final technical reports will be retained at the implementing institute and it will be confidential document until the sponsor approves release for publication.

Names and signature of investigator(s)

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Principal Investigator

(P. P. Srivastava)
Co-Principal Investigator

(K. K. Jain)
Co-Principal Investigator

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Annexure - I

Objectives :

Objectives as stated in the Contract Research Project proposal from JK Agri Genetics Limited, Hyderabad.

1. To evaluate the genetically modified cotton seed (JKC 738 Bt.), Event 1, containing Cry 1Ac (truncated) gene, as a feed ingredient for Indian major carp, *Cirrhinus mrigala*.

2. To study the comparative growth and survival on feeding of given samples of cotton seed as :
   - A : Bt. Cotton variety JKC 738 (with Cry 1Ac [truncated] gene) (Plate-1) **(TGC).**
   - B : Bt. Cotton variety JKC 738 (without Cry 1Ac [truncated] gene) (Plate-2) **(NTGC).**
   - C : Parental Control Cotton Seed (Parental control) (Plate-3) **(PCC).**
   - D : Control Cotton Cake (Laboratory control) (Plate-4) **(CCC)**

Purpose :

This contract research was designed to assess whether raw cotton seed meal (Plate-1) derived from Bt. Gene (JKC 738 Bt., Event 1 with Cry 1Ac [truncated] gene) incorporated cotton plant is as safe and nutritious for growth of the Indian major carp mrigal (*Cirrhinus mrigala*) as the meal derived from the non-Bt. gene (without Cry 1Ac [truncated] gene) raw parental cotton-seed meal (Plate-2,3).

In the present study the assessment was carried out between Transgenic cotton (TGC) and Non-Transgenic Cotton (NTGC) in terms of growth, survival and biochemical composition and histopathological studies of Indian major carp (*Cirrhinus mrigala*). As told by JK Agri Genetics the insect protected cotton lines have been modified to express the protein from *Bacillus thuringiensis* which

has insecticidal activity against certain insect pests. The Bt-protein is specific to the targeted insect pests of cotton. The processed cotton seed meal will be incorporated into the fish feed on an iso-nitrogenous basis in a manner analogous to current practices.

Molecular Biology of the Plant and Transformation Methods

Description of the transformed plant materials

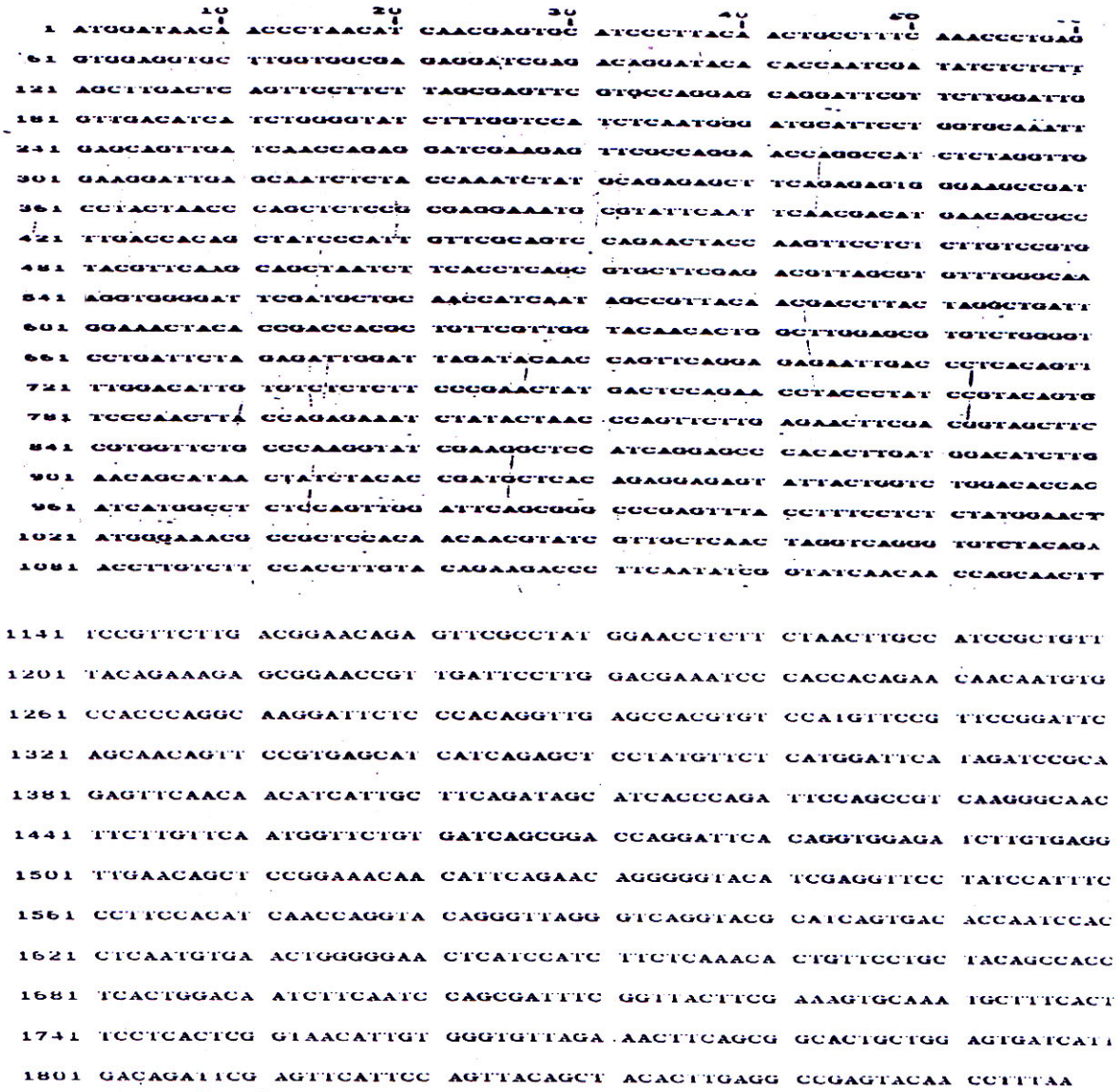
Cotton (*Gossypium hirsuitum*, tetraploidy, belongs to the family Malvaceae) var. JKC-730 and JKC-738 were modified to express the insecticidal protein of Cry1Ac gene (truncated) from the bacteria *Bacillus thuringiensis* for controlling the lepidopteron insect pests. Based on the Containment Green House data and other laboratory generated data through ELISA, insect bioassay study, plant morphological study, etc., JKC 738 (Event-1) found to be superior than all the three events of JKC 730. Besides, presence of single copy gene and kanamycin being selectable marker, JKC 738 is being used either directly as one of the parental line for breeding program or backcrossed to other suitable parental lines to make several JKAL Cotton hybrids namely **JK-Varun Bt, JKCH-666 Bt, , JK-Durga Bt, JKCH-99 Bt, JKCH-226 Bt and JKCH-634 (Ishwar) Bt** were modified to express the insecticidal protein of Cry1Ac gene from the parental Bt line, JKC-738 Bt.

Source of the gene and the cloning strategy followed

(i). Cry1Ac gene : Truncated Source : BREF – BIOTEK, IIT, Kharagpur vide following references.

a. Indian J. Exptl. Biol Vol. 29, 1002-1009 (1991)

Proc. Natl. Acad. Sci. (USA) 92: 2111-2116 (1997)



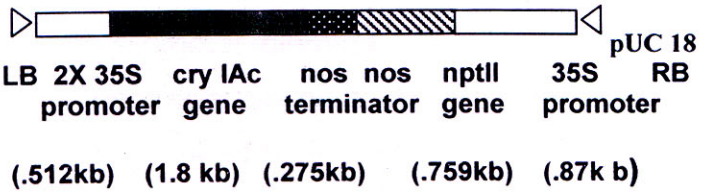
(ii) Agrobacterium T-DNA border sequences:

Source: pTi Ach5 strain of Agrobacterium tumefacieus. The border sequences of RB and LB of the T-DNA were cloned in the lab and their potentials were judged in the laboratory (Ref: Ind. J. Exptl. Biol. Vol 29, pp 991-1001 (1991).

Cloning Strategy:

The chimeric genes were cloned in pUC18 vector and used for biolistic gene delivery system.

Diagram of Cry1Ac Construct 1:



Construct 2:

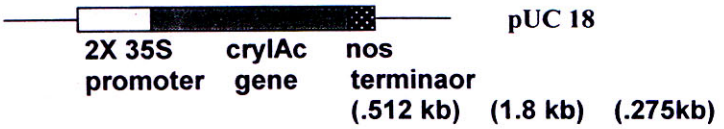
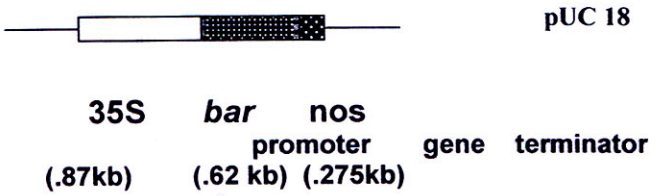


Diagram of *bar* gene construct 3:



Characteristics of the plant expression vector

- Constitutive plant promoter CaMV 35s element has been made use for plant expression.
- A modified CaMV 35s promoter has been used to express the truncated Cry1Ac gene (vide construct 1 & 2), where as the classical CaMV 35s promoter has been used to express the *npt II* gene and *bar* gene in plant (vide construct 1 & construct 3).

Characteristics of the inserted genes with sequence details

The sequences of the reconstructed Cry1Ac gene have been indicated in item (i).

The other bacterial genes, viz., *npt II* and *bar* maintained their native gene sequences.

Characteristics of the vectors and the transformation system employed with description of sequences used.

- The CaMV 35s promoter element has been modified in the laboratory. Two doses of enhancer element of the CaMV 35s promoter has been added to the core element of the CaMV 35s promoter making up for a 512 bp promoter element. This modified promoter has been used to express the Cry1Ac gene.
- For *npt II* and gene, the native CaMV 35s element has been used for plant expression.
- Transformation has been carried out with the help of a particle gun delivery system (BioRad 1000/He Biolistic gun). In case of *bar* gene as selectable marker, co transformation principle has been maintained with Cry1Ac gene. The transformation protocol has been novel and the transfer of the alien genes have been accomplished directly to the cotton cultivars.

Protein Data

Bt Cotton seed provided by JK Agri Genetics Ltd. to CIFE, Mumbai, contains 37.50µ Bt protein /gm of seeds. This estimation is (a mean of individual 5 seeds) made using ELISA kits procured from ENVIROLOGIX, USA.

A. ***Test Material*** : The test material was defined as a cotton meal derived from Bt-gene (JKC 738 Cry 1Ac [truncated] gene) cotton, ID # as Bt. cotton variety TGC, JKC 738 (Plate-1).

B. ***Control Material*** : The control material was defined as the cottonseed meal derived from the control, same germplasm cotton line (without Cry 1Ac [truncated] gene) and ID # as non-Bt. Cotton variety NTGC without Cry 1Ac [truncated] JKC 738 (Plate-2).

C. ***Parental Control Material*** : The parental control material was identified as the Hybrid cotton seed with Bt-gene (JK Bt./JKCH Bt.) and ID # as PCC (Plate-3).

D. ***Laboratory Control Material*** : The laboratory control material was identified as the cotton seed cake from market and its ID # mentioned as CCC (Plate-4).

Samples of cottonseed meal of the test and control materials were evaluated for anti-nutrient contents to determine gossypol level. They were kept frozen and were powdered to 0.5 mm (500 micron) size particle using Cyclotec (1093 sample mill, Tecator Foss, Sweden) and used for feed preparation.

Scope :

To evaluate the effect of TGC (with HD 73 Cry 1Ac [truncated] gene) cotton on the growth of carp fish, mrigal in comparison to NTGC (without Cry 1Ac [truncated] gene) and their use in aquaculture nutritional practices with safety measures.

Annexure - II

Material and Methods :

Experimental diets (Table-1) were formulated to contain crude protein @ 34.0-35.1%. Experimental diets were formulated by substituting cotton seed meals (JKC 738 with Cry 1Ac [truncated] gene) @ 10%, 20% and 30% (F_1 contained gossypol 0.059%; F_2 contained gossypol 0.126% and F_3 contained gossypol 0.238%). The other three NTGC incorporated feeds F_4, F_5 and F_6 contained cotton seed meals (JKC 738 without HD 73 Cry 1Ac [truncated] gene) @ 10%, 20% and 30% (F_4 contained gossypol 0.045%; F_5 contained gossypol 0.139% and F_6 contained gossypol 0.272%). The gossypol contents of parental control cotton PCC was used @ 10%, 20% and 30% in F_7, F_8 and F_9 respectively. The gossypol contents in F_7, F_8 and F_9 were 0.062%, 0.127% and 0.217% respectively. Similarly, control cotton cake gossypol was estimated in all the three feeds (D_{10}, D_{11} and D_{12}) and the contents were recorded as 0.071%, 0.142% and 0.263% respectively. The gossypol contents in cotton seeds and all the twelve feeds are estimated and tabulated in table 2 & 4 respectively, following the analytical methods "Ba 8-78 Official Methods" and recommended practices of 'American Oil Chemists Society', 4th Edition Firetone, D. Ed. AOCS, Champagne, IL, USA, 1989. The basal ingredient used in the nine feeds are Soybean meal (Plate-5), Rice bran (Plate-6), Maize powder (Plate-7), Gram Hull (Plate-8), Wheat bran (Plate-9), Vitamin and mineral mix (Plate-10), Iron supplement (Plate-11), Calcium lactate (Plate-12), Vegetable oil (Plate-13) on the fish, Indian major carp (Plate-14) using TGC (JKC 738) three feeds (F_1-F_3) were prepared with graded levels of TGC @ 10%, 20% and 30% (Plate-15, 16 and 17). Similarly, NTGC (JKC 738) cotton meal was used to prepare three feeds (F_4-F_6) containing NTGC @ 10%, 20% and 30% (Plate-18, 19 and 20). The parental control cotton feeds (F_7-F_9) were made using hybrid cotton seed (PCC) without Transgenic input @ 10%, 20% and 30% (Plate-21, 22 and 23). The control cotton feeds (F_{10}-F_{12}) were made using market control cotton cake

(CCC) @ 10%, 20% and 30% (Plate-24, 25 and 26). The feed preparation was performed by using Twin-Screw Extruder (BTPL, Kolkata) (Plate-27). Feed is prepared (Plate-28) after proper mixing of ingredients (Plate-27).

A. Survival :

The survival percentage recorded during the experimentation.

B. Water quality :

The physico-chemical parameters estimated fortnightly and results are shown in Table-7.

C. Proximate composition :

All the samples of cotton meal, feed (F_1-F_{12}) and experimental fishes were analysed by AOAC (1989) (Table-2, 3 and 8). The fishes used as whole for the analysis.

D. Growth studies :

Food conversion (feed : weight gain), feed efficiency, weight gain, specific growth rate, protein efficiency ratio, dry matter digestibility and crude protein digestibility were recorded (Table-10) following AOAC (1989). Further, the proximate composition of fishes after feeding for 70 days, has been estimated using standard methods and the results are recorded in Table-8.

Table-1 : Per kilogram composition of ingredients for *Cirrhinus mrigala* feeds

Feed No.	Product Code	Tech. Code	TGC	NTGC	PCC	CCC	SBM	RB	GH	MP	WB	VO	Ca-Lactate	Iron-Suppl.**	VM+MM*	TOTAL
F_1	L No.17491 JKC-738	TGC_{10}	100	-	-	-	400	150	83.75	110	60	50	25	1.25	20	1000
F_2	L No.17491 JKC-738	TGC_{20}	200	-	-	-	325	100	108.75	110	60	50	25	1.25	20	1000
F_3	L No.17491 JKC-738	TGC_{30}	300	-	-	-	250	100	83.75	110	60	50	25	1.25	20	1000
F_4	L No.17355 JKC-738	$NTGC_{10}$	-	100	-	-	390	150	93.75	110	60	50	25	1.25	20	1000
F_5	L No.17355 JKC-738	$NTGC_{20}$	-	200	-	-	320	100	113.75	110	60	50	25	1.25	20	1000
F_6	L No.17355 JKC-738	$NTGC_{30}$	-	300	-	-	242	100	91.75	110	60	50	25	1.25	20	1000
F_7	L No.2360 NHH-44	PCC_{10}	-	-	100	-	399	150	84.75	110	60	50	25	1.25	20	1000
F_8	L No.2360 NHH-44	PCC_{20}	-	-	200	-	321	100	112.75	110	60	50	25	1.25	20	1000
F_9	L No.2360 NHH-44	PCC_{30}	-	-	300	-	248	100	85.75	110	60	50	25	1.25	20	1000
F_{10}	-	CCC_{10}	-	-	-	100	393	150	90.75	110	60	50	25	1.25	20	1000
F_{11}	-	CCC_{20}	-	-	-	200	317	100	116.75	110	60	50	25	1.25	20	1000
F_{12}	-	CCC_{30}	-	-	-	300	247	100	86.75	110	60	50	25	1.25	20	1000

TGC = Transgenic cotton seed;
CCC = Control cotton seed cake;
GH = Gram hull;
VO = Vegetable oil;
NTGC = Non-Transgenic cotton seed;
SBM = Soybean meal;
MP = Maize powder;
*VM+MM = Vitamin mix + Mineral mix
PCC = Parental control cotton;
RB = Rice bran;
WB = Wheat bran;
** = Iron supplement

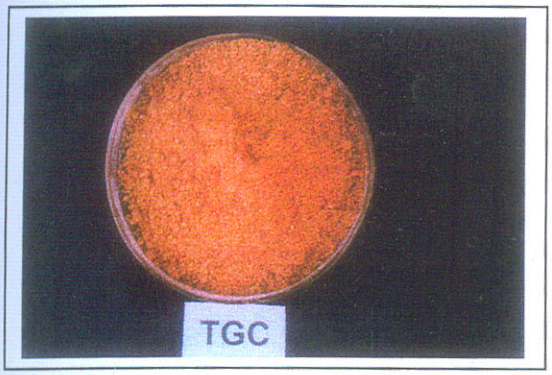


Plate-1 : Transgenic cotton seed meal

Plate-2 :Non-Transgenic cotton seed meal

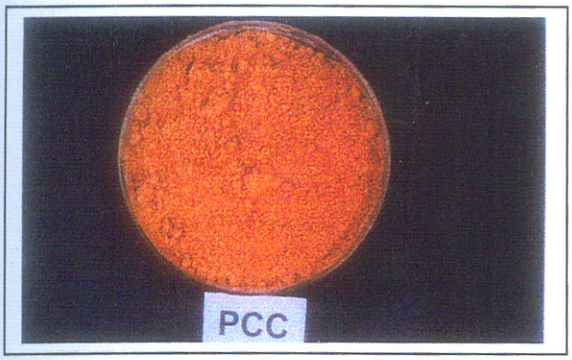


Plate-3 : Parental control cotton seed meal

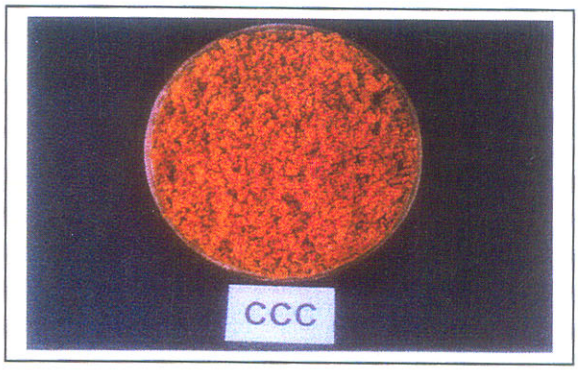


Plate-4 : Laboratory control cotton cake meal

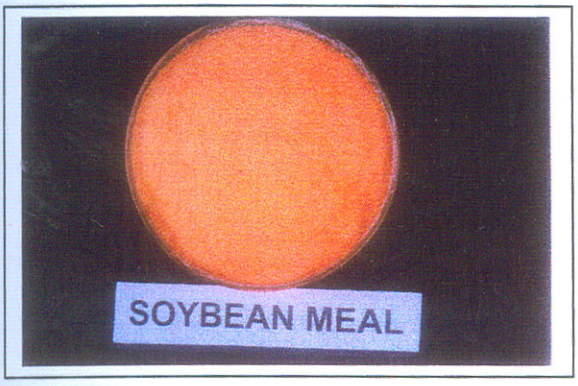


Plate-5 : Soybean Meal

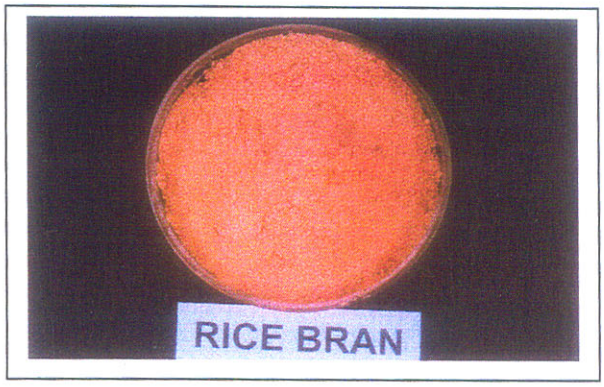


Plate-6 : Rice Bran

Plate-7 : Maize Powder (powdered)

Plate-8 : Gram Hull (Powered)

Plate-9 : Wheat Bran (Powered)

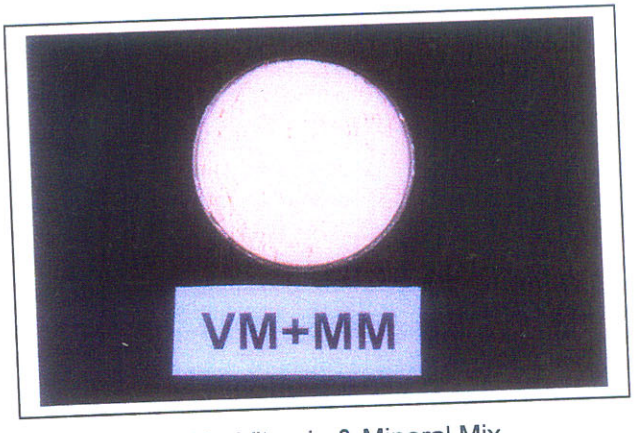


Plate-10 : Vitamin & Mineral Mix.

Plate-11 : Iron-supplement (Powered)

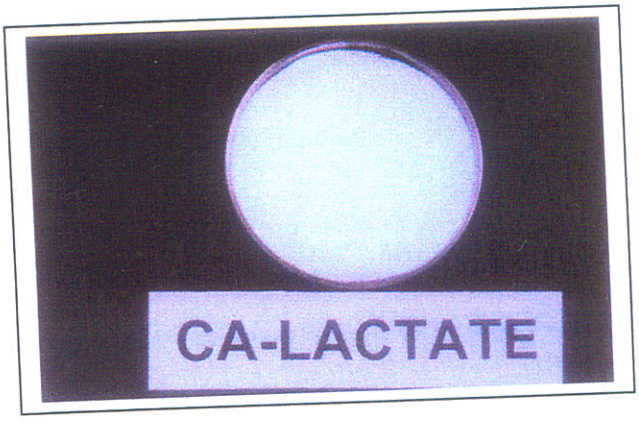


Plate-12 : Calcium-lactate (Mineral)

Plate-13 : Vegetable Oil

Plate-14 : *Cirrhinus mrigala* fingerling

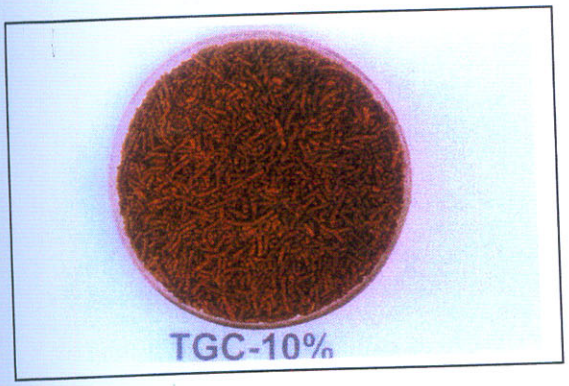


Plate-15 : Extruded feed containing TGC-10%

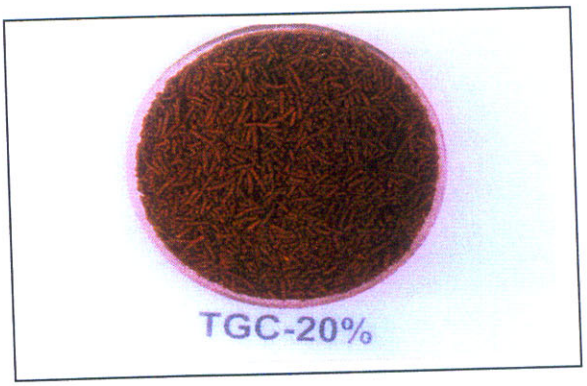


Plate-16 : Extruded feed containing TGC-20%

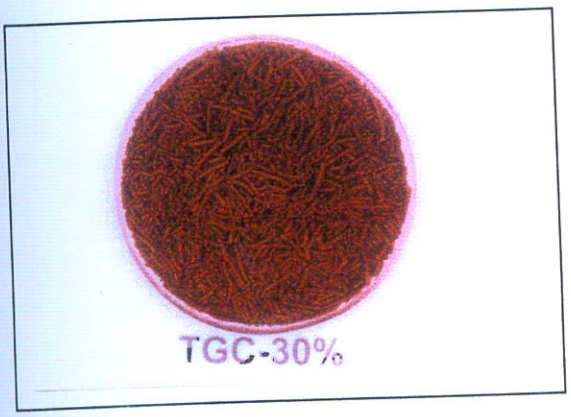


Plate-17 : Extruded feed containing TGC-30%

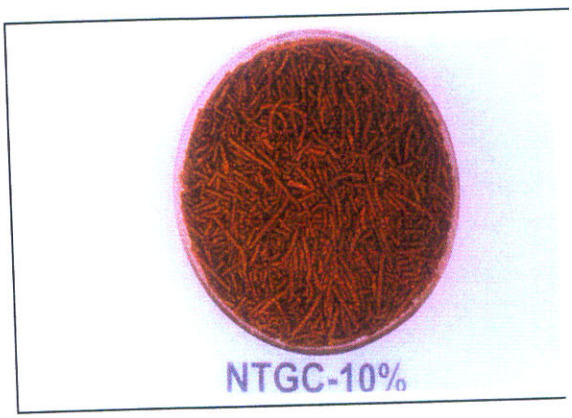


Plate-18 : Extruded feed containing NTGC-10%

Plate-19 : Extruded feed containing NTGC-20%

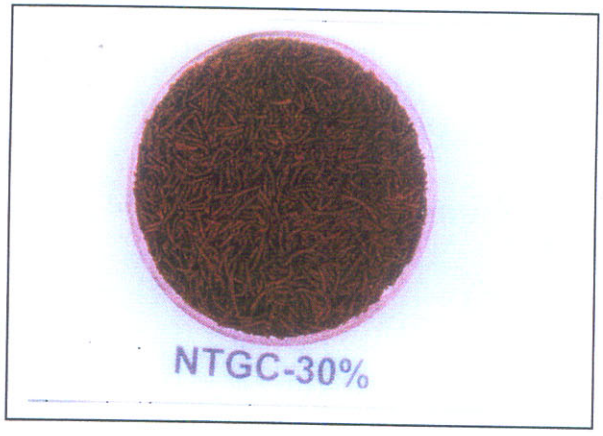


Plate-20 : Extruded feed containing NTGC-30%

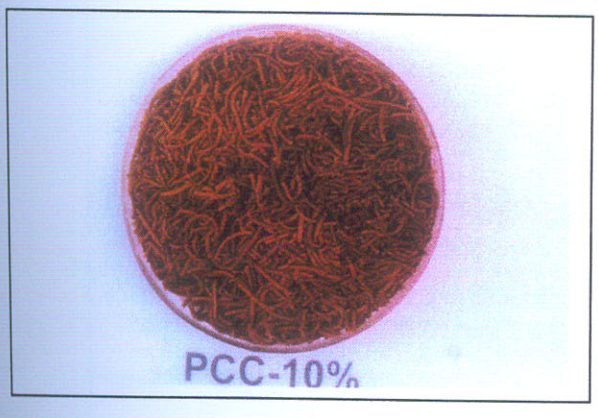


Plate-21 : Extruded feed containing PCC-10%

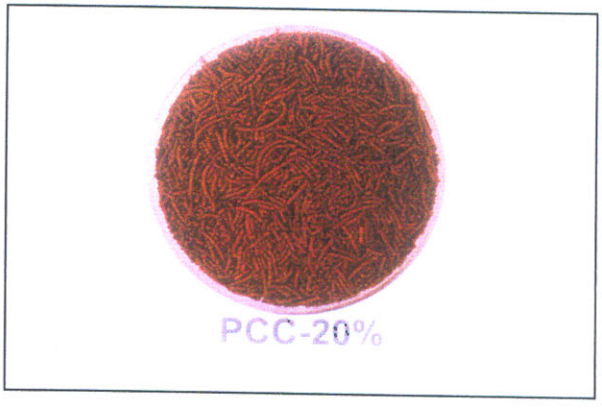


Plate-22 : Extruded feed containing PCC-20%

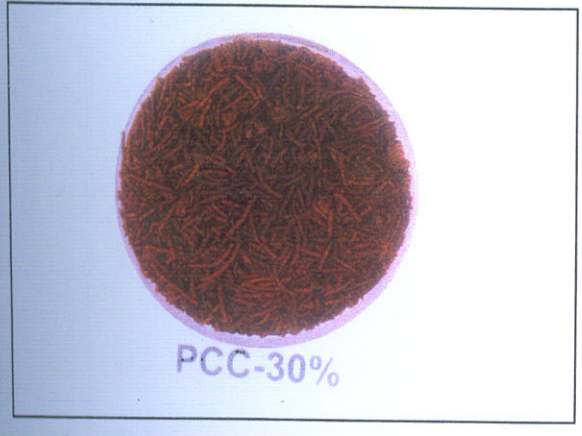


Plate-23 : Extruded feed containing PCC-30%

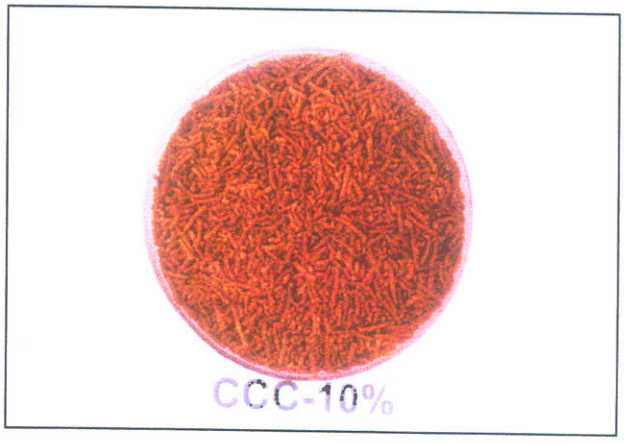


Plate-24 : Extruded feed containing CCC- 10%

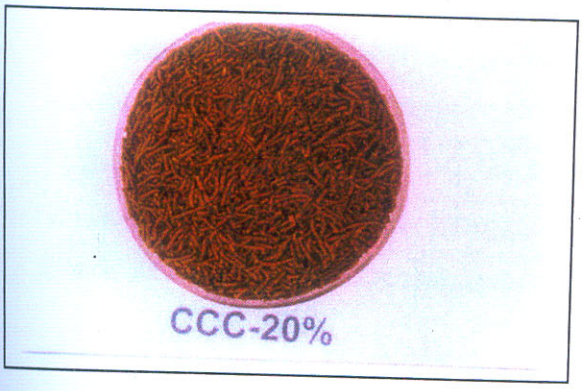


Plate-25 : Extruded feed containing CCC-20%

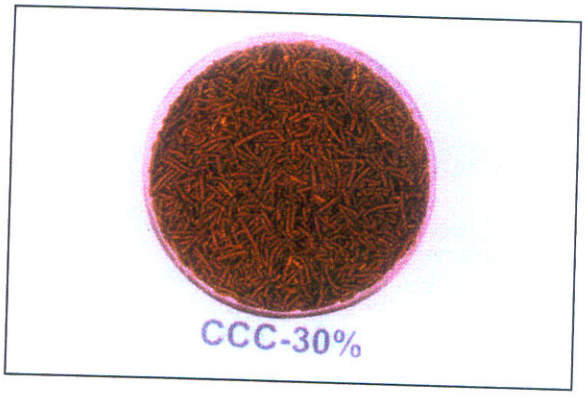


Plate-26 : Extruded feed containing CCC-30%

Plate-27 : Mixing of ingredients

Plate-28 : Feed preparation

Plate-29:Fish Stocking

Plate-30: Scientists demonstrating proximate analysis steps

E. ***Mineral Analysis*** :

The metals like Cadmium (Cd), Cobalt (CO), Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn) and Magnesium (Mg) were estimated using Atomic Absorption Spectrophotometer (ECIL, Hyderabad, Model No. AAS 4129). The wavelength for maximum absorbancy for 'Cd', 'Co', 'Cu', 'Fe', 'Mn', 'Zn' and 'Mg' used as 229.1 nm, 241.3 nm, 325.0 nm, 248.5 nm, 280.1 nm, 214.0 nm and 285.2nm respectively. Results are shown in Table-11 and 12.

F. ***Histological studies*** :

The gill, liver, intestine and kidney were collected from untreated fishes on day 1 and samples from fishes, fed with 12 feeds, were collected (after 70 days) and preserved in 10% formaldehyde solution. The tissues were processed and sections were cut at 6 μ and staining was done using Haematoxylin and Eosin. Histopathological studies were made using Olympus microscope (Model CS31RBSF, Olympus Optics Co. Ltd., Philippines)

Table 2 : Biochemical composition of cotton seeds and cake

Cultivar name	Lipid (%)	Protein (%)	Ash (%)	Carbo-Hydrate (%)	Gross Energy K-cal (per 100g)	Gossypol (%)
JKC 738 (with Cry 1Ac [truncated] gene)	22.8±0.73	26.5±0.42	3.51±0.22	49.5±4.30	431.5±30.2	1.49±0.09
JKC 738 (without Cry 1Ac [truncated] gene)	20.7±0.41	27.1±1.51	3.75±0.16	50.26±3.18	428.4±22.1	1.52±0.11
NHH-44	21.3±1.72	28.1±1.39	3.6±0.21	48.32±2.75	422.5±11.75	1.56±0.17
-	8.5±2.10	30.6±2.10	4.2±0.39	54.1±3.32	401.2±17.39	1.72±0.12

Table 3 : Composition of feeds (F_1-F_{12})

Sr.No.	Feed	Moisture* (%)	Lipid* (%)	Protein* (Nx6.25)	Ash* (%)	Carbo-hydrate* (%)	Gross Energy* (K-cal per 100g)
1.	F_1	6.8	3.0	34.3	8.7	56.3	351.6
2.	F_2	6.5	5.4	34.7	9.2	54.5	348.7
3.	F_3	7.2	9.1	35.1	10.1	51.6	352.5
4.	F_4	7.8	2.5	34.2	10.2	51.3	349.3
5.	F_5	8.1	4.4	34.8	8.9	52.4	342.5
6.	F_6	8.2	9.0	35.0	9.2	50.0	347.1
7.	F_7	8.3	3.0	34.5	9.0	53.2	350.2
8.	F_8	7.5	5.8	34.6	8.9	50.1	343.8
9.	F_9	8.2	9.2	34.8	9.3	49.9	347.4
10.	F_{10}	8.2	3.1	34.0	9.7	54.3	340.7
11.	F_{11}	9.6	4.0	34.2	10.1	55.3	342.8
12.	F_{12}	8.8	5.6	34.8	10.4	52.7	350.5

*All values are mean of two determinations and are on dry weight basis.

Table 4 : Total Gossypol contents in the TGC, NTGC, PCC and CCC cotton seeds incorporated feeds

Sr.No.	Feed	Containing cotton seed (%)	Total Gossypol* (%)
1.	F_1	10	0.059
2.	F_2	20	0.126
3.	F_3	30	0.238
4.	F_4	10	0.045
5.	F_5	20	0.139
6.	F_6	30	0.272
7.	F_7	10	0.062
8.	F_8	20	0.127
9.	F_9	30	0.217
10.	F_{10}	10	0.071
11.	F_{11}	20	0.142
12.	F_{12}	30	0.263

*All values are mean of two determinations and are on dry weight basis.

All the nine feed containing cotton meal (F_1-F_9) and three control feeds (F_{10}-F_{12}) were added iron supplement to avoid and/or reduce the affect of gossypol (as it holds the iron content of the body). The composition of iron supplement & vitamin and mineral mixture composition are tabulated in Table 5 and 6 respectively.

Table 5 : Composition of iron supplement (Raricap Forte, Johnson & Johnson, India)

Caplet	Complex	Quantity	Quantity of Iron	Quantity of Calcium	Folic Acid
Raricap Forte	Ferrous calcium citrate	556 mg	50 mg	72 mg	0.3 mg

Table 6 : Vitamins and Mineral feed supplements

Sr.No.	Components	Quantity per 2.5 kg
1.	Vitamin A	55,00,000 IU
2.	Vitamin D3	11,00,000 IU
3.	Vitamin B2	2,000 mg
4.	Vitamin E	750 mg
5.	Vitamin K	1,000 mg
6.	Vitamin B6	1,000 mg
7.	Vitamin B12	6 mcg
8.	Calcium panthothenate	2,500 mg
9..	Niacinamide	10 gm
10.	Choline chloride	150 gm
11.	Manganese	27,000 mg
12.	Iodine	1,000 mg
13.	Iron	7,000 mg
14.	Zinc	5,000 mg
15.	Copper	2,000 mg
16.	Cobalt	650 mg
17.	Calcium	500 g
18.	Phosphorous	300 g
19.	L-lysine	10 g
20.	DL-methionine	10 g
21.	Selemium	50 ppm
22.	Satewari	2,500 mg
23.	Carriers	C.S.

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Testing System and Procedure :

The experiments were conducted in 300 L capacity round plastic pools (Plate-29) with continuous aeration and containing 200 L of water. The water quality were analysed and tabulated in Table-7 and found with in the normal limits. Each pool was stocked with fingerling *Cirrhinus mrigala* (Avg. length, 4.4 ± 0.30 to 4.8 ± 0.2 cm and avg. weight, 0.80 ± 0.01 to 1.04 ± 0.02 g) @ 50 fishes per pool (Plate-29). Each feed was given in the two replications @ 8% body weight and the quantity of feed was adjusted every fortnight as per the weight gain. The feeding experiment was conducted for five fortnight (70 day, 14 day x 5). After each fortnight the 10 fishes were sampled and were put for various biochemical analysis. Digestibility analysis was carried out by feed matter collection method.

Data Collection and Proximate analysis of fish and feed :

At the end of 70th day feeding experiment, a minimum of five fish from each plastic pool were taken for the purpose of pooled tissues analysis and final length and weight of individual specimen. Proximate composition of fish were carried out in the Nutrition Laboratory using Soxtec system (Model : HT-2,1045, Sweden) for lipid analysis (Plate-30); Kjeltec system (model : 2200 Kjeltec Auto Distillation Extraction Unit, Tecator, Sweden) for protein analysis ; Muffle furnace (Expo, Mumbai) for ash contents Semi-micro Calorimeter (Parr, USA 1425 model) for gross energy and oven (Newtronic, Mumbai) for moisture content. Growth, survival, feed conversion and fish body proximate composition were subjected to one-way analysis of variance and Duncan's multiple range test to determine treatment differences ($P<0.05$). A student's t-test was used to statistically evaluate any differences in measured parameters between fish fed diets containing Bt. cotton (JKC-738) and non-Bt. cotton (JKC-738) and non-Bt. cotton Steele and Torrie, 1960), and to their treatment group, using SPSS statistical package.

Programme of work with phasing milestones :

The experiment will be carried out in 300L (3½ ft. depth) plastic pools with 8 inches of water level. After stabilizing fish samples, the fish was stocked @ 50 fish each species per pool. Sampling/ Data on growth parameters was carried on 2nd week, 4th week, 6th week, 8th week and at the end of 10h week samples of water, and fish tissues were preserved quickly to analyse the water quality, tissue histology and biochemical composition beside the growth evaluation. Total 24 plastic pools tanks will be used following RBD system to analyse data statistically.

Experimental design:

(TGC+RFI) -	Transgenic cotton (TGC) inclusion along with other Routine feed ingredients (RFI).	: **10 %, 20%,** and **30 %**	= 3
(NTGC+RFI)-	Non-Transgenic cotton (NTGC) inclusion along with other Routine feed ingredients (RFI).	: **10 %, 20%,** and **30 %**	= 3
(PCC+RFI) -	Parental control cotton (PCC) inclusion along with other Routine feed ingredients (RFI).	: **10%, 20%,** and **30%**	= 3
(CCC+RFI) -	Laboratory control cotton (CCC) alongwith other routine feed ingredients (RFI).	: **10%, 20%,** and **30%**	= 3
	Replication		= 2
	Total pools		**= 24**

Sampling :

(i) Water samples : On initial, every fortnight and at the end of 70 days.

(ii) Experimental design and Fish tissue sampling as follows (Every fortnight) :

(A)		**POOL**		**POOL**
	1	TGC 10%	4	TGC 30%
(TGC+RFI)	2	TGC 30%	5	TGC 20%
+ fish @ 50 per pool	3	TGC 20%	6	TGC 10%
(B)		**POOL**		**POOL**
	7	NTGC 10%	10	NTGC 30%
(NTGC+RFI)	8	NTGC 30%	11	NTGC 20%
+ fish @ 50 per pool	9	NTGC 20%	12	NTGC 10%
(C)		**POOL**		**POOL**
	13	PCC 10%	16	PCC 30%
(PCC+RFI)	14	PCC 30%	17	PCC 20%
+ fish @ 50 per Pool	15	PCC 20%	18	PCC 10%

(D)

	POOL		POOL	
	19	CCC 10%	22	CCC 30%
(CCC+RFI)	20	CCC 30%	23	CCC 20%
+ fish @ 50 per Pool	21	CCC 20%	24	CCC 10%

Sampling days per pool:

Days	POOL NUMBER REPLICATES WITH CONDITION			
0	1-6 **(TGC + RFI)**	7-12 **(NTGC + RFI)**	13-18 **(PCC + RFI)**	19-24 **(CCC + RFI)**
14	1-6 **(TGC + RFI)**	7-12 **(NTGC + RFI)**	13-18 **(PCC + RFI)**	19-24 **(CCC + RFI)**
28	1-6 **(TGC + RFI)**	7-12 **(NTGC + RFI)**	13-18 **(PCC + RFI)**	19-24 **(CCC + RFI)**
42	1-6 **(TGC + RFI)**	7-12 **(NTGC + RFI)**	13-18 **(PCC + RFI)**	19-24 **(CCC + RFI)**
56	1-6 **(TGC + RFI)**	7-12 **(NTGC + RFI)**	13-18 **(PCC + RFI)**	19-24 **(CCC + RFI)**
70	1-6 **(TGC + RFI)**	7-12 **(NTGC + RFI)**	13-18 **(PCC + RFI)**	19-24 **(CCC + RFI)**

TGC + RFI = Transgenic cotton seed + Remaining Feed Ingredient
NTGC + RFI = Non-Transgenic cotton seed + Remaining Feed Ingredient
PCC + RFI = Parental control cotton seed + Remaining Feed Ingredient
CCC + RFI = Market control cotton cake + Remaining Feed Ingredient

All the proximate composition and various growth parameters was carried out after completion of the experimentation at CIFE, Mumbai.

Annexure-III

Results :

A. Survival :

The data collected from the 70 days feeding study showed no statistically significant differences in the survival of Indian major carp (*Cirrhinus mrigala*) fed diets containing TGC cotton seed as treatment 'A' compared to fish fed diet containing NTGC cotton seed as treatment 'B' parental control cotton seed at 'C' and laboratory control 'D' containing market control cotton alongwith other ingredients. In all the 24 plastic pools all the 50 fishes were healthy and even after sampling of 10 samples from each pool at every fortnight the 50, 40, 30, 20 and 10 fishes survived on day 14, 28, 42, 56 and 70 days respectively. Thus, zero mortality was recorded.

B. Water quality :

The water quality during the experimental period was within normal limits and shown in Table-7.

Table-7 : Water quality parameters during experimentation

Para-meters / Tank	Temp. °C	pH	Alkalinity (mg/l)	Chlorides (mg/l)	Hardness (mg/l)	DO (mg/l)	Ammonia (NH_4^+-N)	Nitrite (NO_2N) (mg/l)	Phosphate (PO_4-P) (mg/l)	Total organic matter (mg/l)
F_1	25-27	7.5±0.1	270±6	70±0.4	310±2.1	6.2±0.2	0.19±0.02	0.04±0.002	0.11±0.005	12.5±0.2
F_2	25-27	7.8±0.2	271±5	74±0.3	318±5.1	8.4±0.1	0.18±0.01	0.05±0.003	0.12±0.006	14.3±0.2
F_3	25-27	7.9±0.1	274±3	75±0.3	315±7.3	7.2±0.2	0.17±0.02	0.05±0.004	0.10±0.003	13.5±0.1
F_4	25-27	7.7±0.2	278±5	71±0.2	316±2.6	6.9±0.1	0.18±0.01	0.05±0.002	0.12±0.005	14.7±0.3
F_5	25-27	7.8±0.3	279±2	69±0.6	320±1.8	7.0±0.2	0.20±0.02	0.06±0.002	0.11±0.005	15.1±0.2
F_6	25-27	7.9±0.2	281±3	71±0.4	319±7.5	7.3±0.1	0.21±0.02	0.08±0.005	0.12±0.007	13.8±0.3
F_7	25-27	7.7±0.2	285±5	68±1.0	325±3.9	7.4±0.2	0.22±0.01	0.09±0.007	0.11±0.016	12.5±0.1
F_8	25-27	7.9±0.1	286±3	69±0.6	321±2.8	7.1±0.1	0.23±0.03	0.08±0.003	0.12±0.003	11.9±0.2
F_9	25-27	7.6±0.2	279±2	68±0.3	330±3.5	7.2±0.2	0.21±0.02	0.05±0.001	0.10±0.009	12.2±0.1
F_{10}	25-27	7.6±0.1	268±4	69±0.2	310±3.1	6.8±0.2	0.19±0.01	0.05±0.001	0.13±0.001	13.7±0.4
F_{11}	25-27	7.5±0.2	271±3	67±0.3	309±1.7	6.9±0.1	0.21±0.01	0.04±0.002	0.12±0.005	12.9±0.2
F_{12}	25-27	7.4±0.3	278±4	68±0.1	311±1.5	6.7±0.3	0.20±0.02	0.05±0.002	0.11±0.003	11.9±0.3

Mean ± SE

C. Proximate Composition :

The proximate composition of cotton meal feeds (F_1-F_{12}) and experimental fishes analysed by AOAC (1989) are shown in Table-2, 3, 4, 8 and 10.

The cotton seeds contains Gossypol contents as 1.49 ± 0.09, 1.52 ± 0.11, 1.56 ± 0.17 and 1.72 ± 0.12 in TGC. NTGC, PCC and CCC samples (Table-2) respectively. The gross energy of prepared feeds (F_1-F_{12}) ranged between 342.5 to 352.5 K.cal/100 g (Table-3). After mixing the cotton seed (TGC, NTGC, PCC) and cotton cake in feed F_1-F_{12} @ 10%, 20% and 30% of both the samples the gossypol contents recorded as 0.059%, 0.126%, 0.238%, 0.045%, 0.139%, 0.272%, 0.062%, 0.127%, 0.217%, 0.071%, 0.142% and 0.263% in F_1, F_2, F_3,F_4, F_5, F_6, F_7, F_8,F_9, F_{10}, F_{11} and F_{12} respectively (Table-4).

The proximate composition of experimental fishes (of every fortnight) are shown in Table-8 and 10. The moisture contents ranged, in dried samples, from 8.8% to 10.1% in first fortnight, 7.9% to 10.4% second fortnight samples, 7.8% to 10.5% in third fortnight samples, 7.1% to 8.8% in fourth fortnight and 8.1% to 9.2% in fifth fortnight i.e. last samples. The protein contents (on dry weight basis) ranged between 38.1% to 46.9% during the experimental sampling (Table-8). The ash and carbohydrate contents ranged from 8.1% to 12.9% and 5.9% to 15.3% respectively (on dry matter basis). The gross energy contents of the fishes ranged from 429.7 K.cal/100g to 522.9 K.cal/100g (Table-8).

D. Growth studies :

The average weight gain, growth increment, aggregate length and weight increment, final weight gain, specific growth rate, feed conversion, feed efficiency and protein efficiency ratio are shown in Table-9 and 10 respectively.

Table-8 : Proximate composition of experimental fishes (Dry matter basis)

Feed/Day	Moisture %	Lipid %	Protein (N*6.25) %	Ash %	Carbo-hydrates %	Gross Energy K.calories Per 100g
Initial	9.1	21.1	40.2	11.7	7.1	432.7
F_1/14	8.8	20.2	46.5	1.5	12.2	431. 8
F_2/14	10.0	22.2	42.6	8.9	15.3	429.7
F_3/14	9.7	23.5	43.5	.2	10.5	436.2
F_4/14	10.2	19.7	41.8	9.3	14.7	435.6
F_5/14	10.1	21.3	45.3	10.5	11.2	436.2
F_6/14	8.8	22.5	44.8	11.3	10.2	440.5
F_7/14	9.2	23.4	46.9	10.8	14.3	442.7
F_8/14	9.3	19.7	43.5	12.9	11.8	447.3
F_9/14	10.1	18.7	44.5	10.7	14.5	448.5
F_{10}/14	10.2	19.2	41.3	12.1	13.8	430.2
F_{11}/14	9.9	19.8	42.8	11.2	14.3	430.7
F_{12}/14	8.9	19.4	45.2	10.8	15.1	436.4
F_1/28	9.3	25.1	42.8	9.2	10.5	476.3
F_2/28	7.9	26.4	45.7	9.5	11.3	478.5
F_3/28	8.1	27.1	43.5	10.2	10.5	472.8
F_4/28	9.3	24.3	44.6	9.1	10.2	470.3
F_5/28	10.4	25.1	48.3	8.9	10.7	468.5
F_6/28	10.1	24.6	42.6	9.0	9.3	470.9
F_7/28	8.9	23.9	42.5	9.2	9.0	481.3
F_8/28	9.2	24.7	41.8	9.5	9.5	468.6
F_9/28	9.3	24.1	43.5	11.5	12.7	469.3
F_{10}/28	9.5	24.5	44.5	10.7	13.2	430.7
F_{11}/28	10.1	24.7	44.3	11.3	12.8	444.5
F_{12}/28	10.3	24.6	43.8	12.5	13.4	460.1
F_1/42	8.9	31.3	43.8	9.8	7.2	487.5
F_2/42	9.1	30.1	42.3	9.2	9.8	498.8
F_3/42	7.8	30.6	40.1	9.2	9.4	436.3
F_4/42	7.9	30.8	41.2	8.9	9.8	510.3
F_5/42	8.8	31.5	43.1	8.7	10.2	515.6
F_6/42	8.5	32.3	42.7	8.5	11.5	499.7
F_7/42	8.4	34.1	41.0	8.6	10.7	502.1
F_8/42	8.4	33.5	42.5	8.1	11.3	488.9
F_9/42	8.3	33.7	42.8	8.2	12.1	496.6
F_{10}/42	10.1	32.1	41.2	9.1	7.9	488.7
F_{11}/42	10-5	30.1	40.5	9.2	7.8	478.8
F_{12}/42	9.8	29.7	39.8	9.8	7.2	472.5

Contd./----

Feed/Day	Moisture* %	Lipid* %	Protein* (N*6.25) %	Ash* %	Carbo-hydrates* %	Gross Energy* K.calories Per 100g
F_1/56	7.8	31.1	41.8	9.1	12.1	515.7
F_2/56	7.2	32.1	42.5	9.2	10.1	508.2
F_3/56	7.3	32.4	43.7	9.6	8.2	492.5
F_4/56	7.5	31.6	44.5	9.0	7.1	490.3
F_5/56	7.1	30.5	44.2	8.8	5.9	499.3
F_6/56	7.2	30.7	40.8	8.9	8.6	437.5
F_7/56	7.3	31.3	43.5	8.9	7.2	488.8
F_8/56	7.1	31.4	44.6	9.0	7.8	483.4
F_9/56	7.2	37.5	41.8	9.3	9.1	485.6
F_{10}/56	7.9	30.2	40.1	9.1	9.7	479.6
F_{11}/56	8.2	31.2	38.1	9.3	10.1	481.5
F_{12}/56	8.8	30.5	37.5	9.1	11.3	477.8
F_1/70	9.1	28.8	44.2	9.2	10.2	515.5
F_2/70	9.0	30.2	43.8	9.3	11.1	522.9
F_3/70	9.2	31.5	42.9	8.8	10.9	511.7
F_4/70	8.8	28.6	41.5	9.0	9.9	507.6
F_5/70	8.3	28.5	42.6	8.8	10.0	511.6
F_6/70	8.5	27.9	42.5	8.9	10.5	515.2
F_7/70	9.1	28.9	41.8	9.1	10.3	501.2
F_8/70	9.2	30.5	42.5	9.2	10.7	500.7
F_9/70	9.0	31.2	43.8	8.8	10.8	498.1
F_{10}/70	8.1	29.8	40.8	8.9	10.2	490.1
F_{11}/70	8.7	30.5	40.1	9.0	9.2	470.1
F_{12}/70	9.2	30.2	40.3	8.6	9.8	478.3

*All values are mean of two determinations and are on dry weight basis.

Table 9 : Fortnightly growth increment in terms of average length (cm) and average weight (g) in *Cirrhinus mrigal* (mrigala)

Tank No. (Feed)	Replicate	29/10/2004		12/10/2004		26/11/2004		10/12/2004		24/12/2004		07/01/2005	
		L (cm) ± SE (Initial)	W (g) ± SE) (Initial)	L (cm) ± SE	W (g) ± SE)	L (cm) ± SE	W (g) ± SE)	L (cm) ± SE	W (g) ± SE)	L (cm) ± SE	W (g) ± SE)	L (cm) ± SE	W (g) ± SE)
1 (TGC-10)	I	4.4±0.4	0.80±0.01	5.2±0.4	1.43±0.11	6.1±0.2	2.01±0.07	6.6±0.2	3.71±0.22	7.3±0.1	4.56±0.33	8.1±0.1	6.70±0.19
	II	4.4±0.4	0.80±0.01	5.1±0.3	1.47±0.09	6.0±0.2	2.08±0.04	6.5±0.1	3.70±0.17	7.2±0.2	4.59±0.30	8.1±0.2	6.72±0.11
2 (TGC-20)	I	4.5±0.3	0.83±0.03	5.3±0.1	1.82±0.04	6.3±0.1	2.51±0.13	6.7±0.2	3.89±0.11	7.1±0.1	4.75±0.10	8.0±0.3	6.58±0.72
	II	4.5±0.3	0.83±0.03	5.3±0.2	1.80±0.10	6.3±0.2	2.52±0.12	6.9±0.1	3.91±0.10	7.1±0.2	4.78±0.15	8.0±0.2	6.61±0.28
3 (TGC-30)	I	4.6±0.1	0.92±0.03	5.4±0.2	2.68±0.21	6.4±0.1	2.61±0.08	6.7±0.1	4.12±0.36	7.6±0.1	5.21±0.11	8.7±0.1	8.14±0.32
	II	4.6±0.1	0.92±0.03	5.3±0.1	2.70±0.13	6.4±0.2	2.60±0.06	6.8±0.1	4.15±0.28	7.5±0.2	5.37±0.17	8.8±0.2	8.17±0.42
4 (NTGC-10)	I	4.5±0.4	0.86±0.06	5.0±0.3	1.56±0.17	6.3±0.1	2.10±0.05	6.8±0.1	3.68±0.16	7.4±0.1	5.01±0.30	7.9±0.3	5.96±0.34
	II	4.5±0.4	0.86±0.06	5.1±0.2	1.59±0.12	6.2±0.1	2.08±0.11	6.7±0.1	3.67±0.19	7.3±0.2	4.99±0.06	7.9±0.2	5.99±0.21
5 (NTGC-20)	I	4.7±0.2	1.00±0.07	5.2±0.3	1.74±0.10	6.3±0.2	2.35±0.03	6.8±0.2	3.82±0.13	7.5±0.2	5.03±0.21	8.0±0.1	6.24±0.52
	II	4.7±0.2	1.00±0.07	5.3±0.1	1.76±0.02	6.2±0.1	2.38±0.10	6.8±0.1	3.83±0.15	7.5±0.1	5.04±0.17	8.0±0.1	6.29±0.66
6 (NTGC-30)	I	4.8±0.2	1.04±0.02	5.1±0.4	1.81±0.14	6.1±0.2	2.59±0.05	6.5±0.2	3.86±0.15	7.3±0.2	5.28±0.41	8.5±0.2	7.70±0.20
	II	4.8±0.2	1.04±0.02	5.1±0.2	1.79±0.03	6.1±0.1	2.63±0.01	6.7±0.3	3.86±0.11	7.4±0.3	5.31±0.32	8.4±0.3	7.74±0.50
7 (PCC-10)	I	4.4±0.3	0.83±0.02	5.0±0.1	1.49±0.08	6.0±0.2	2.00±0.16	6.6±0.1	3.69±0.05	7.1±0.2	4.92±0.26	7.9±0.1	5.56±0.48
	II	4.4±0.3	0.83±0.02	5.0±0.4	1.41±0.05	6.0±0.1	2.09±0.15	6.8±0.2	3.67±0.04	7.1±0.1	4.95±0.23	7.8±0.2	5.59±0.37
8 (PCC-20)	I	4.6±0.2	0.99±0.02	5.1±0.3	1.82±0.07	6.0±0.2	2.30±0.17	6.9±0.2	3.72±0.07	7.2±0.1	5.02±0.47	8.0±0.1	6.09±0.42
	II	4.6±0.2	0.99±0.02	5.2±0.1	1.80±0.05	6.1±0.1	2.32±0.12	6.8±0.4	3.74±0.10	7.3±0.2	5.00±0.31	8.0±0.1	6.14±0.28
9 (PCC-30)	I	4.4±0.3	0.85±0.01	5.3±0.2	1.88±0.03	6.0±0.2	2.69±0.11	6.6±0.2	3.88±0.15	7.1±0.3	5.13±0.22	8.1±0.2	6.43±0.22
	II	4.4±0.3	0.85±0.01	5.2±0.3	1.85±0.02	6.0±0.2	2.66±0.06	6.6±0.1	3.89±0.06	7.2±0.2	5.14±0.17	8.1±0.3	6.46±0.13
10 (CCC-10)	I	4.6±0.2	0.88±0.02	5.0±0.2	1.50±0.04	6.1±0.2	2.02±0.01	6.7±0.2	3.52±0.14	7.0±0.1	4.17±0.19	7.8±0.2	5.10±0.27
	II	4.6±0.2	0.88±0.02	5.2±0.1	1.48±0.02	6.1±0.2	1.99±0.03	6.8±0.3	3.48±0.08	7.0±0.2	4.18±0.16	7.8±0.1	5.12±0.41
11 (CCC-20)	I	4.5±0.1	0.85±0.02	5.1±0.2	1.69±0.09	6.2±0.1	2.17±0.05	6.9±0.1	3.57±0.12	6.9±0.4	4.22±0.11	7.9±0.2	5.39±0.48
	II	4.5±0.1	0.85±0.02	5.1±0.2	1.71±0.13	6.2±0.1	2.19±0.02	6.9±0.2	3.60±0.11	6.9±0.2	4.33±0.15	7.9±0.3	5.43±0.61
12 (CCC-30)	I	4.5±0.3	0.87±0.04	5.2±0.1	1.78±0.18	6.1±0.3	2.50±0.11	6.9±0.1	3.81±0.17	7.1±0.1	4.71±0.18	8.1±0.1	6.17±0.04
	II	4.5±0.3	0.87±0.04	5.2±0.2	1.76±0.15	6.1±0.2	2.49±0.08	6.9±0.2	3.76±0.19	7.3±0.2	4.73±0.26	8.0±0.2	6.20±0.29

Table 10 : Growth performance of *Cirrhinus mrigala* fed with varying levels of TGC, NTGC,PCC and CCC for 70 days

Sr. No.	Parameters	Initial	TGC			NTGC			PCC			CCC		
			10%	20%	30%	10%	20%	30%	10%	20%	30%	10%	20%	30%
1.	Initial body weight (g)	-	0.80±0.01	0.83±0.03	0.92±0.03	0.86±0.06	1.00±0.07	1.04±0.02	0.83±0.02	0.99±0.02	0.85±0.01	0.88±0.02	0.85±0.02	0.87±0.04
2.	Final body weight (g)	-	6.71±0.17^{a}	6.59±0.02^{a}	8.15±0.35^{b}	5.97±0.41^{a}	6.26±0.33^{a}	7.71±0.59^{c}	5.58±0.40^{a}	6.11±0.26^{a}	6.45±0.51^{a}	5.11±0.30^{d}	5.41±0.51^{d}	6.19±0.05^{a}
3.	Specific growth rate (SGR) %	-	0.59±0.01^{a}	0.58±0.04^{a}	0.73±0.02^{b}	0.53±0.01^{a}	0.48±0.02^{b}	0.53±0.02^{a}	0.61±0.01^{c}	0.51±0.03^{a}	0.49±0.02^{b}	0.52±0.01^{a}	0.63±0.22^{c}	0.58±0.07^{a}
4.	Feed conversion ratio (FCR)	-	2.81±0.13^{a}	2.75±0.07^{a}	2.70±0.03^{a}	2.58±0.02^{a}	2.68±0.04^{a}	2.70±0.10^{a}	2.55±0.12^{a}	2.60±0.12^{a}	2.68±0.12^{a}	2.69±0.11^{a}	2.76±0.18^{a}	2.89±0.06^{a}
5.	Feed efficiency ratio (FER)	-	0.36±0.01^{a}	0.36±0.02^{a}	0.37±0.01^{a}	0.39±0.02^{a}	0.37±0.02^{a}	0.37±0.01^{a}	0.39±0.01^{a}	0.38±0.02^{a}	0.37±0.03^{a}	0.37±0.02^{a}	0.36±0.01^{a}	0.34±0.03^{a}
6.	Protein efficiency ratio (PER)	-	1.17±0.02^{a}	1.15±0.01^{a}	1.11±0.03^{a}	1.15±0.03^{a}	1.21±0.04^{b}	1.16±0.02^{a}	1.20±0.02^{b}	1.19±0.02^{a}	1.21±0.02^{b}	1.16±0.07^{a}	1.19±0.05^{a}	1.13±0.02^{a}
7.	Feed Intake (g)	-	18.86±0.81	18.26±0.66	22.32±0.36	15.52±2.24	17.51±0.09	20.58±0.28	14.82±02.6	16.51±1.20	17.72±0.75	14.59±1.02	15.20±0.88	18.18±01.22
Final carcass composition (Dry matter basis)														
1.	Moisture (%)$^{\$}$	9.1	9.1	9.0	9.2	8.8	8.3	8.5	9.1	9.2	9.0	8.1	8.7	9.2
2.	Dry matter (%)$^{\$}$	90.9	90.9	91.0	90.3	90.8	91.7	91.5	90.9	90.8	91.0	91.9	91.3	90.8
3.	Lipid (%)$^{\$}$	21.1	28.8	30.2	31.5	29.0	28.5	27.9	28.9	30.5	31.2	29.8	30.5	30.2
4.	Crude protein (%)$^{\$}$	40.2	44.2	43.8	42.9	41.5	42.6	42.5	41.8	42.5	43.8	40.8	40.1	40.3
5.	Ash (%)$^{\$}$	11.7	9.2	9.3	8.8	9.0	8.8	8.9	9.1	9.2	8.8	8.9	9.0	8.6
6.	Carbohydrate (%)$^{\$}$	7.1	10.2	11.1	10.9	9.9	10.0	10.5	10.3	10.7	10.8	10.2	9.2	9.8
7.	DM Digestibility (%)$^{\$}$	53.5	49.7	52.8	56.2	54.1	55.1	51.7	51.3	50.5	50.72	49.7	48.5	51.2
8.	CP Digestibility (%)$^{\$}$	74.2	70.5	71.7	72.6	75.2	73.8	72.3	72.5	76.2	70.5	68.7	69.2	72.3
9.	Gross energy$^{\$}$ (K.cal/100 g)	432.7	515.5	522.9	511.7	507.6	511.6	515.2	501.2	500.7	498.1	490.1	470.1	478.3

- Same alphabet as superscript in a row demonstrate no significant change ($P>0.05$) whereas different alphabet shows significant change ($P<0.05$) in 'a' to 'b', 'b' to 'c', 'c' to 'd' and $P<0.01$ in 'a' to 'c', 'a' to 'd')
- Mean ± SE
- $^{\$}$All values are mean of the determinations and are on dry weight basis.

Specific growth rate (SGR) of fishes fed with TGC cotton supplemented feeds (F_1, F_2 and F_3) ranged between 0.58 ± 0.04% to 0.73 ± 0.02% (Table-10). Similarly, SGR of NTGC ((F_4, F_5 and F_6) fed fishes recorded as 0.48 ± 0.02% to 0.53 ± 0.02%, however, SGR of parental control cotton seed ranged 0.49 ± 0.02 to 0.61 ± 0.01 and laboratory control cotton cake from 0.52 ± 0.01 to 0.63 ± 0.22. The feed efficiency ratio of F_1 to F_{12} recorded as 0.36 ± 0.01, 0.36 ± 0.02, 0.37 ± 0.02, 0.39 ± 0.02, 0.37 ± 0.02, 0.37 ± 0.01, 0.39 ± 0.01, 0.38 ± 0.01, 0.37 ± 0.03, 0.37 ± 0.02, 0.36 ± 0.01 and 0.34 ± 0.03 respectively (Table-10). The protein efficiency ratio (PER) of feed F_1 to F_{12} ranged between 1.11 ± 0.03 to 1.21 ± 0.04. The highest protein efficiency recorded in NTGC fed fishes followed by PCC, TGC and CCC fed fishes (Table-10).

E. Mineral Analysis :

The metal contents of feeds (F_1-F_{12}) and fish carcass recorded and results are shown in Table-11 and 12. In feed Cd, Co, Cu, Fe, Mn, Zn and Mg ranged between 1.059 $\mu g.g^{-1}$ to 3.126 $\mu g.g^{-1}$, 0.986 $\mu g.g^{-1}$ to 14.579 $\mu g.g^{-1}$, 0.762 $\mu g.g^{-1}$ to 1.159 $\mu g.g^{-1}$, 450.213 $\mu g.g^{-1}$ to 2175.029 $\mu g.g^{-1}$, 65.226 $\mu g.g^{-1}$ to 104.319 $\mu g.g^{-1}$, 73.522 $\mu g.g^{-1}$ to 100.288 $\mu g.g^{-1}$ and 3120.871 $\mu g.g^{-1}$ to 5678.238 $\mu g.g^{-1}$ respectively (Table-11).

Similarly, metal contents of the carcass recorded as 2.188 $\mu g.g^{-1}$ to 3.198 $\mu g.g^{-1}$, 5.212 $\mu g.g^{-1}$ to 14.751 $\mu g.g^{-1}$, 0.262 $\mu g.g^{-1}$ to 2.189 $\mu g.g^{-1}$, 200.512 $\mu g.g^{-1}$ to 906.782 $\mu g.g^{-1}$, 8.178 $\mu g.g^{-1}$ to 36.038 $\mu g.g^{-1}$, 245.812 $\mu g.g^{-1}$ to 418.382 $\mu g.g^{-1}$ and 789.538 $\mu g.g^{-1}$ to 2475.310 $\mu g.g^{-1}$ for Cd, Co, Cu, Fe, Mn, Zn and Mg respectively (Table-12).

Table 11 : Mineral composition of the Feeds F_1 to F_{12}

Sr.No.	Feed	Cadmium ($\mu g.g^{-1}$)	Cobalt ($\mu g.g^{-1}$)	Copper ($\mu g.g^{-1}$)	Iron ($\mu g.g^{-1}$)	Manganese ($\mu g.g^{-1}$)	Zinc ($\mu g.g^{-1}$)	Magnesium ($\mu g.g^{-1}$)
1	F_1	3.126	14.506	1.022	2175.029	80.201	76.826	3241.283
2	F_2	1.792	6.238	1.159	1715.528	78.159	73.522	3454.628
3	F_3	1.369	14.579	1.062	917.578	104.319	77.516	3474.519
4	F_4	1.826	7.361	1.051	1874.238	78.226	100.288	3120.871
5	F_5	1.626	9.198	1.003	1278.583	74.513	90.862	3588.602
6	F_6	1.598	13.156	1.056	1854.312	67.428	86.519	5346.875
7	F_7	1.987	7.502	0.828	1546.728	70.516	70.326	5452.029
8	F_8	1.136	9.952	0.762	786.123	78.519	88.188	5678.238
9	F_9	1.059	0.986	0.783	450.213	80.228	79.392	4654.129
10	F_{10}	2.217	1.021	0.782	1545.312	65.226	83.412	4635.300
11	F_{11}	2.202	1.008	0.791	871.416	68.192	80.716	4575.186
12	F_{12}	2.192	1.006	0.831	435.198	71.169	84.190	4852.383

Table-12 : Mineral composition content of the carcass of the fishes fed with F_1 to F_{12} for 70 days.

Sr.No.	Fishes fed Feeds	Cadmium ($\mu g.g^{-1}$)	Cobalt ($\mu g.g^{-1}$)	Copper ($\mu g.g^{-1}$)	Iron ($\mu g.g^{-1}$)	Manganese ($\mu g.g^{-1}$)	Zinc ($\mu g.g^{-1}$)	Magnesium ($\mu g.g^{-1}$)
1	In	2.226	5.212	0.262	417.154	11.728	317.602	1503.370
2	F_1	3.198	14.751	0.498	409.287	36.038	334.188	2475.310
3	F_2	2.678	9.623	0.281	370.006	9.515	301.305	1160.179
4	F_3	2.512	10.875	0.372	398.198	8.362	245.812	1278.538
5	F_4	2.188	8.289	2.189	392.507	10.401	380.619	1148.583
6	F_5	3.099	6.728	0.312	248.219	11.732	342.518	1203.526
7	F_6	2.876	7.008	0.412	906.782	10.519	357.509	789.538
8	F_7	2.689	6.989	0.399	348.718	14.072	418.382	1410.588
9	F_8	2.663	8.838	0.375	200.512	8.178	271.813	1103.789
10	F_9	3.128	14.513	0.286	407.501	16.518	342.483	1283.518
11	F_{10}	2.872	11.712	0.299	400.395	14.521	342.712	1240.223
12	F_{11}	2.769	12.519	0.315	390.348	11.712	341.619	1205.853
13	F_{12}	2.868	10.862	0.326	388.356	10.862	330.861	1309.628

F. Histopathological studies :

After feeding TGC, NTGC, PCC and CCC for 70 days the histological studies have been carried out and results are shown in Plate-31 to 82.

Gills :

The histological structure of gills of control mrigal, *Cirrhinus mrigala* was the same as in other freshwater carps. The respiratory lamellae are covered by an epithelial layer, which is usually two cells thick. The lamellar flood sinus are lined and spanned by pillar cells. The filaments between gill lamellae are covered by thick stratified epithelium of mucous cells, chloride cells, pilaster cells and blood cells scattered in the interlamellar epithelium. Equally spaced secondary gill lamellae and intact cellular layers are the characteristics features of the normal structure of the gills (Plate-31). .

Fishes fed with TGC incorporated feed (F_1, F_2 and F_3) for 70 days showed some alterations in the general structure of gill. In F_1 (10% Transgenic cotton, TGC) fed fishes showed enlargement of cartilagenous cells (Plate-32). Fishes fed with F_2 (20% TGC seed) demonstrated some damages in primary gill lamellae (Plate-33) characterized by loss of secondary lamella in few cases. Furthermore, fishes fed with F_3 (30% TGC cotton seed) showed some damages to primary gill lamellae (Plate-34) evidenced by loss of secondary lamellae at the lease of the filaments and increased vascularity at the basement.

Fishes fed with Non-Transgenic cotton (NTGC) incorporated feeds for 70 days (F_4, F_5 and F_7) also showed some changes in the general structure of gill. F_4 (10% NTGC seed) fed fishes showed enlargement of cartilaginous cells. . Fishes fed with F_5 (20% NTGC seed) showed (Plate-35) furthermore, enlarged cartilaginous cells (Plate-36). On feeding F_6 (30% NTGC seeds) the gill lamellae of *C. mrigala* is showing absolutely normal structure with some enlarged cartilaginous cells (Plate-37).

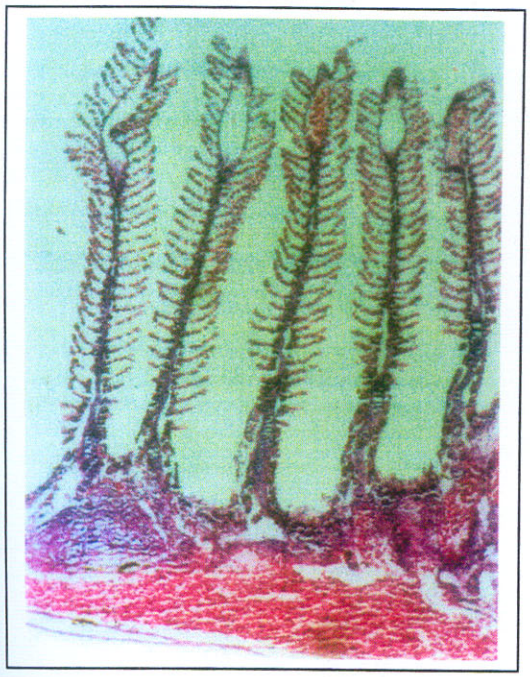

Plate-31 : Control gill of *Cirrhinus mrigala* **60X (H/E)**

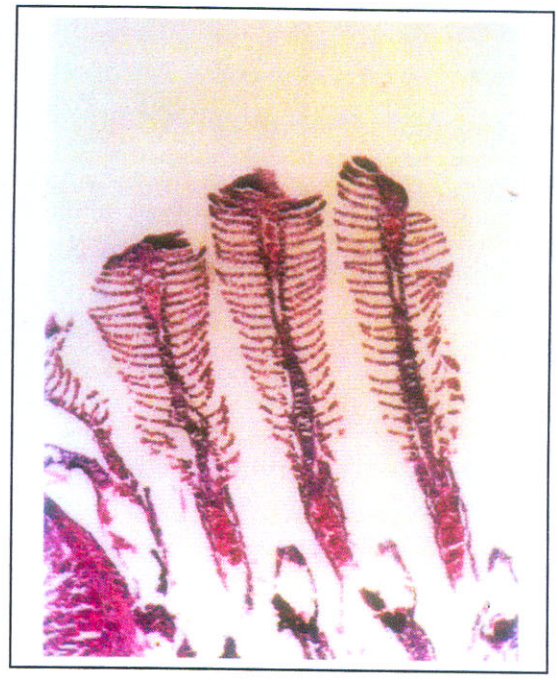

Plate-32 : Gill of *Cirrhinus mrigala* fed with TGC-10% incorporated feed **60X (H/E)**

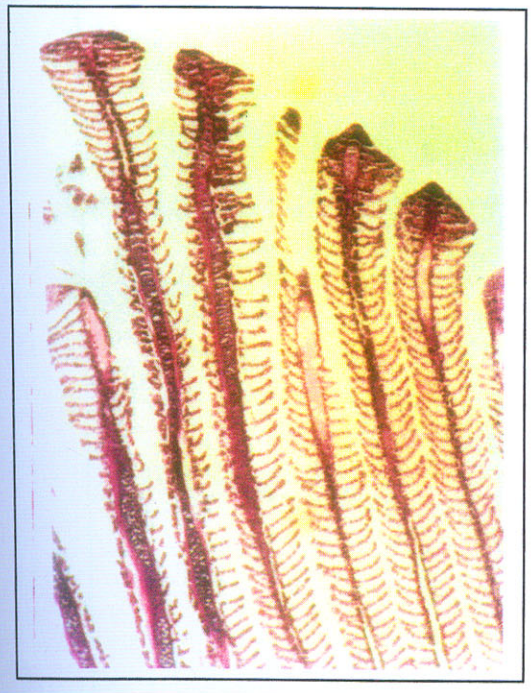

Plate-33 : Gill of *Cirrhinus mrigala* fed with TGC-20% incorporated feed **60X (H/E)**

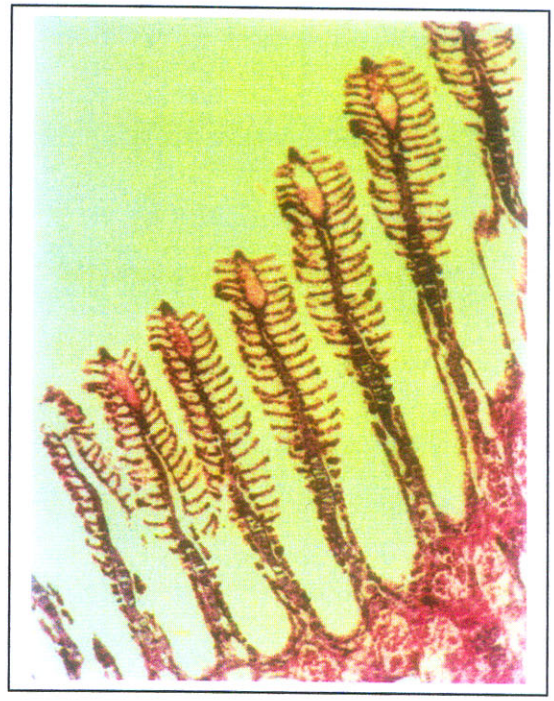

Plate-34 : Gill of *Cirrhinus mrigala* fed with TGC-30% incorporated feed **60 X (H/E)**

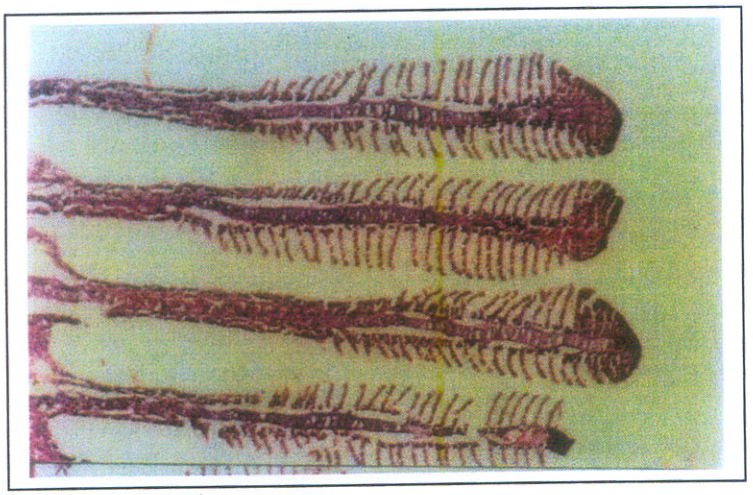

Plate-35 : Gill of *Cirrhinus mrigala* fed with NTGC-10% incorporated feed **60X (H/E)**

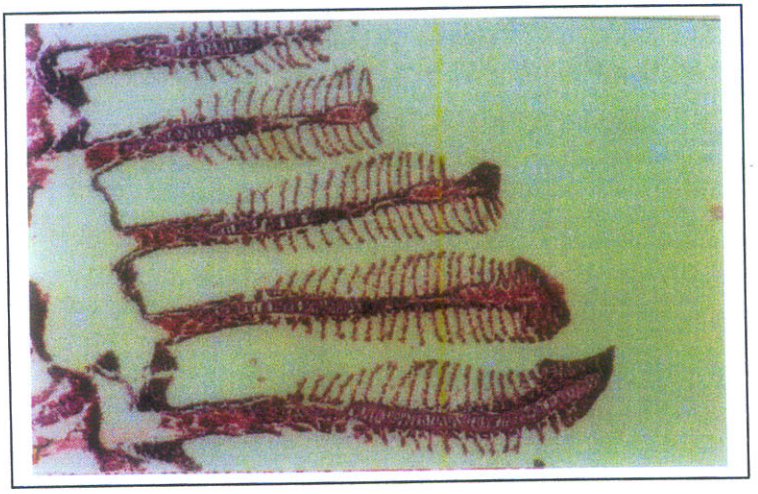

Plate-36 : Gill of *Cirrhinus mrigala* fed with NTGC-20% incorporated feed **60X (H/E)**

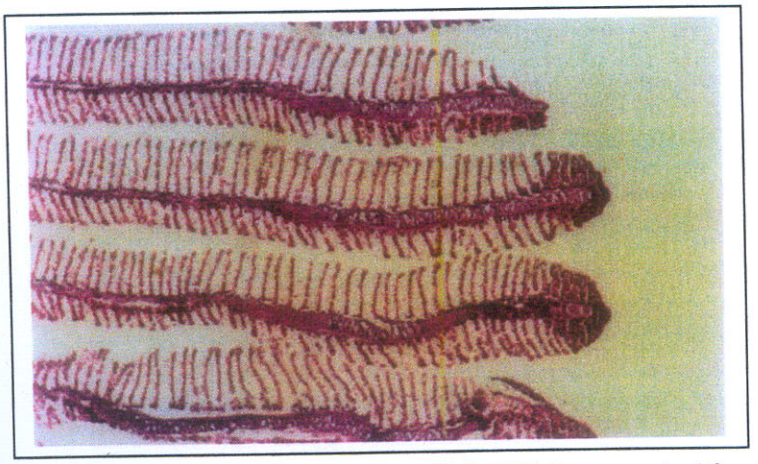

Plate-37 : Gill of *Cirrhinus mrigala* fed with NTGC-30% incorporated feed **60X (H/E)**

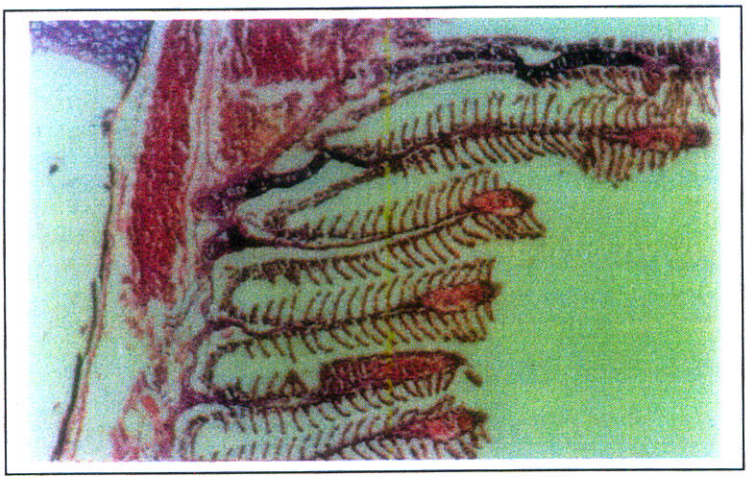

Plate-38 : Gill of *Cirrhinus mrigala* fed with PCC-10% incorporated feed **60X (H/E)**

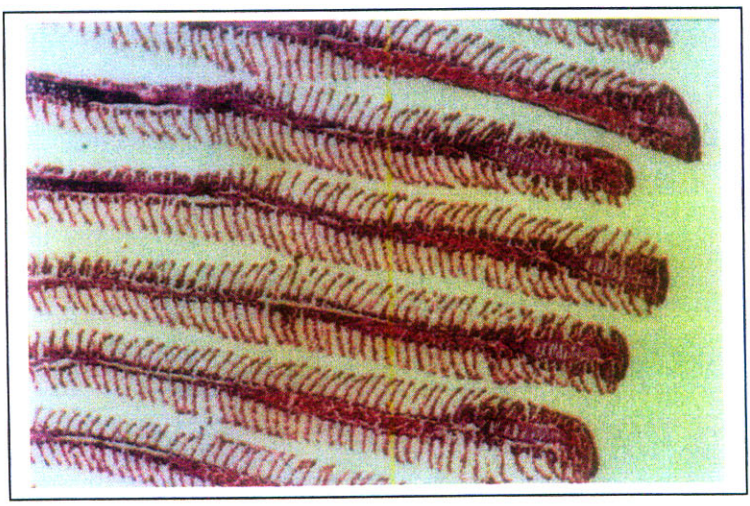

Plate-39 : Gill of *Cirrhinus mrigala* fed with PCC 20% incorporated feed **60X (H/E)**

Plate-40 : Gill of *Cirrhinus mrigala* fed with PCC-30% incorporated feed **60X (H/E)**

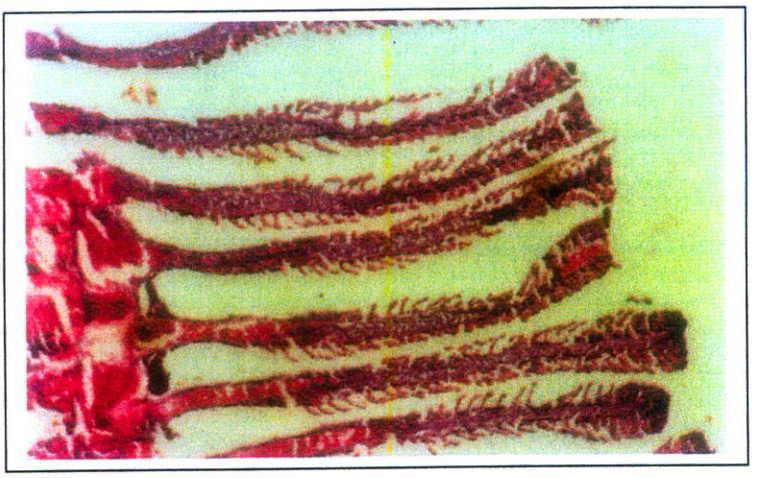

Plate-41 : Gill of *Cirrhinus mrigala* fed with CCC-10% incorporated feed **60X (H/E)**

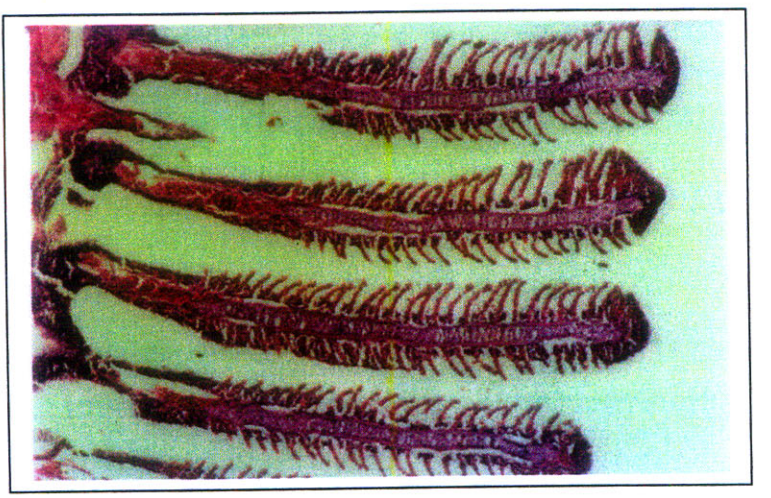

Plate-42 : Gill of *Cirrhinus mrigala* fed with CCC 20% incorporated feed **60X (H/E)**

Plate-43 : Gill of *Cirrhinus mrigala* fed with CCC-30% incorporated feed **60X (H/E)**

Fishes fed with Parental Cotton Seed (PCC) incorporated feeds (F_7, F_8 and F_9) for 70 days demonstrated some alterations. In F_7 (10% PCC) showing normal structure of gill (Plate-38) fishes fed with F_8 (20% PCC) demonstrated normal structure (Plate-39). Fishes, fed with feed F_9 (30% PCC) showing enlargement of cartilaginous cells and damage of secondary gill lamellae with hyperaemic condition at the lease of the filaments (Plate-40).

Fishes fed with Control Cotton Cake (CCC) incorporated feeds (F_{10}, F_{11} and F_{12}) for 70 days demonstrate some alterations. F_{10} (10% CCC) showed marked loss of secondary gill lamellae (Plate-41) and oedematous change in the branchial arch. However, 20% CCC (F_{11}) and 30% (F_{12}) CCC feeding trial showed massive destruction of the secondary lamellae exhibiting deformed structure (Plate 42 & 43).

Liver :

The control (Plate-44) liver of mrigal, *Cirrhinus mrigala* showed no abnormality in the structure. The experimental feeding trials impact on liver histopathology are shown in Plate 45-56.

Fish fed with 10% NTGC feed exhibited extensive loss of hepatic cells with marked areas of vacuolation. Histological features of liver tissue appeared as a network with scanty hepatocellular contents (Plate 48-50). In comparison the 20% and 30% NTGC fed fish showed lesser areas of vacuolation. Similarly, PCC 30% incorporated feed showed more hepatocellular disintegration in comparison to 10% and 20% PCC incorporated feed (Plate 51-53). Liver of mrigal, *Cirrhinus mrigala* fed with CCC showed no abnormality in the structure (Plate 54-56).

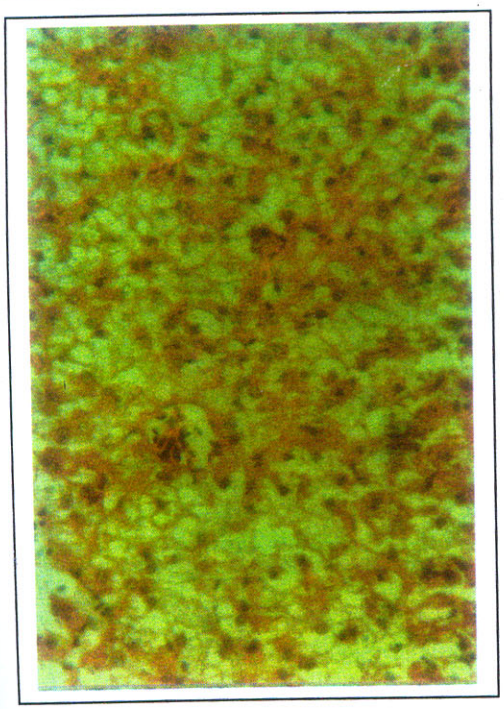

Plate-44 : Control liver of *Cirrhinus mrigala* fed with **60X (H/E)**

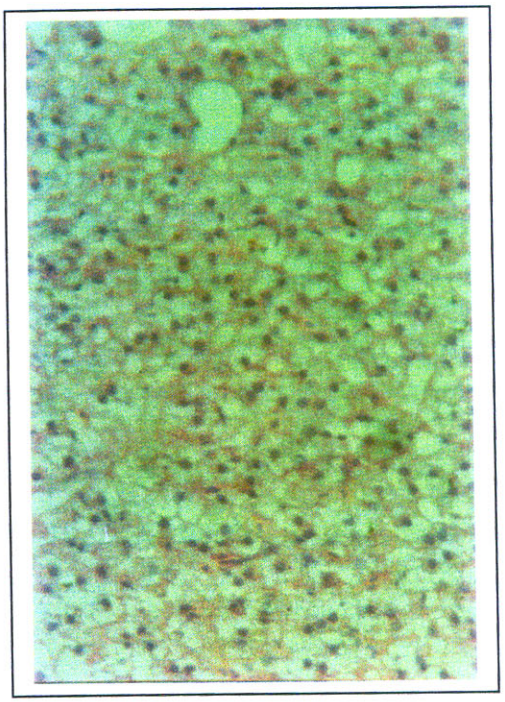

Plate-45 : Liver of *Cirrhinus mrigala* fed with TGC-10% incorporated feed **160X (H/E)**

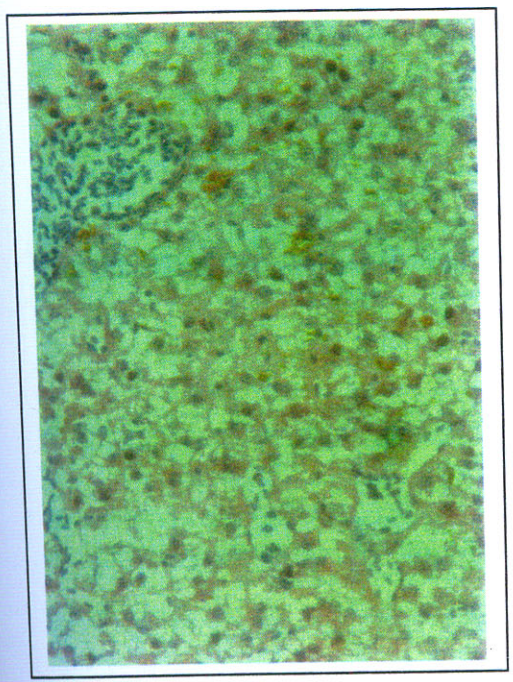

Plate-46 : Liver of *Cirrhinus mrigala* fed with TGC-20% incorporated feed **160X (H/E)**

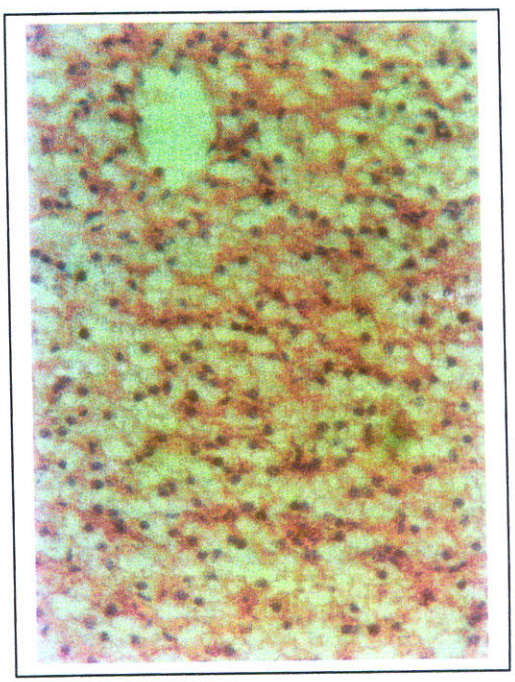

Plate-47 : Liver of *Cirrhinus mrigala* fed with TGC-30% incorporated feed **160X (H/E)**

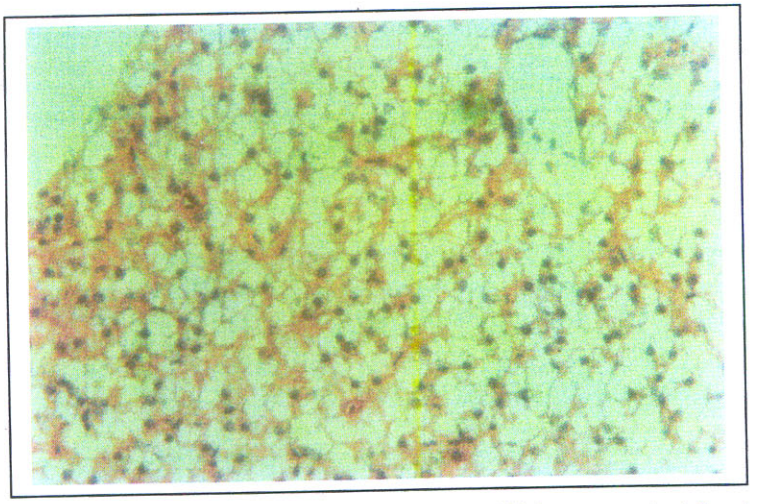

Plate-48 : Liver of *Cirrhinus mrigala* fed with NTGC-10% incorporated feed **160X (H/E)**

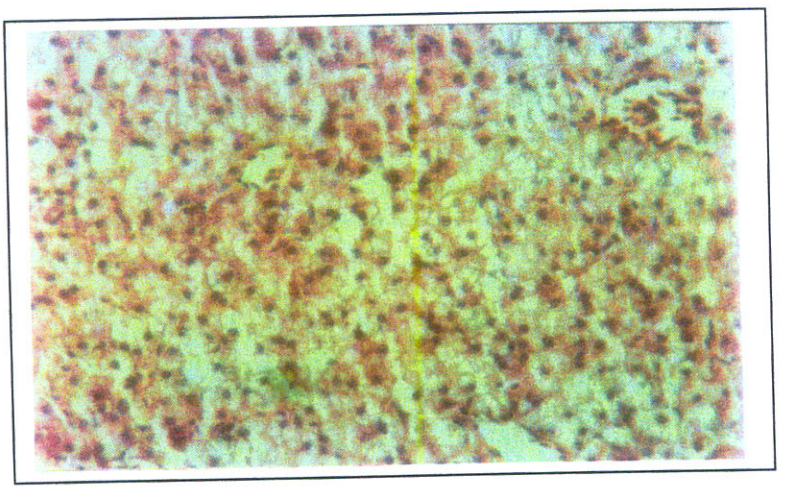

Plate-49 : Liver of *Cirrhinus mrigala* fed with NTGC-20% incorporated feed **160X (H/E)**

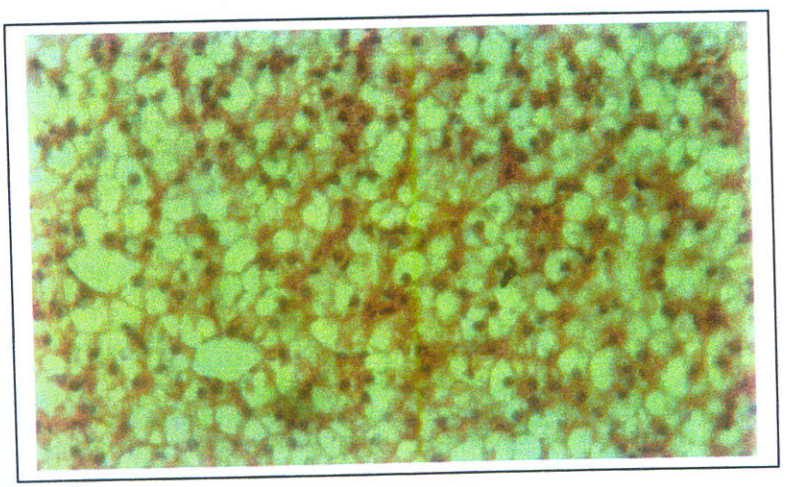

Plate-50 : Liver of *Cirrhinus mrigala* fed with NTGC-30% incorporated feed **160X (H/E)**

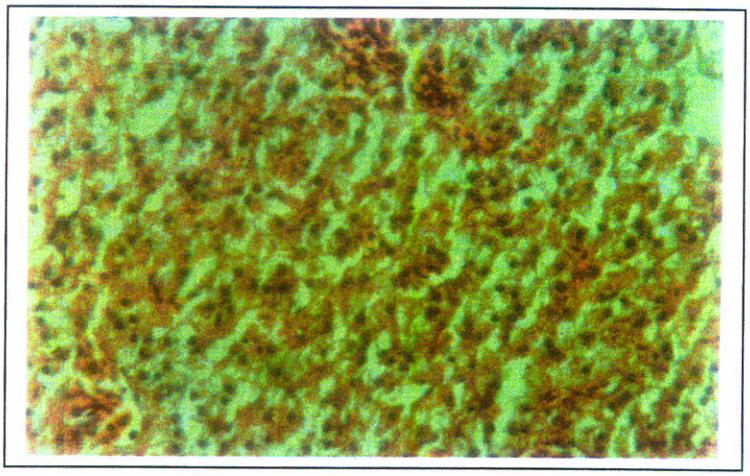

Plate-51 : Liver of *Cirrhinus mrigala* fed with PCC-10% incorporated feed **160X (H/E)**

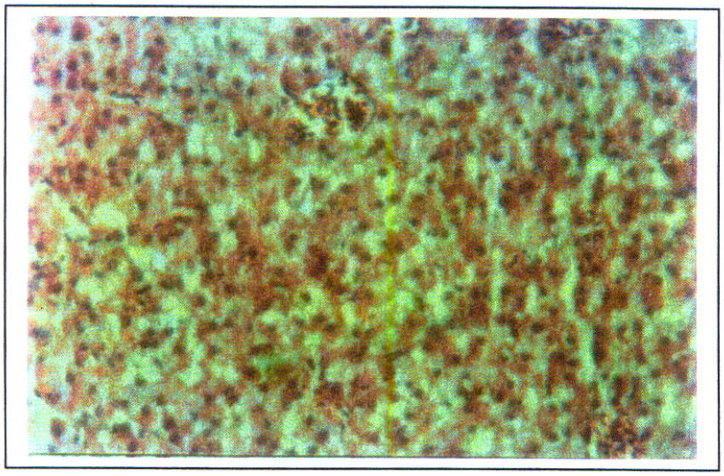

Plate-52 : Liver of *Cirrhinus mrigala* fed with PCC-20% incorporated feed **160X (H/E)**

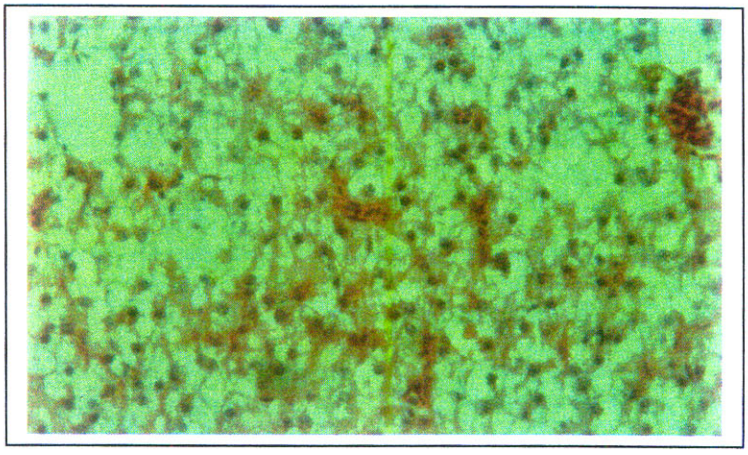

Plate-53 : Liver of *Cirrhinus mrigala* fed with PCC-30% incorporated feed **160X (H/E)**

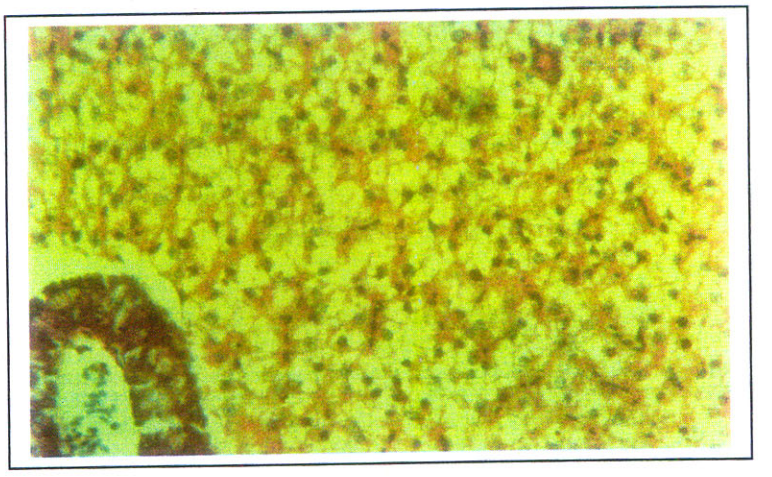

Plate-54 : Liver of *Cirrhinus mrigala* fed with CCC-10% incorporated feed **160X (H/E)**

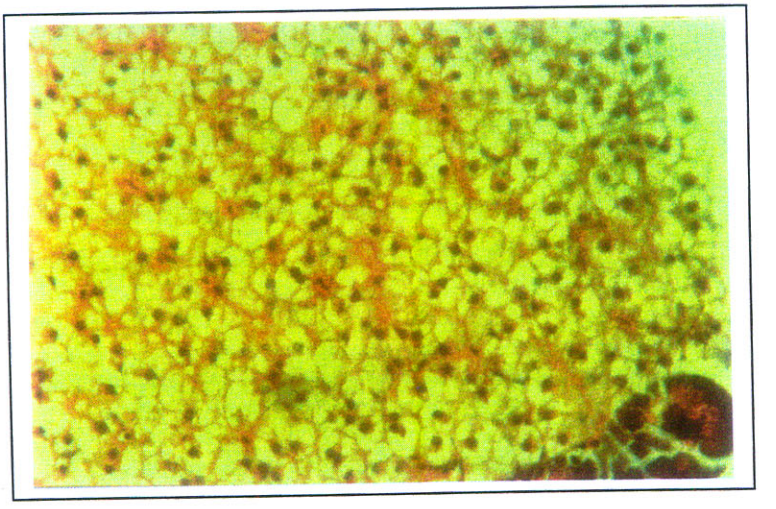

Plate-55 : Liver of *Cirrhinus mrigala* fed with CCC-20% incorporated feed **160X (H/E)**

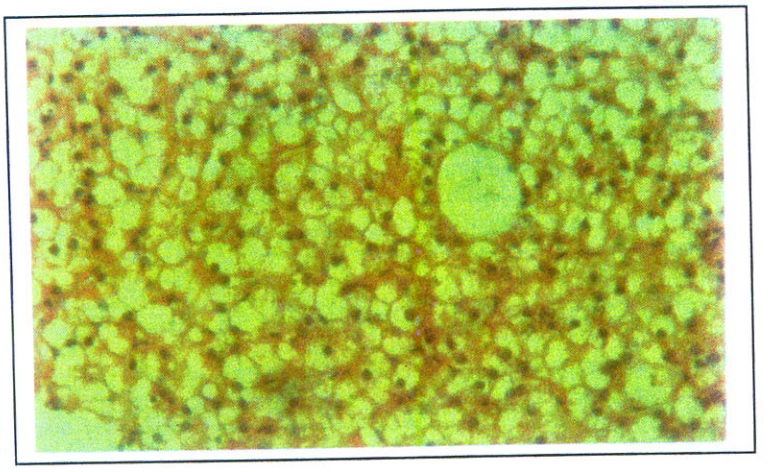

Plate-56 : Liver of *Cirrhinus mrigala* fed with CCC-30% incorporated feed **160X (H/E)**

Intestine :

The control (Plate-57) fish intestine showing normal appearance of villi.

Fishes fed with TGC incorporated feeds (F_1, F_2 and F_3) and NTGC incorporated diets (F_4, F_5 and F_6) showed no change in general histopathological structures (Plate-58 to 63).

Fishes fed with PCC feeds (F_7 and F_8) showed villi (Plate-64, 65 and 66), were same as in fishes fed with CCC (F_{10}, F_{11} and F_{12}) shows elongation of lumen in villi has been recorded (Plate-67, 68 & 69).

Kidney :

The histopathological changes in kidney has been taken up for studies after feeding for 70 days with TGC feeds (F_1, F_2 and F_3), NTGC feeds (F_4, F_5 and F_6), PCC feeds (F_7, F_8 and F_9) and CCC feeds (F_{10}, F_{11} and F_{12}). Results are shown in Plate-70 to 82.

The control kidney showing well vascularized glomerular capsule and renal tubules (Plate-70).

Fishes fed with TGC feeds (F_1, F_2 and F_3) showing some alterations in the kidney tissue. F_1 (TGC-10% cotton seed) fed fishes have almost normal structure except space in Bowman's capsule (Plate-71). The space in F_2 (TGC-20% cotton seed) showing more spaces in Bowman capsule (Plate-72).

Plate-57 : Control Intestine of *Cirrhinus mrigala* fed with **60X (H/E)**

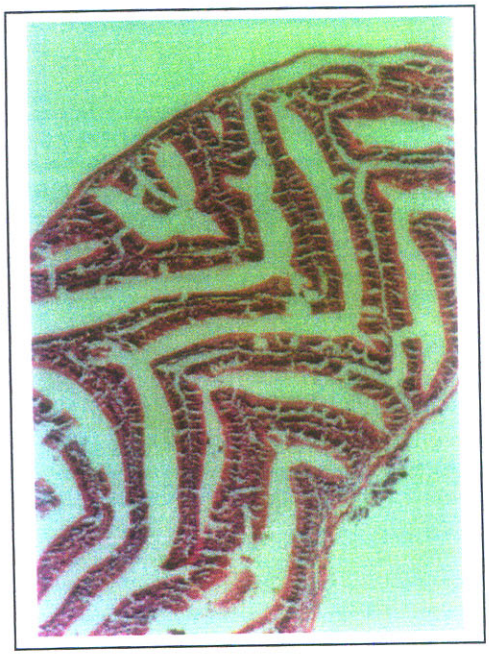

Plate-58 : Intestine of *Cirrhinus mrigala* fed with TGC-10% incorporated feed **160X (H/E)**

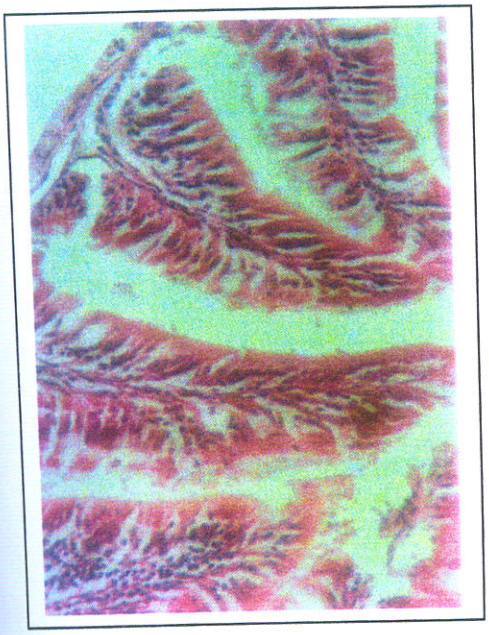

Plate-59 : Intestine of *Cirrhinus mrigala* fed with TGC-20% incorporated feed **160X (H/E)**

Plate-60 : Intestine of *Cirrhinus mrigala* fed with TGC-10% incorporated feed **160X (H/E)**

Plate-61 : Intestine of *Cirrhinus mrigala* fed with NTGC-10% incorporated feed **160X (H/E)**

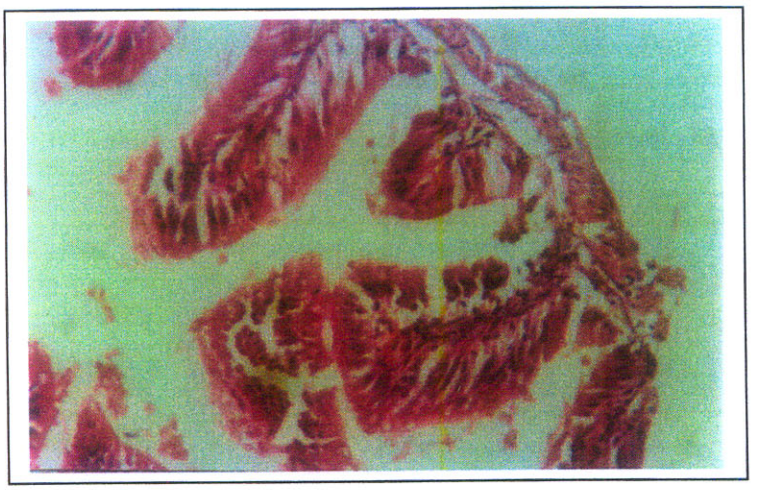

Plate-62 : Intestine of *Cirrhinus mrigala* fed with NTGC-20% incorporated feed **160X (H/E)**

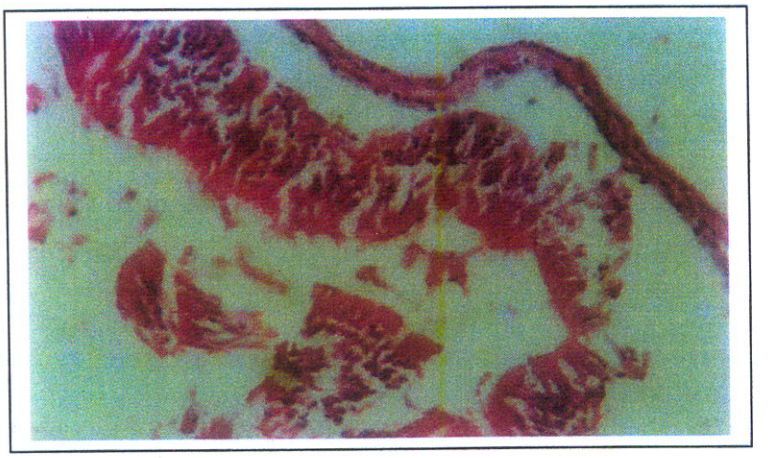

Plate-63 : Intestine of *Cirrhinus mrigala* fed with NTGC-30% incorporated feed **160X (H/E)**

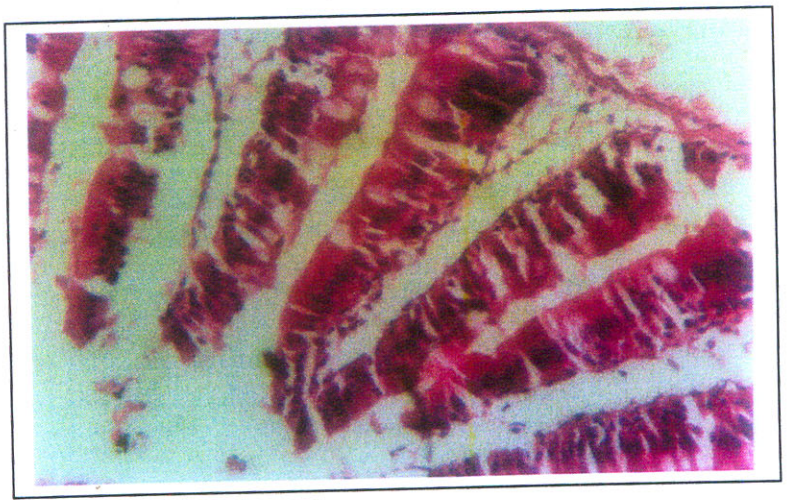

Plate-64 : Intestine of *Cirrhinus mrigala* fed with PCC-10% incorporated feed **160X (H/E)**

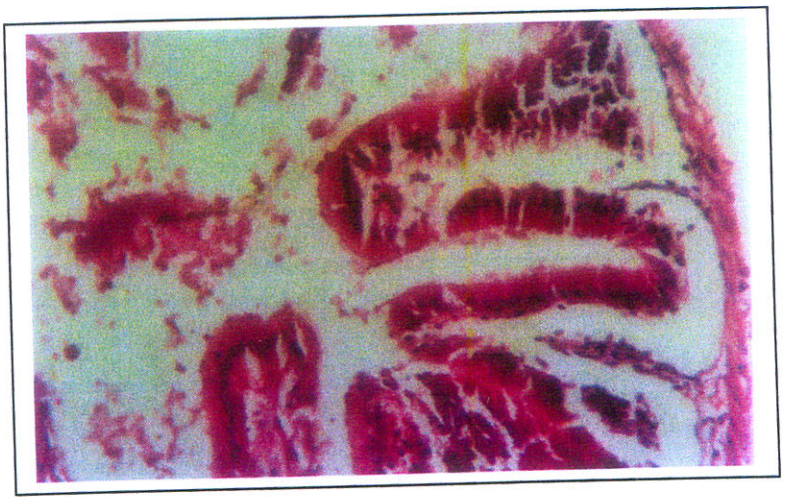

Plate-65 : Intestine of *Cirrhinus mrigala* fed with PCC-20% incorporated feed **160X (H/E)**

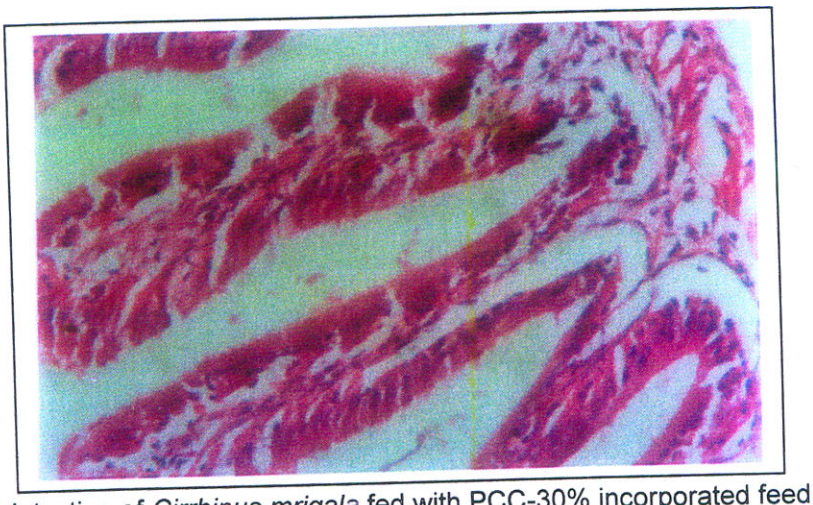

Plate-66 : Intestine of *Cirrhinus mrigala* fed with PCC-30% incorporated feed **160X (H/E)**

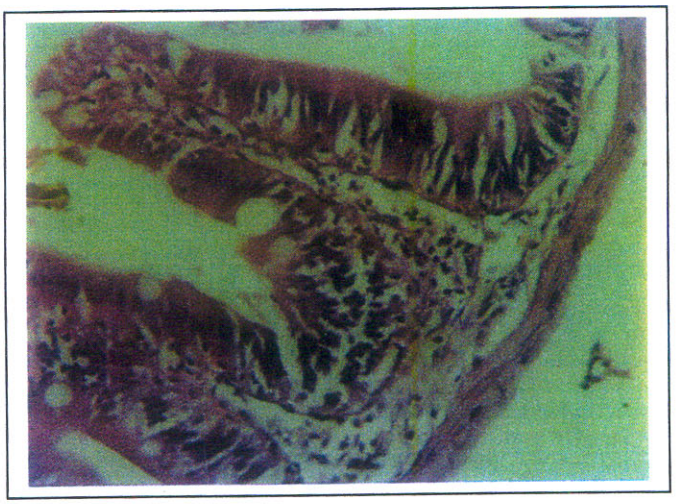

Plate-67 : Intestine of *Cirrhinus mrigala* fed with CCC-10% incorporated feed **160X (H/E)**

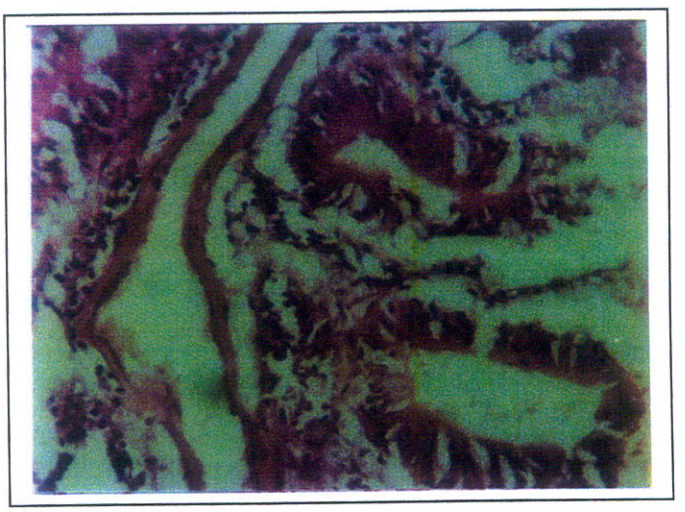

Plate-68 : Intestine of *Cirrhinus mrigala* fed with CCC-20% incorporated feed **160X (H/E)**

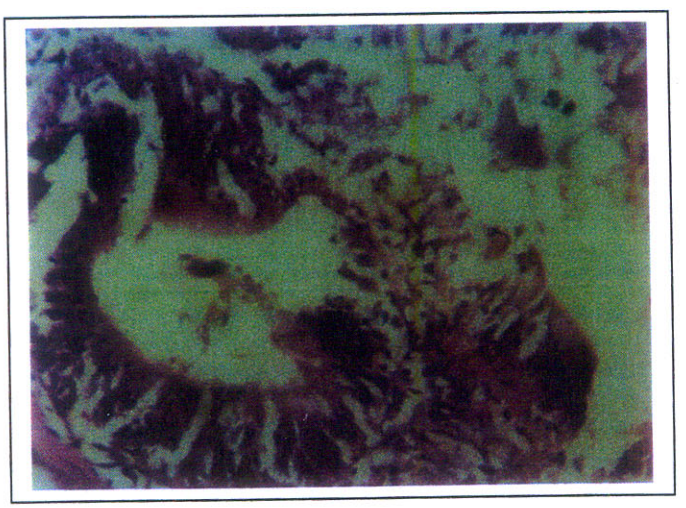

Plate-69 : Intestine of *Cirrhinus mrigala* fed with CCC-30% incorporated feed **160X (H/E)**

In fishes fed with F_3 (TGC-30% cotton seed) showing some necrotic patches (Plate-73).

Feeding the fishes with NTGC (F_4, F_5 and F_6) there are some changes at cellular levels. On feeding F_4 (NTGC-10% cotton seed), there was increased in space in the glomarulus mostly due to shrinkage of the capillary tuft (Plate-74). The renal tubules showed some loss of renal epithelium (Plate-75) on feeding F_5 (NTGC-20% cotton seed). However, fishes on feeding with F_6 (NTGC-30% cotton seeds) did not show any appreciable change in kidney tissues (Plate-76).

Feeding with Parental Cotton Cake (PCC) feeds (F_7, F_8 and F_9) the alterations in general structure was noticed and shown in Plate-77, 78 and 79.

Feeding with F_7 (10% PCC) there was not much difference recorded and structures appeared normal except shrinkage of the capillary tuft in the glomeruli (Plate-77). However, feeding with F_8 (20% PCC) the renal tubules exhibited oedematous swelling (Plate-78). And in the F_9 (30% PCC) fed fishes the kidney tissue showed small necrotic patches in the renal tubules (Plate-79).

Feeding the fishes with Control Cotton Cake (CCC) incorporated feeds (F_{10}, F_{11} and F_{12}) showed minor tissue alterations. Feeding with F_{10} (CCC-10%) showed loss of interstitial tissues at many places (Plate-80). Feeding with F_{11} (CCC-20%) showed similar changes (Plate-81). Similarly on feeding F_{12} (CCC-30%) necrotic patches could be seen in the tubular wall (Plate-82).

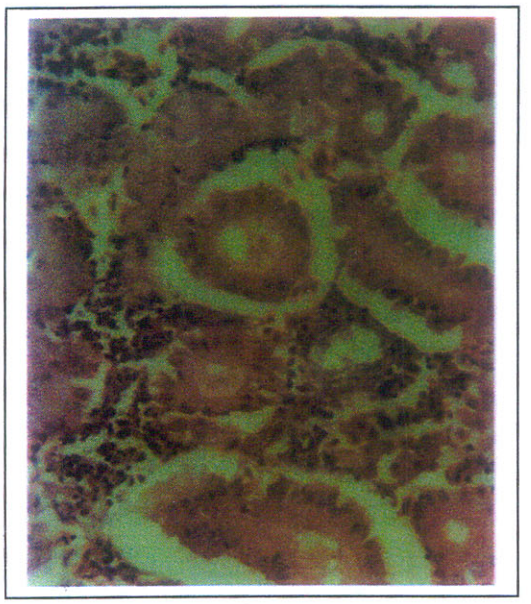

Plate-70 : Control Kidney of *Cirrhinus mrigala* fed with **60X (H/E)**

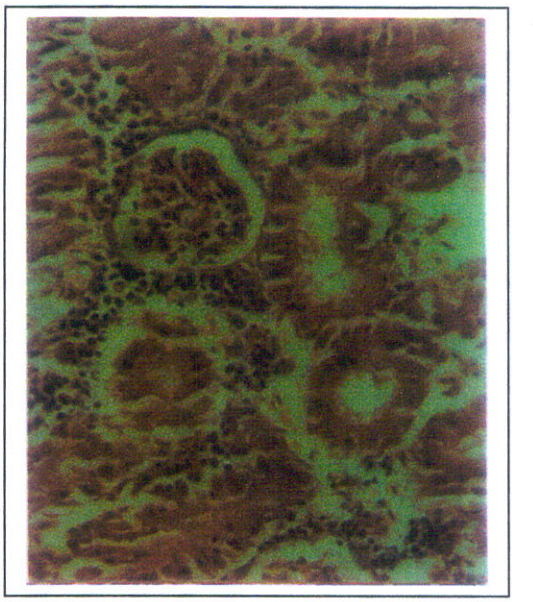

Plate-71 : Kidneyof *Cirrhinus mrigala* fed with TGC-10% incorporated feed **160X (H/E)**

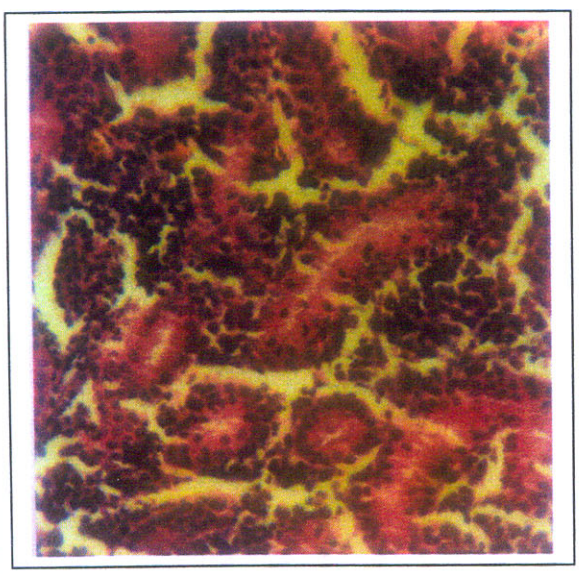

Plate-72: Kidney of *Cirrhinus mrigala* fed with TGC-20% incorporated feed **160X (H/E)**

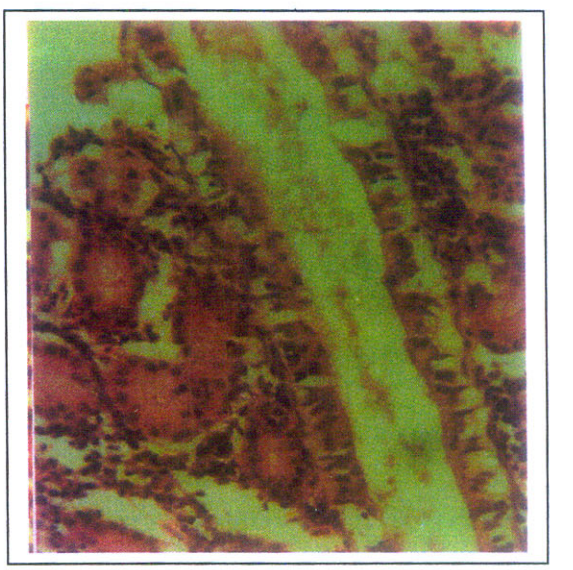

Plate-73: Kidney of *Cirrhinus mrigala* fed with TGC-10% incorporated feed **160X (H/E)**

Plate-74 : Kidney of *Cirrhinus mrigala* fed with NTGC-10% incorporated feed **160X (H/E)**

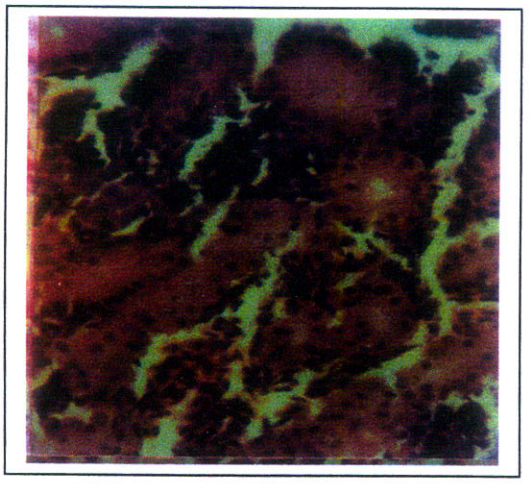

Plate-75 : Kidney of *Cirrhinus mrigala* fed with NTGC-20% incorporated feed **160X (H/E)**

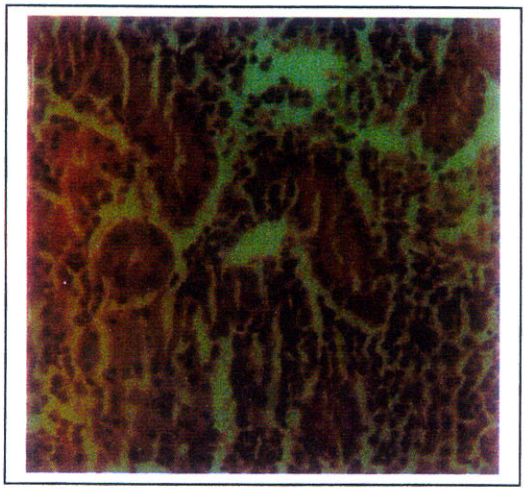

Plate-76 : Kidney of *Cirrhinus mrigala* fed with NTGC-30% incorporated feed **160X (H/E)**

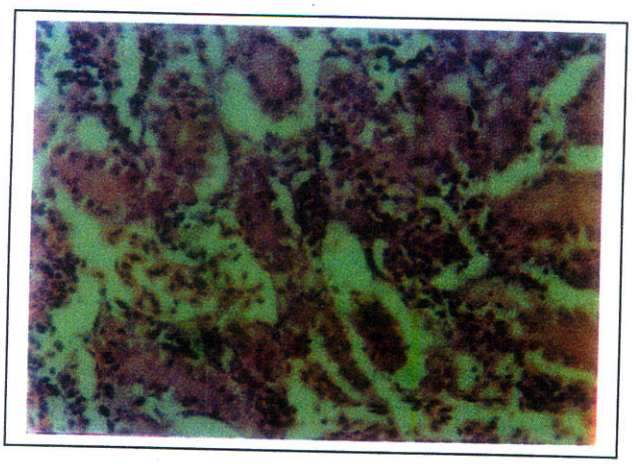

Plate-77 : Kidney of *Cirrhinus mrigala* fed with PCC-10% incorporated feed **160X (H/E)**

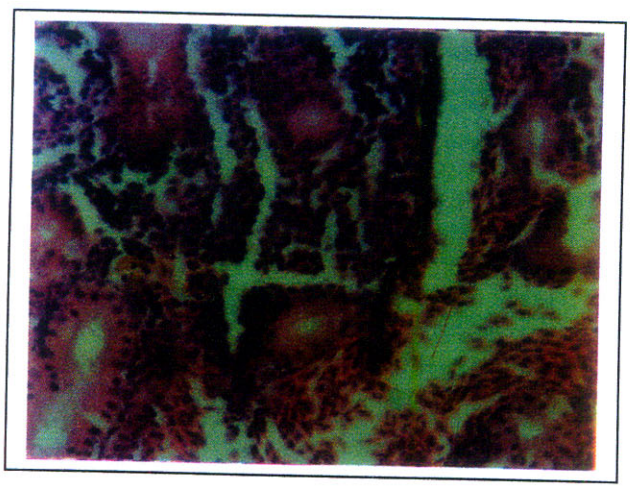

Plate-78 : Kidney of *Cirrhinus mrigala* fed with PCC-20% incorporated feed **160X (H/E)**

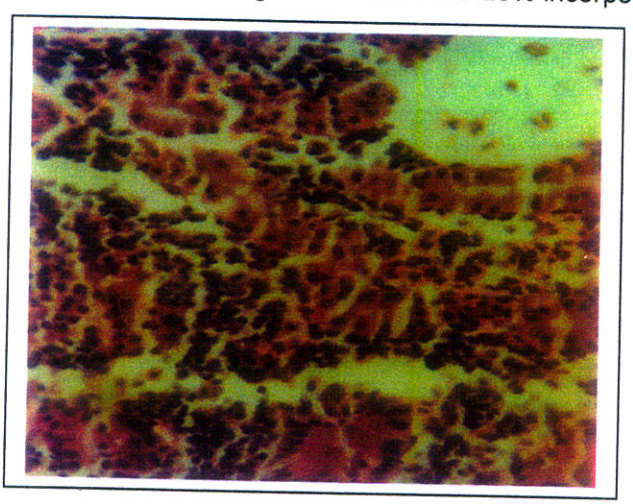

Plate-79 : Kidney of *Cirrhinus mrigala* fed with PCC-30% incorporated feed **160X (H/E)**

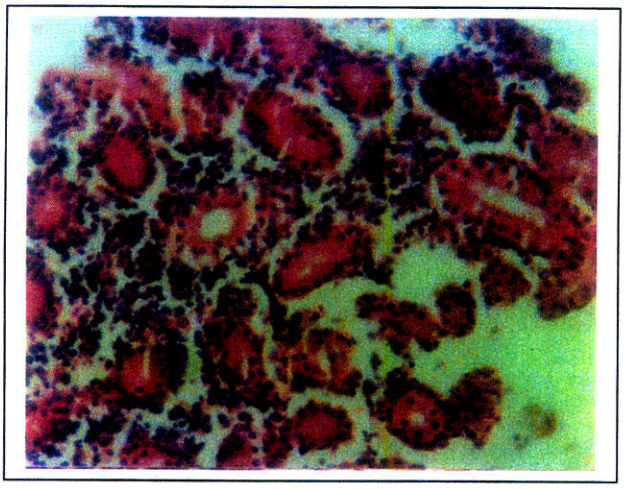

Plate-80 : Kidney of *Cirrhinus mrigala* fed with CCC-10% incorporated feed **160X (H/E)**

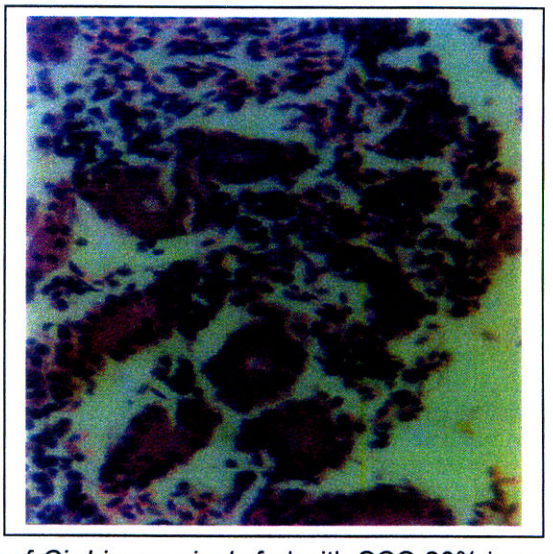

Plate-81 : Kidney of *Cirrhinus mrigala* fed with CCC-20% incorporated feed **160X (H/E)**

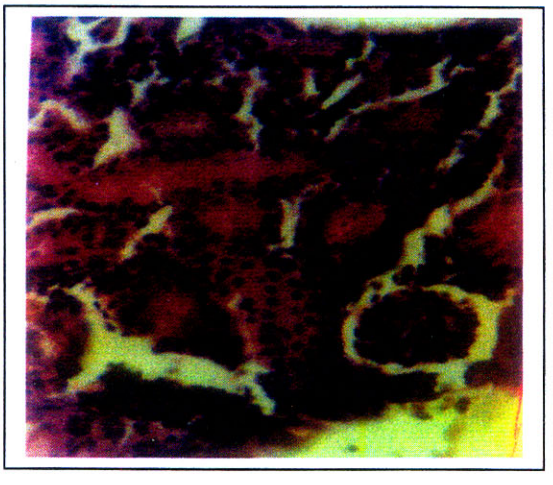

Plate-82 : Kidney of *Cirrhinus mrigala* fed with CCC-30% incorporated feed **160X (H/E)**

ANNEXURE-IV

Conclusion Summarizing the Results

1. Composition of the isoproteinaceous feeds (F_1-F_{12}) are summarized in Table-1 and ingredients are shown in Plate-1 to 26.
2. Proximate composition of two cotton hybrids TGC, NTGC, PCC and CCC shown in Table-2. Seeds and cake have gossipol @ 1.49 ± 0.09%, 1.52 ± 0.11%, 1.56 ± 0.17% and 1.72 ± 0.12% respectively. And the gross energy estimated as 431.5 ± 30.2 K cal/100 g and 428.4 ± 22.1 K.cal/100 g, 422.5 ± 11.75 K cal/100 g and 401.2 ± 17.39 K cal/100 g. Lipid content of TGC (JKC 738 with Cry 1Ac [1845 bp] gene) has more lipid content than NTGC, PCC and CCC recorded as 22.8 ± 0.73%, 20.7 ± 0.41%, 21.3 ± 1.72% and 8.5 ± 0.46% respectively.
3. All the feeds (F_1-F_{12}) contain protein between 34.0 – 35.1% (Nx6.25) and gross energy ranged from 342.5 K.cal/100 g to 352.5 K.cal/100 g (Table-3).
4. The gossypol content of feeds (F_1-F_{12}) recorded as 0.059%, 0.126%, 0.238%, 0.045%, 0.179, 0.272%, 0.062%, 0.127%, 0.217%, 0.071%, 0.142% and 0.263% respectively after incorporating TGC 10%, 20% and 30% (F_1-F_3) and NTGC @ 10%, 20% and 30% (F_4-F_6), PCC @ 10%, 20% and 30% (F_7-F_9) and CCC @ 10%, 20% and 30% (F_{10}-F_{12})(Table-4).
5. The water quality parameters are exhibited in Table-7 and values are within permissible range of aquaculture practices.
6. The proximate composition of experimental fishes are presented in Table-8 and 10. There is no statistical significant difference ($P>0.05$) in carcass gross energy contents and protein contents of experimental fishes ($P>0.01$).
7. Growth parameters are shown in Table-9 to 10. Feed efficiency ranged between 0.34 ± 0.03 to 0.39 ± 0.02; protein efficiency ranged between 1.11 ± 0.03 to 1.21 ± 0.04. However, lowest protein efficiency (1.11 ± 0.03) recorded in TGC–30% feed (F_3) and best (1.21 ± 0.04) recorded in NTGC-20%. The growth results are significant of 99% confidence limit ($P<0.01$) in comparison to control feed (F_9-F_{12}).

8. Final gross energy (GE) of fish ranged between 470.1 K.cal/100 g to 515.5 K.cal/100 g. Lowest GE recorded in PCC - 20% and highest GE recorded in PCC – 30%. Overall no reduced gross energy is recorded in NTGC fed fishes in comparison to CCC fed fishes (Table-10).
9. Specific growth rate (SGR) ranged from 0.48 $\pm$ 0.02% to 0.73 $\pm$ 0.02%. Lowest (0.48 $\pm$ 0.02%) recorded in NTGC-20% and highest recorded in TGC-30% (0.73 $\pm$ 0.02%). Among the four groups TGC showed overall higher ($P<0.05$) SGR followed by CCC, PCC & NTGC fishes (Table-10).
10. The dry matter digestibility ranged from 48.5% to 56.2% and crude protein digestibility ranged from 68.7% to 76.2%. No significant difference recorded among the groups ($P>0.05$) (Table-10)
11. After 70 days feeding trial in plastic pool maximum final weight recorded in TGC-30% (8.15 $\pm$ 0.35g) followed by NTGC-30% (7.71 $\pm$ 0.59g) ($P<0.01$). However, lowest final weight recorded in CCC-10% (5.11 $\pm$ 0.30g).
12. The mineral contents of feed as well as fish carcass demonstrate arbitrary concentrations and not shows any pattern (Table-12 and 13).
13. Histopathological alterations have been recorded in all the four groups (TGC, NTGC, PCC and CCC) in gill, liver, intestine and kidney and results are shown in Plate-31 to 43, Plate-44 to 56, Plate-57 to 69 and Plkate-70 to 82 for gill, liver intestine and kidney respectively.

CONCISE SUMMARY

The genetically modified cotton seed (TGC) containing Cry-IAc [truncated] gene, in comparison to Bt.cotton variety without Cry-IAc gene (NTGC), parental control cotton seed (PCC) and laboratory control cotton cake (CCC) shows similar growth pattern and there was no significant difference ($P>0.05$) among FCR, FER and PER of these three varieties (TGC, NTGC & PCC) on feeding to fish mrigala (*Cirrhinus mrigal*) for 70 days. The TGC, NTGC and PCC feeds (F_1-F_3, F_4-F_6 and F_7-F_9) are compared with CCC incorporated feed (F_{10}-F_{12}) on the basis of isocaloric and isoproteinaceous feeds in terms of fish growth responses, and histopathological alterations in gill, liver, intestine and kidney tissues in an Indian major carp, mrigal (*Cirrhinus mrigala*).

EXPLANATION OF PHOTOGRAPHS

Inside cover page

1. Harvesting of fish fry.
2. Collection of fish samples.
3. Segregation of fish fry.
4. Collecting of *Cirrhinus mrigala* fish fry in to bucket.
5. Fish stock of *Cirrhinus mrigala.*
6. Fish sampling / counting at farm.
7. Transportation of fish fry.
8. Stocking of fish in plastic pool.

Inside back cover page

9. Conditioning of fish sample.
10. Transporting of fish sample to oxygen packing center.
11. Conditioning of the fish in bigger FRP tank.
12. Filling of ambient water in fish transportation bag.
13. Oxygen packing of fish fry.
14. Keeping oxygen packed fish fry in to plastic bucket.
15. Keeping oxygen packed fish fry in to bigger container.
16. Transportation of fish from farm to laboratory.
17. Stocking of fish after acclimatization.

Cover page

Photo of cotton seed plant.

Back page

Field of cotton seed plantation.
Cotton seed meal (TGC, NTGC and PCC)
Analytical laboratory of the Institute.
Fish stock of *Cirrhinus mrigala*