Comparison of broiler chicken performance when fed diets containing cottonseed from Bollgard II cotton with the *cry1Ac* and *cry2Ab2* genes (*cry-X* gene), parental cotton line or commercial cotton

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Study Centre
Avian Nutrition & Feed Technology Division
Central Avian Research Institute
Izatnagar (UP), 243 122. India.

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Maharashtra Hybrid Seeds Company Ltd. (Mahyco)
Resham Bhavan, 4th floor
78, Veer Nariman Road
Mumbai-400 020.

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Project Leader

Dr A. K. Shrivastav
Principal Scientist & Head, NFT

Associates

Dr T. S. Johri
Director

Dr A. B. Mandal
Principal Scientist

Dr A.V. Elangovan
Senior Scientist

Technical Assistance

Anurag K. Johri
Sarabmeet Kaur

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Comparison of broiler chicken performance when fed diets containing cottonseed from Bollgard II cotton with the *cry1Ac* and *cry2Ab2* genes (*cry-X* gene), parental cotton line or commercial cotton

Summary

A new cotton plant, Bollgard II cotton, has been produced using biotechnology methods to insert the *cry2Ab2* gene from *Bacillus thuringiensis* var. *kurstaki* into the Bollgard cotton genome. As a result, Bollgard II cotton produces both the Cry1Ac and the Cry2Ab2 proteins that have insecticidal activity against Lepidopteran insect pests of cotton. The combination of both proteins in one cotton plant provides an additional tool to delay the development of pest resistance to the Cry1Ac protein in Bollgard cotton, because the Cry2Ab2 protein is a different Bt protein class than Cry1Ac. The Bollgard II traits have been introduced into Indian cotton varieties using traditional plant breeding methods to provide Indian cotton growers an additional tool to protect cotton from lepidopteran insect damage. As a requirement for commercial development of the Bollgard II product, a study to evaluate the nutritional value of diets containing Bollgard II cottonseed, parental and commercial lines of cottonseed on chicken performance and carcass yield was conducted in a 42 day feeding study.

The Bollgard II, parental control and commercial cottonseeds obtained from the Mahyco were first processed with solvent to obtain oil extracted meals. The total and free gossypol (%) contents of cottonseeds were 0.68 and 0.52 in Bollgard II cottonseeds (MRC 6301 BG II, F_2), 0.64 and 0.47 in parental control cottonseeds (MRC 6301 Non-BG II F_2), 0.63 and 0.49% in the commercial cottonseed variety (NHH-44, F_2). The total and free gossypol contents were 0.62 and 0.08, 0.54 and 0.05, and 0.61 and 0.07% in meals processed (solvent extraction) from MRC 6301 BG II, MRC 6301 Non-BG II or NHH-44 cottonseeds, respectively. Day-old unsexed broiler chicks (n=280) were divided into 28 groups of 10 each. Seven dietary treatments (iso-nitrogenous, 22% CP and 19.5% CP for 0-3 and 3-6 weeks and iso-caloric, 2900 kcal ME/kg from 0-6 weeks) were formulated viz., D1 (non-cotton control, soybean meal-SBM based), D2 and D3, commercial cotton (NHH-44) CSM at 10% of diet with and without additional iron), D4 and D5 (MRC 6301 BG II cottonseed CSM with and without additional iron), and D6 and D7 (MRC 6301 Non-BG II cottonseed CSM with or without additional iron at 2 ppm for

every 1 ppm of free gossypol, respectively). The limiting amino acids lysine, methionine, cystine, threonine and arginine were similar in all the diets in both growth phases (0-3 weeks starting and 3-6 weeks finishing phases). Each dietary treatment was offered to four replicated groups of birds up to 6 weeks of age. The birds were reared in battery cages with brooding, feeding and watering facility. Other management practices and the vaccination schedule remained similar for all birds. Birds were weighed at weekly intervals with feed intake measured and recorded during the sample interval. A nitrogen retention trial (3 day collection period) was conducted the 6th week. At the end of 6th week, 8 birds per treatment (2 birds / replicate) were sacrificed to study certain blood bio-chemicals, carcass traits, and development of digestive and immune organs.

Body weight gain, feed intake, feed conversion efficiency, and protein and energy utilization efficiency did not differ statistically (P<0.05) either at starting (0-3 wk) or growing (3-6 weeks) and overall (0-6 weeks) growth phase. Dry matter digestibility, nitrogen intake (g/bird/day or per unit energy intake) and nitrogen retention (either expressed as percent of N-intake, g/bird/day or g/unit energy intake) remained statistically similar in all the diets. The different blood biochemical constituents, viz. serum protein, albumin and globulin, uric acid, total cholesterol and hemoglobin did not differ (P<0.05) across dietary treatments. The consumption of cottonseed meals did not influence the mortality of birds. Feather loss, blood loss, yield of giblet, gizzard and liver were not different (P<0.05) across dietary treatments. However, eviscerated yield in the broilers fed NHH-44 diets was significantly higher (P<0.01) than the other treatment groups. All treatment groups had similar eviscerated yield as compared to soybean meal based control except for the MRC 6301 non-BG II + Fe group which was lower and the NHH-44 group which was higher. Also, the yield of heart was statistically higher from the NHH-44 commercial cottonseed meal fed group compared to the soybean meal control, NHH-44 + Fe and MRC 6301 Non-BG II groups. No statistical differences across treatment were observed in the major cut up parts. Likewise, the development of digestive organs was not affected by dietary treatments except for the weight of the small intestine which was lighter in the birds fed the NHH-44 and NHH-44 + Fe groups as compared to the MRC 6301 BG II, MRC 6301 BG II + Fe and MRC 6301 NonBGII + Fe groups. The yield of bursa and thymus was significantly higher (P<0.005) in MRC 6301

BG II, MRC 6301 BG II + Fe, MRC 6301 NonBGII, and MRC 6301 NonBGII + Fe groups as compared to the NHH-44 fed group. Overall, performance, carcass characteristics, nitrogen retention, organ weights and blood biochemical constituents were not different in birds fed cottonseed meal from Bollgard II cotton compared to the non-transgenic cotton. This indicates that Bollgard II cotton is as safe and nutritious to chickens as commercial cotton with low free gossypol.

Introduction

Cotton is the leading plant fiber crop produced in the world and the most important in India. In addition, cottonseed provides an important source of oil for human consumption and cottonseed and processed cottonseed meal for animal feed. India ranks number one in the world for total area planted to cotton, but the country is ranked third in total cotton produced. One major limitation to cotton production in India is damage by insect pests. The cotton crop is damaged by about 130 species of insects of which the Lepidopteran insects are the most important. Conventional chemical pesticides have been used to control these pests. However, use of these pesticides is costly to the grower, often pose environmental hazards, and have limited efficacy due to development of resistance in target pest populations.

As an effective and environmentally superior approach to control these insect pests, Bollgard cotton was produced by insertion of a gene from a naturally occurring bacterium, *Bacillus thuringiensis* subsp. *kurstaki*, into the chromosome of cotton. This enabled production of the Cry1Ac protein in the cotton plant, and this protein is active against lepidopteran insect pests. Bollgard cotton has been adopted broadly by growers worldwide, including India, since commercial introduction in 1996. The Cry1Ac protein in Bollgard cotton provides effective protection from feeding damage by lepidopteran insect pests, and growers using Bollgard cotton typically apply significantly less insecticide to control these pests, realize higher yields, and achieve greater profitability using these improved Bollgard cotton varieties as compared to conventional cotton varieties.

Bollgard II cotton has been produced using particle acceleration plant transformation procedures to insert the *cry2Ab2* insect control gene from *Bacillus thuringiensis* var.

Deleted: *cry2Ab*

kurstaki into the Bollgard cotton genome. Therefore, Bollgard II cotton contains two proteins, Cry1Ac and Cry2Ab, that have insecticidal activity against Lepidopteran insect pests of cotton. Bollgard II cotton provides equivalent or increased control of the major insect pests of cotton with additional control of sporadic pests, such as beet and fall armyworm. Furthermore, combining the Cry2Ab protein with the Cry1Ac protein provides an additional tool to delay the development of lepidopteran pest resistance to the Cry1Ac protein in Bollgard cotton, as Cry2 is a different Bt protein class than Cry1Ac. The Bollgard II traits were introduced into Indian cotton using traditional plant breeding methods to provide Indian cotton growers an additional tool to protect the cotton crop from lepidopteran insect damage.

Bollgard cotton is now cleared by the government of India for propagation in the central and southern cotton zones. As a requirement for commercial development of the Bollgard II cotton product, a study to evaluate the nutritional value of diets containing Bollgard II cottonseed, parental and commercial lines of cottonseed on chicken performance and carcass yield was conducted in a 42 day feeding study.

Review of Literature

Contradictory reports are available indicating the safe/effective level of cottonseed meal in broiler chicken diets (Phelps, 1966; Waldroup, 1981). Raw cottonseed meals (CSM) beyond 4-5% in diet significantly reduce average weight gains and feed intake in broiler chickens (Atuahene *et al.* 1986; Flemming 1996). On the other hand, broilers fed on diets between 10 and 20% raw cottonseed meal had similar body weight, feed intake and feed conversion efficiency (Walkins *et al.*, 1993; Watkins and Waldroup, 1995; Golian, 1994; Phelps 1966). Reports show depressed weight gains and/or feed intakes when free gossypol levels fed to poultry were between 140-756 ppm (0.014-0.076%; Milligan and Bird, 1951), 200-400 ppm (0.02-0.04%; Richardson and Blaylock, 1950), 240-360 ppm (0.024-0.036%; Heywang *et al.*, 1952), or greater than 480 ppm (0.048%; Lipstein and Bornstein, 1964) or 600 ppm (0.06%; Couch *et al.*, 1955). Supplementation of iron at 2 ppm for every 1 ppm of free gossypol, to this diet improved the gain in weight and feed intake significantly from that with the iron un-supplemented group.

Deleterious effects of gossypol in blood and bone of chicks characterized by reduction of haemoglobin levels, red blood cells and bone marrow cells have also been reported (Rigdon *et al.*, 1958). No pathological changes in organs such as liver, kidney, intestine and testes of rats were observed by Song *et al.* (1996) when cottonseed meal (gossypol content <0.02%), containing protein from Bt cotton was fed.

In an earlier experiment, conducted in this Institute (Johri *et al.* 2001, Elangovan *et al.* 2003), broiler chickens received solvent extracted CSM processed from Bt cotton (D2, 1753g & D3, 1638g) & Parental Non-BT (D4, 1653g & D5, 1687g), with or without additional Fe, grew at the same rate as observed in soybean meal based diet (D1, 1676g). The feed intake and feed conversion efficiency (feed: gain) in these dietary treatments (Bt, non-Bt cotton based diets) also did not differ significantly ($P>0.05$) from control diet. A similar observation was also observed in dietary treatments (D8 & D9) containing solvent extracted cottonseed meal of commercial cottonseed. However, a decrease ($P<0.05$) in body weight gain and feed intake was observed in D6 containing national check CSM with high gossypol content. Addition of Fe in the diet (D7) improved ($P<0.05$) feed intake and weight gain but not to the extent as observed in diets containing Bt, parental non-Bt, and commercial cotton CSM or soybean control. However, CSM did not affect feed conversion efficiency in any treatment with or without the addition of iron. The carcass characteristics in terms of dressing percentage, liver weight and heart weight were not significantly ($P>0.05$) different between the treatments. The eviscerated yields emanated from diets containing either Bt, non-Bt or commercial CSM were statistically similar to the soybean control diet. However, eviscerated yield of broilers fed national check CSM with or without iron supplementation was lower ($P<0.05$) than Bt cotton with Fe supplementation and commercial cotton CSM. The conclusions of this study were that solvent extracted Bt, parental non-Bt and commercial cottonseed meal can be included at 10% in soybean meal based broiler diet replacing soybean meal and rice bran without additional iron.

Materials And Methods

The proposed research work was conducted at the Avian Nutrition and Feed Technology Division, Central Avian Research Institute, Izatnagar, India. The biological trial involving 280 broiler chicks was conducted from 4th March to 15th April 2003. The detailed materials and methods employed in the study are presented as follows:

Selection of chicks and experimental design

The day-old unsexed broiler chicks (commercial strain, Hubbard, n=300) were procured from a local hatchery situated at Haldwani, Uttaranchal and were vaccinated against Ranikhet (day1) and Infectious Bursal Disease (14 and 35 days of age). Two hundred and eighty (280) thrifty day-old unsexed, chicks were selected, wing banded, weighed and randomly distributed into 28 groups of 10 chicks each. The experiment was conducted following completely randomized design having 7 dietary treatments with 4 observations (replicates) in each.

Housing and Brooding

The broiler chicks from their first day of age were housed in battery cages. The cages were fitted with feeder, waterer and dropping trays along with electrical brooding arrangement. Standard management practices for brooding, vaccination schedule and light to encourage feed intake were followed. However, supplemental light was provided during night hours so that 24 hours light was available to encourage feed intake. The brooder temperature was maintained at 35°C during 1st week of age and gradually reduced to 25°C at 4 weeks of age, thereafter, only light was provided according to the local commercial practices.

Selection of feed ingredients

The feed ingredients, other than cottonseed meals, were procured all at once in one lot from feed store of Central Avian Research Institute. They were analyzed for proximate composition, calcium, phosphorus and amino acid content. MRC 6301 Bollgard II cotton (BG II), MRC 6301 Non-Bollgard II parental cotton (Non-BG II) and non-transgenic commercial variety (NHH-44) cottonseed samples, supplied by Maharashtra Hybrid Seeds Company Ltd. (Mahyco), New Delhi, were processed in the laboratory for preparation of their respective solvent extracted meals (undecorticated). The ground seeds were expelled applying pressure and residual oil was extracted in laboratory using petroleum ether as the fat solvent. The crude protein and gossypol contents of different cottonseed meals are given in Table 1.

Feeds and feeding

During the entire growth period, extra care was exercised to ensure efficient feeding and watering of chicks. They were supplied fresh underground drinking water daily *ad lib*. Four dietary treatments (isonitrogenous, 22% crude protein and isocaloric, 2900 kcal ME/kg for starting phase i.e. 0-3 weeks and 19.5% crude protein 2900 kcal ME/kg for finishing phase i.e. 3-6 weeks of age) were formulated following standard specifications (Table 2). Seven dietary treatments formulated were viz., D1 (control, soybean meal-SBM based), D2 and D3, commercial (NHH-44) CSM at 10% of diet with and without additional iron ($FeSo4$, $7H2O$ reagent grade), D4 and D5 (BG II CSM with and without additional iron), and D6 and D7 (Parental Non-BG II CSM with or without additional iron at 2ppm for every 1ppm of free gossypol, respectively). The limiting amino acids lysine, methionine, cystine, threonine and arginine were similar in all the diets in both growth phases (0-3 weeks starting and 3-6 weeks finishing phases).

Each dietary treatment was formulated (Table 2) and compounded separately for starting and (0-3 weeks of age) and finishing phase (3-6 weeks of age) and offered in mash form to 4 replicated groups in both phases of production i.e. starting and finishing phases. Before compounding the experimental diets, the ingredients were ground to 2.4 mm (screen size), weighed as per feed formulae and added successively, by and by, in the whole lot. The supplements viz., mineral, vitamin, lysine, methionine and threonine were premixed in maize before mixing in the whole lot to ensure uniform mixing. The mixing was done in vertical feed mixer and mixed for 6-7 minutes to achieve uniform mixing.

Record of Body weight changes and feed intake

Body weight of individual broiler chicks was recorded weekly, while the feed intake of chicks, allotted in replicates, was recorded at weekly interval up to 6 weeks of age. The mortality of birds was recorded as and when it occurred, weighed and sent for postmortem examination. The feed conversion ratio was calculated on the basis of unit feed consumed to unit body weight gain for each replicate separately. The body weights of dead birds were recorded as and when occurred and the gain was taken into consideration for calculation of feed conversion ratio. The metabolizable energy efficiency (ME intake, kcal/g gain) and protein efficiency (protein intake/ gain) were calculated.

Dry matter digestibility and nitrogen retention trials

A digestibility and nitrogen retention trial (3 day collection period) was conducted during the 6[th] week. All excreta collection method was followed as described by Hill and Anderson (1958). Every effort was made to avoid spillage of feed during the collection period as well as during entire trial. During the collection period, total quantity of feed and total excreta voided during 24 hour periods were collected daily, weighed, mixed thoroughly and a representative sample of excreta was pooled for consecutive three days and dried in a hot air oven with exhaust facility at 60°C until a constant weight was obtained. The dried excreta, feed and feed residue samples were ground and processed for nitrogen analysis (AOAC, 1990) to calculate nitrogen retention.

Carcass traits

At the end of the 6[th] week, two birds (one male and one female) were picked randomly from each replicate (i.e. 4 male and 4 female broiler chicks per each treatment). They were starved for 12-hr (only drinking water was supplied *ad libitum)*, and were sacrificed as per standard procedure for evaluation of carcass characteristics including the yield of defeathered weight, eviscerated weight, different digestive and immune organs viz., liver, heart, proventriculus, gizzard, spleen, bursa, thymus, and abdominal fat. The weight and length of small intestine, large intestine and caecum were also recorded. The carcass was cut up to standard parts that included back, breast, thigh, drum stick, neck and wings. The various parameters were then expressed in terms of percentage of live weight.

Laboratory analyses

The representative samples of feed ingredients were analyzed for proximate composition (moisture, crude protein, ether extract, crude fibre, total ash & nitrogen free extract), and phosphorus following standard techniques (AOAC, 1990) and calcium (Talapatra *et al.* 1940). For calculation of amino acids in diets, the analyzed values of amino acids (Llames and Fontaine 1994) for maize, soybean meal and de-oiled rice bran were used, but for cottonseed meals, the reported values were adjusted according to the total protein content. The processed cottonseed meal samples were analyzed for total and free gossypol content following standard techniques (AOCS, 1989). The nutrient composition of the feedstuffs is given in **Table 1** while the calculated nutrient composition for the starting and finishing feeds are presented in **Table 2**.

Statistical analysis

The data were subjected to analyses of variance following one-way classification of completely randomized design (Snedecor and Cochran, 1989). The means of different dietary treatments were tested for statistical significance using Duncan's multiple range tests (Duncan, 1955).

Results And Discussion

Body weight gain, Feed intake and Feed conversion ratio

The weekly live weights of chicks in different dietary treatments have been depicted in Table 3 and Figure 1. The body weight gains at 0-3 weeks and 0-6 weeks of age are given in Table 5. The live weight at 6 weeks of age is also depicted in graphical form (Fig. 3) Body weight gain did not differ statistically ($P<0.05$) either at starting (0-3 wk), growing (3-6 wk), or overall growth phase (0-6 weeks of age). The rapidly growing chicks are highly sensitive to anti-nutrients or toxicants in feed which may be manifested by growth depression. BG II, Non-BG II CSM and NHH-44 commercial CSM when included at 10% of the diet did not depress body weight either at 0-3 or 0-6 weeks of age, indicating no toxic effects in birds. Addition of Fe in the diets (D3, D5 & D6) containing BG II, Non-BG II CSM and NHH-44 commercial CSM also did not prove beneficial which further confirmed that the lower level of free gossypol does not require iron supplementation. Johri *et al.* 2001 and Elangovan *et al.* 2003 reported no beneficial effects of iron supplementation for Bt or non-Bt cotton with low gossypol. However, they did report improved growth of broilers when receiving iron supplemented diets containing 0.002% free gossypol. Moreover, in the present experiment all the diets contained similar energy, limiting amino acids, calcium and available phosphorus. Therefore, the broilers responded to all the diets equally.

Feed intake data at different weeks of age are given in Table 4 and Fig. 2 & 4, while Table 5 depicts feed intake and feed conversion efficiency in different phases. Neither feed intake nor the efficiency of feed utilization (Fig. 5) was affected due to dietary treatments. These results clearly indicate that palatability of feed was not altered due to inclusion of any cottonseed meal (BG II, non-BG II or commercial cottonseed meal) in diet. Feed conversion efficiency and protein and energy utilization efficiency (Table 6) did not differ statistically (P<0.05) either at starting (0-3 wk), growing (3-6 wk) or overall growth phase (0-6 weeks of age).

Contradictory reports are available indicating the safe/effective level of inclusion of cottonseed meal in poultry diets. Raw cottonseed meals beyond 4-5% in diet significantly reduced average weight gains and feed intake in broiler chickens. (Atuahene *et al.* 1986; Flemming *et al.* 1996). On the other hand broilers fed diets between 10 and 20% had similar body weight, feed intake and feed conversion efficiency (Watkins and Waldroup, 1995; Golian, 1994; Phelps, 1996). Therefore in the present study, all the meals were tested at 10% level replacing soybean meal. Inclusion of cottonseed meal (10% of the diet) caused no depression in body weight gain or feed intake as compared to when all soybean meal was used. Similarly BT, Parental Non-BT and commercial cottonseeds containing low free gossypol levels (Elangovan *et al.*, 2003) did not exert depression of growth or feed intake. Song *et al.* (1996) also did not observe any adverse effect on body weight gain and feed utilization in rats or quails fed diets with cottonseed meals from BT-transgenic cotton plants in 28 and 8 day toxicity trials. The high amount of fiber, gossypol, or cyclopropene fatty acids have been attributed to lower feed intake and body weight gain in cottonseed meal based diets (Phelps *et al.* 1965). In the earlier work (Elangovan *et al.* 2003), depression of growth and feed intake in diet containing national check variety of cottonseed meal was due to higher content of free gossypol (0.44%) in it contributing to 0.044% in diet. Reports have shown depressed weight gains and/or feed intakes when free gossypol levels fed to poultry were between 140-756 ppm (0.014-0.076%; Milligan and Bird, 1951), 200-400 ppm (0.02-0.04%; Richardson and Blaylock, 1950), 240-360 ppm (0.024-0.036%; Heywang *et al.*, 1952), or greater than 480 ppm (0.048%; Lipstein and Bornstein, 1964) or 600 ppm (0.06%; Couch *et al.* 1855). Therefore, in the present experiment, as all the dietary treatments were balanced properly with similar concentration of nutrients to meet the requirements of birds, it is envisaged that either the known factor gossypol, because of its low level, or the gene/protein that has been incorporated into cottonseed BG II did not exert any deleterious effect on growth as well as nutrient utilization.

Nutrient utilization

The dry matter digestibility (DMD), nitrogen intake and nitrogen retention are presented in Table 7. The dry matter digestibility, nitrogen intake (g/bird/day or per unit energy intake) and nitrogen retention (either expressed as percent of N- intake, g/bird/day or g/unit energy intake) remained statistically similar in all the diets. The apparent metabolizable energy (AMEn) contents were adjusted by adding fat and altering the levels of de-oiled rice bran. The dry matter digestibility remained similar in the present experiment indicating that the energy availability was not influenced by addition of cottonseed meals, as a strong correlation existed between available energy and dry matter digestibility in chicken (Mandal & Pathak, 1996). The nitrogen intake per unit energy intake remained almost similar in all the diets indicating that due consideration on calorie-protein ratio during ration formulation. Though nitrogen retention as percent of intake was numerically lower in cottonseed fed groups, nitrogen retained as gram/bird or per unit energy intake remained similar among all treatment groups indicating that neither the protein nor the energy was limiting.

Blood biochemical constituents

The blood biochemical constituents viz. serum protein, albumin and globulin, uric acid, total cholesterol and hemoglobin are presented in Table 8. The different blood biochemical constituents did not differ statistically due to dietary treatments. The similar concentration of serum protein, albumin, globulin and uric acid in all the groups indicated similar protein and amino acid metabolism status in birds fed different types of cottonseed meal.

Carcass characteristics

Feather loss, blood loss, yield of giblet, gizzard and liver were not altered due to the dietary treatments (Table 9). However, significantly higher $P<0.01$ eviscerated yield was recorded in NHH fed broilers as compared to the other treatment groups. Except in treatment groups containing non-BG+Fe or NHH, all had eviscerated yield statistically similar to soybean meal based control. The yield of heart also differed statistically being higher in commercial cottonseed meal fed group (NHH) when compared to the control, NHH+Fe and non-BGII groups. The major cut up parts did not differ due to treatments. In the earlier work (Elangovan, 2003), carcass characteristics in terms of dressing percentage and giblets yield were not significantly (P>0.05) different due to either BT, non-BT, commercial cottonseed meal or control SBM diets, similarly the yield of different organs when expressed on unit live weight also did not differ significantly (P>0.05). Atuahene *et al.* (1986) found significant differences among treatment means for dressing percentage, liver and viscera weights, along with depressed weight gain and decreased feed intake in experimental diets containing 5, 7.5 or 10% cottonseed meal.

Development of digestive and immune organs

The development of digestive organs, as envisaged by the yield (% of body weight) of proventriculous, gizzard, length and weight of small and large intestine, ceaca, liver, etc., was not affected by dietary treatments except NHH and NHH+Fe fed groups had lighter small intestines than BGII, BGII+Fe and non-BGII+Fe groups. The yield of immune organs such as Bursa was significantly higher for BGII and thymus yield was significantly lower (P<0.05) in control and NHH fed group. The weight of the spleen and the PHAP response, an index of cell mediated immunity, did not differ due to dietary treatments.

Mortality

The mortality of birds was 5, 1, 1, 2, 3, 1 and 4 in dietary treatments D1 to D7, respectively. However, the pathological changes observed on post-mortem examination of dead birds during the experimental period were not attributed to the dietary treatments. The results indicated that the cottonseed meals with gossypol content (0.05-0.08% in meal) did not influence the mortality of birds. Contradictory reports are also available on the effect of gossypol on mortality pattern. Increasing levels of gossypol have shown to be correlated with increased mortality in some trials (Lillie and Bird, 1950; Couch *et al.* 1955) but not in others (Milligan and Bird, 1951; Eagle and Davies, 1957).

Conclusion

Overall growth performance, feed utilization efficiency, nitrogen retention, carcass characteristics, weights of digestive and immune organs and blood biochemical constituents were not different in birds fed diet with 10% transgenically modified cotton (BGII) seed meal from those fed maize-soybean meal based diet or nongenetically modified cotton seed meal. This indicates that Bollgard II cotton is as safe and nutritious to chickens as commercial cotton with low free gossypol.

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Table 1. Protein & Gossypol Content (% on dry weight basis)

Type of cottonseed	Protein		Gossypol, total		Gossypol, free	
	Seeds	Meal	Seeds	Meal	Seeds	Meal
BG II (MRC 6301)	18.5	30.0	0.68	0.62	0.52	0.08
Non BG II (MRC 6301)	16.4	28.1	0.64	0.54	0.47	0.05
Commercial, NHH44	17.4	31.8	0.63	0.61	0.49	0.07

Table 2. Ingredient and chemical composition of starting (0-3 weeks) and finishing (3-6 weeks) diets (%, as such basis)

Ingredient	Starter (0-3 wk)				Finisher (3-6 wk)			
	SBM Control	NHH-44	BGII	nonBG II	SBM Control	NHH-44	BGII	non BG II
Maize	62.5	59.1	59.1	59.1	64.5	65.61	65.61	65.61
De-oiled rice bran	3.2	0	0	0	8.2	1.5	1.5	1.5
Soybean meal	31	26.5	26.5	26.5	24	19.5	19.5	19.5
NHH-44	-	10	-	-	-	10	-	-
BGII			10		-	-	10	-
nonBGII				10	-	-	-	10
Vegetable oil	0	1	1	1	0	0	0	0
Limestone	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
DCP	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Trace mineral	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin premix	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Lysine	0	0.09	0.09	0.095	-	0.09	0.09	0.09
Methionine	0.12	0.125	0.125	0.13	0.1	0.1	0.1	0.1
Threonine	-	0.03	0.03	0.03	-	0.03	0.03	0.04
Total	100	100	100	100	100	100	100	100
Nutrient composition								
ME,kcal/kg	2906	2910	2910	2911	2904	2904	2904	2904
CP, %	21.83	21.99	21.99	21.89	19.41	19.51	19.51	19.40
Lysine,%	1.20	1.20	1.20	1.20	1.01	1.01	1.01	1.00
Methionine,%	0.50	0.50	0.50	0.50	0.46	0.45	0.45	0.45
Threonine,%	0.89	0.89	0.89	0.89	0.79	0.79	0.79	0.79
Cystine,%	0.41	0.40	0.40	0.40	0.38	0.36	0.36	0.36
Arginine,%	1.41	1.43	1.43	1.46	1.18	1.21	1.21	1.23
Calcium, %	1.02	1.02	1.02	1.02	1.01	1.00	1.00	1.00
Av.Phosphorous,%	0.48	0.48	0.48	0.48	0.48	0.47	0.47	0.47

Trace mineral premix supplied mg / kg diet: Mg. 300; Mn,55; I. 0.4; Fe. 56; Zn, 30; Cu. 4. The vitamin premix supplied per kg diet :Vit.A, 8250 IU; Vit.D_3,. 1200 ICU; Vit.K, 1mg; Vit.E, 40 IU; Vit. B_1, 2mg; Vit. B_2 4mg; Vit.B_{12}, 10mcg; niacin, 60mg; pantothenic acid, 10mg; choline, 500mg.

Ferrous sulphate was added in treatments containing cotton seed meals to achieve additional iron @ 2:1 to free gossypol

DCP: dicalcium phosphate; ME: metabolizable energy; CP: crude protein

Table 3. Live weight (g/bird) of broilers at different age (weeks) fed dietary treatments

Treatments	Age in weeks						
	0	I	II	III	IV	V	VI
SBM Control	43.5	107.8	225.8	432.9	769.8	1160	1537
NHH-44	43.5	115.1	228.8	432.1	728.5	1117	1442
NHH-44+ Fe	43.4	111.2	222.7	436.4	711.9	1066	1447
BGII	43.5	114.3	233.2	448.4	775.6	1177	1522
BGII+ Fe	43.6	113.6	227.8	427.5	738.9	1168	1510
Non-BGII	43.5	118.2	234.1	439.9	760.2	1194	1496
Non-BGII + Fe	43.5	102.7	226.2	429.4	695.8	1085	1471
SEM	0.05	1.58	2.36	3.81	7.93	14.01	13.54

SEM: standard error of the mean

Table 4. Feed intake of broilers at different age (g/weeks) fed dietary treatments

Treatments	Age in weeks					
	I	II	III	IV	V	VI
SBM Control	88.6	214.3	379.2	600.5	796.2	799.6
NHH-44	97.7.6	220.1	388.2	590.4	866.9	859.9
NHH-44+ Fe	94.6	219.2	373.9	552.9	818.6	742.8
BGII	98.7	226.2	392.6	627.3	863.1	943.5
BGII+ Fe	97.1	224.8	389.0	664.0	736.6	958.4
Non-BGII	101.8	232.3	382.5	648.4	933.7	911.1
Non-BGII + Fe	93.6	216.7	398.9	607.6	865.8	968.8
SEM	1.37	2.27	5.13	10.86	33.93	23.08

SEM: standard error of the mean

Table 5. Live weight gain, feed intake and feed conversion ratio (FCR) in dietary treatments

Treatments	Body weight gain (g/bird)			Feed Intake (g/bird)			FCR		
	0-3 wk	3-6wk	0-6wk	0-3wk	3-6wk	0-6wk	0-3 wk	3-6wk	0-6wk
SBM Control	389.5	1096	1493	682	2196	2878	1.76	2.12	1.99
NHH-44	388.6	1006	1398	706	2317	3023	1.82	2.34	2.19
NHH-44+ Fe	397.3	985	1383	688	2114	2802	1.80	2.17	2.05
BGII	412.7	1065	1478	718	2434	3152	1.86	2.35	2.21
BGII+ Fe	389.0	1077	1466	711	2359	3070	1.85	2.21	2.10
Non-BGII	396.4	1057	1452	717	2493	3209	1.81	2.39	2.23
Non-BGII + Fe	385.9	1033	1427	709	2442	3151	1.84	2.36	2.21
SEM	4.06	12.89	13.53	7.08	50.60	53.17	0.01	0.04	0.03
Probability	NS	NS	NS	NS	NS	NS	NS	NS	NS

SEM: standard error of the mean; NS: non significant at P>0,05

Table 6. Energy and protein efficiency in different treatments

Diet	Energy Efficiency (kcal/g gain)			Protein Efficiency (g/g gain)		
	0-3 wk	3-6 wk	0-6 wk	0-3 wk	3-6 wk	0-6 wk
SBM Control	5.12	6.16	5.78	0.38	0.41	0.40
NHH-44	5.29	6.81	6.39	0.40	0.46	0.44
NHH-44+ Fe	5.24	6.30	5.94	0.40	0.42	0.41
BGII	5.42	6.84	6.42	0.41	0.46	0.44
BGII+ Fe	5.39	6.42	6.12	0.41	0.43	0.42
Non-BGII	5.26	6.93	6.48	0.40	0.46	0.44
NonBGII + Fe	5.36	7.11	6.62	0.40	0.47	0.45
SEM	0.04	0.12	0.09	0.003	0.008	0.006
Probability	NS	NS	NS	NS	NS	NS

Table 7. Dry matter metabolizability, nitrogen intake and retention at different treatments

Treatments	DMM	Nitrogen Intake		Nitrogen retained		
		g/day	Per 100 kcal	% of intake	g/ b/d	Per 100 kcal
SBM Control	69.82	3.36	1.070	51.81	1.036	0.554
NHH-44	67.38	3.40	1.073	46.92	0.938	0.504
NHH-44+ Fe	68.03	3.45	1.074	46.63	0.933	0.501
BGII	68.40	3.67	1.074	46.92	0.939	0.504
BGII+ Fe	67.77	3.68	1.074	47.53	0.951	0.510
Non-BGII	67.47	3.52	1.067	46.93	0.939	0.501
Non-BGII + Fe	68.43	3.46	1.067	49.14	0.983	0.524
SEM	0.31	0.05	0.001	0.86	0.017	0.009
Probability[1]	NS	NS	NS	NS	NS	NS

Table 8. Certain blood biochemical constituents in different dietary treatments

Treatments	Serum protein (g/dl)	Serum Albumin (g/dl)	Serum Globulin (g/dl)	Cholesterol (mg/dl)	Hemoglobin (g/dl)	Uric acid (g/dl)
Control	3.37	1.38	1.98	249.38	8.05	4.67
NHH	3.41	1.27	2.15	229.79	7.70	5.67
NHH+ Fe	3.19	1.17	2.02	230.36	7.34	4.35
BGII	3.32	1.18	2.14	239.18	7.22	4.08
BGII+ Fe	3.20	1.25	1.96	220.03	7.31	5.11
Non-BGII	3.28	1.23	2.05	226.73	7.38	4.39
NBGII + Fe	3.28	1.27	2.01	224.59	7.27	4.41
SEM	0.03	0.02	0.03	4.78	0.17	0.17
Probability	NS	NS	NS	NS	NS	NS

Table 9. Carcass traits (% of live weight) in different dietary treatments

Treatments	Feather loss	Blood loss	Eviscerated	Giblet	Gizzard	Heart	Liver
Control	10.40	5.11	72.61[b]	6.68	3.43	0.48[b]	2.77
NHH	9.87	4.71	74.06[a]	7.55	3.86	0.60[a]	3.09
NHH+ Fe	10.45	4.33	71.17[bc]	6.63	3.40	0.47[b]	2.76
BGH	10.31	5.16	71.44[bc]	6.65	3.42	0.52[ab]	2.71
BGH+ Fe	10.30	4.80	71.56[bc]	6.92	3.53	0.55[ab]	2.84
Non-BGH	10.35	4.29	71.70[bc]	6.43	3.29	0.49[b]	2.65
Non BGH + Fe	10.64	5.32	70.23[c]	6.84	3.50	0.52[ab]	2.82
SEM	0.10	0.11	0.23	0.11	0.06	0.01	0.05
Probability	NS	NS	P<0.01	NS	NS	P<0.03	NS

[abc]Means bearing different superscripts in a column differ significantly (P<0.05)

Table 10. Cut up parts (% of live weight) in different dietary treatments

Treatments	Back	Breast	Drum	Neck	Thigh	Wings
SBM Control	15.80	15.75	9.67	4.02[b]	10.02	8.08
NHH-44	17.48	14.99	9.94	5.08[a]	9.37	7.99
NHH+ Fe	16.49	14.63	9.91	4.08[b]	9.44	7.53
BGH	16.74	15.47	9.73	3.56[b]	9.31	8.05
BGH+ Fe	16.78	15.27	9.75	3.91[b]	9.29	7.95
Non-BGH	16.15	16.40	10.03	3.56[b]	9.79	7.86
NonBGH + Fe	15.74	16.53	9.94	3.49[b]	9.25	7.54
SEM	0.18	0.19	0.07	0.12	0.09	0.07
Probability	NS	NS	NS	P<0.004	NS	NS

[ab]Means bearing different superscripts in a column differ significantly (P<0.05)

Table 11. Digestive organs (% of live weight) in different dietary treatments

Treatments	Proventri-culous	SI length	SI. wt	LI length	LI wt.	Cea. L.	Cea. wt.
Control	2.40	8.04	2.82[abc]	2.30	1.08	1.06	0.76
NHH	2.09	8.36	2.55[c]	2.59	0.88	1.19	0.73
NHH+ Fe	2.22	8.45	2.45[c]	2.62	0.91	1.21	0.57
BGH	2.25	8.19	3.08[ab]	2.33	0.75	1.20	0.82
BGH+ Fe	2.36	8.11	3.13[ab]	2.45	0.86	1.03	0.62
Non-BGH	2.10	8.09	2.78[bc]	2.17	0.59	1.03	0.73
NonBGH + Fe	2.16	8.25	3.31[a]	2.26	0.71	1.11	0.74
SEM	0.03	0.97	0.07	0.05	0.04	0.02	0.02
Probability	NS	NS	P<0.004	NS	NS	NS	NS

[abc]Means bearing different superscripts in a column differ significantly (P<0.05)

Table 12. Abdominal fat and immune organs (% of live weight) in different dietary treatments

Treatments	Abdominal fat	Bursa	Spleen	Thymus	PHAP
Control	1.07	0.052[b]	0.174	0.057[bc]	0.78
NHH-44	1.48	0.047[b]	0.167	0.051[c]	0.33
NHH-44+ Fe	1.48	0.050[b]	0.165	0.090[abc]	0.69
BGII	1.37	0.086[a]	0.187	0.105[a]	0.51
BGII+ Fe	1.34	0.059[b]	0.143	0.099[a]	0.68
Non-BGII	1.14	0.054[b]	0.157	0.092[ab]	0.79
NonBGII + Fe	2.29	0.052[b]	0.188	0.114[a]	0.79
SEM	0.13	0.003	0.006	0.006	0.06
Probability	NS	P<0.005	NS	P<0.007	NS

[abc]Means bearing different superscripts in a column differ significantly (P<0.05)

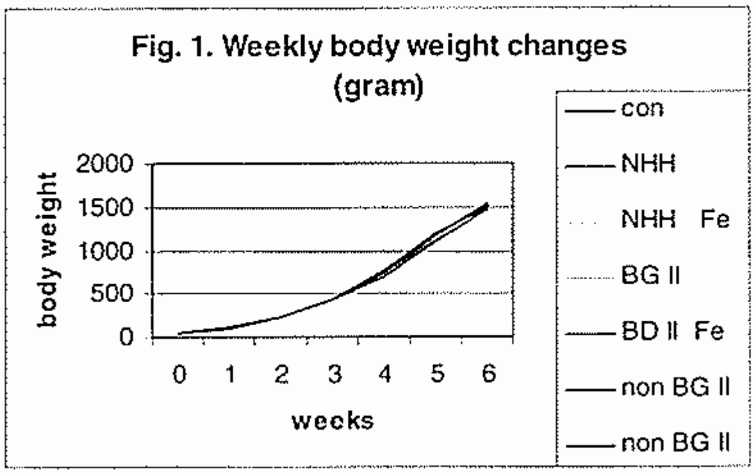


Fig. 1. Weekly body weight changes (gram)
body weight
2000
1500
1000
500
0
0
1
2
3
4
5
6
weeks
con
NHH
NHH Fe
BG II
BD II Fe
non BG II
non BG II

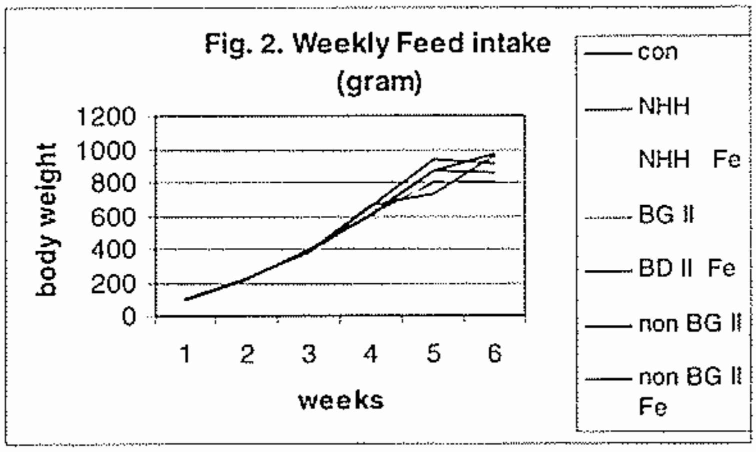


Fig. 2. Weekly Feed intake (gram)
body weight
1200
1000
800
600
400
200
0
1
2
3
4
5
6
weeks
con
NHH
NHH Fe
BG II
BD II Fe
non BG II
non BG II Fe

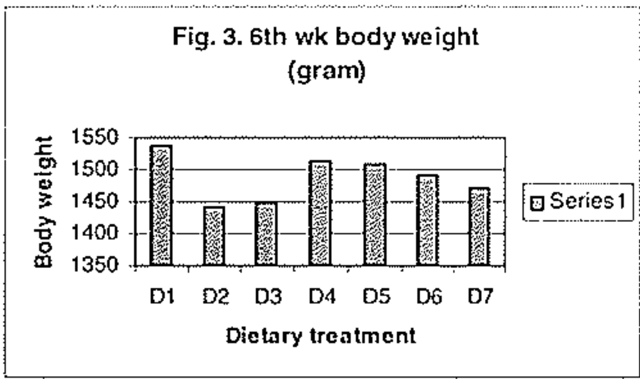


Fig. 3. 6th wk body weight
(gram)
Body weight
1550
1500
1450
1400
1350
D1 D2 D3 D4 D5 D6 D7
Dietary treatment
Series1

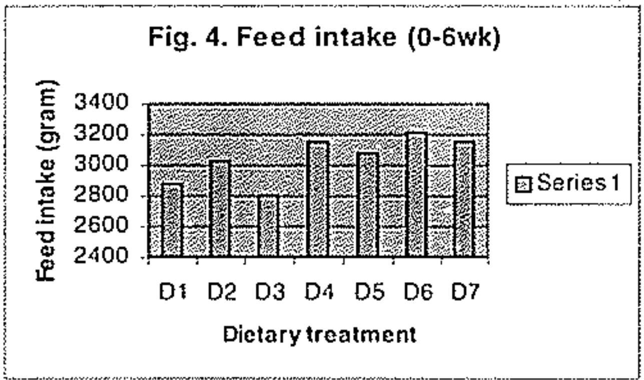


Fig. 4. Feed intake (0-6wk)
Feed intake (gram)
3400
3200
3000
2800
2600
2400
D1 D2 D3 D4 D5 D6 D7
Dietary treatment
Series1

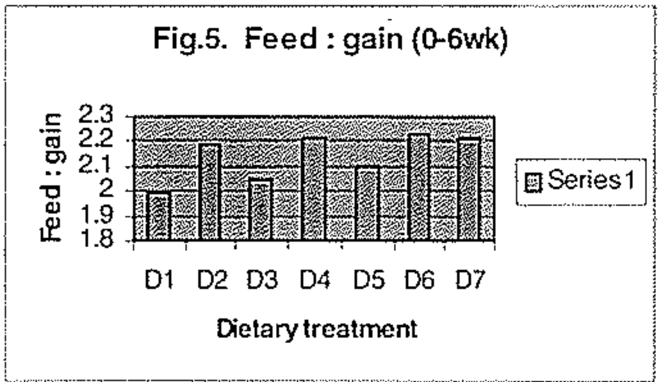


Fig.5. Feed : gain (0-6wk)
Feed : gain
2.3
2.2
2.1
2
1.9
1.8
D1 D2 D3 D4 D5 D6 D7
Dietary treatment
Series1

ANNEXURE 6.3.3(c)

A twenty eight day feeding study of plant/ plant parts to containing Bollgard II ™ cotton to lactating crossbred dairy cows

Effect of feeding cottonseed produced from BG II cotton on feed intake, milk production and composition in lactating crossbred cows.

K.K. Singhal, A.K. Tyagi, Mahindra Singh and Y.S. Rajput
National Dairy Research Institute, Karnal 132 001

ABSTRACT

Twenty crossbred (KS and KF) multiparous cows, housed in well ventilated shed, were fed on concentrate mixture consisting crushed cottonseed (Non Bg II) 40 parts, groundnut cake 15 parts, maize 20 parts, wheat bran 22 parts, common salt 1 part and mineral mixture 2 parts along with green maize fodder *ad lib.* according to their nutritional requirements for 15 days for their adaptation to the cottonseed based diet. Thereafter, cows were divided in two groups of 10 each on the basis of their stage of lactation and milk yield. Group 1 was continued on the same ration and designated as Non Bg II (control) group while cottonseed was replaced with Bg II cottonseed in the concentrate mixture of group 2 and designated as Bg II group. Average voluntary feed intake, milk yield and its composition in the cows of both groups during adaptation period of 15 days was taken as basis for judging the treatment effect of the type of dietary cottonseed. Milk yield and voluntary feed intake were recorded daily while milk samples were collected at 0, 3, 7, 14 , 21 and 28 day of experimental feeding period of four weeks for the analysis of milk composition and to test for the presence of Cry 1Ac and Cry 2Ab proteins. These proteins were also tested in both types of cottonseed, concentrate mixtures based on these seeds and in blood plasma, separated from the blood samples, collected on the 28th day of experimental feeding from all the cows using ELISA method.

The amount of Cry 1Ac and Cry 2Ab protein in Bg II cottonseed was 5.528 and 150.82 μg/g cottonseed on fresh basis, respectively. The corresponding values in cottonseed based Bg II concentrate mixtures were 0.733 and 8.81 μg/g on fresh basis. Concentrate mixture provided to the control and Bg II groups were isonitrogenous and isocaloric. Mean voluntary dry matter intake /100 kg body weight was 3.38 in Bg II group and 3.35 in control group and there was no significant variation between the groups. Cows in both

the groups maintained their body weight during the study and there was no significant affect on the dietary treatment on their body weight. Average milk yield in control and Bg II groups was 11.8 and 12.6 kg/ day, respectively. Average 4% FCM yield/ kg DM intake in corresponding groups was 0.95 and 1.01 and variation between groups for both the parameters was not significant. Mean fat, total solids. SNF contents and somatic cell score (log10 somatic cell counts) in the milk of control group was 4.27, 11.66, 7.45 % and 2.26, respectively and the corresponding value in Bg II group was 4.39, 12.26, 7.81 and 1.76. No significant variation in the milk composition was of both the groups was significant except that for milk protein content, which varied significantly (2.92 vs 3.60%) between the groups when covariate was 3.2, however when covariate was raised to 3.5 or 3.8, there was no statistical difference between the groups. Cry 1Ac and Cry 2 Ab proteins were not detected in milk samples and blood plasma, drawn at various intervals of experimental feeding, Lactating dairy cows of both the groups did not show symptom of any disease, maintained their health and body weight and performed in a similar fashion when fed Bg II and Non Bg II cottonseed, as a source of energy and protein supplement during the four weeks.

TECHNICAL REPORT

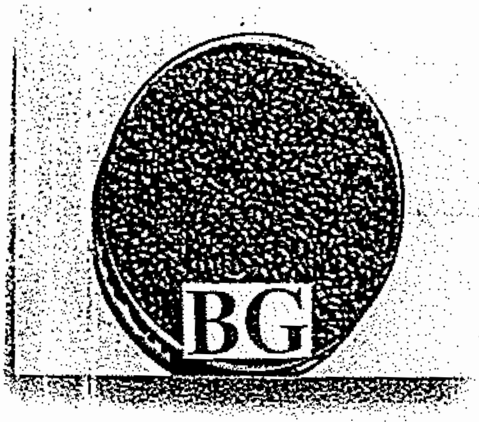
OF

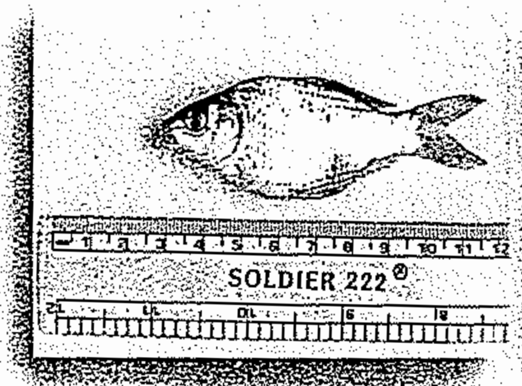
CONTRACT RESEARCH PROJECT

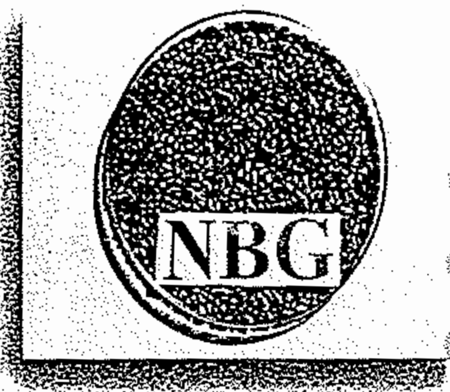
ON

Evaluation of cotton seed meal derived from transgenic Bollgard II cotton hybrids seed containing cry-X gene (*Cry 1Ac* & *Cry 2Ab*) as a feed ingredient for common carp, *Cyprinus carpio* (L.)

28th December, 2002 to 30th October, 2003







ICAR

<u>Performing Laboratory</u>

Aquafeed Laboratory of Fish Nutrition and Biochemistry Division

Central Institute of Fisheries Education

(Deemed University),

Versova, Mumbai-400 061 (MS), India

<u>Laboratory Project ID</u>

Study: ALNUBI-CR-2002-03

Experiment : MOU-CR/CIFE-MAHYCOBOLLGARD/ALNUBI/2002/1

CREDITS

Data Entry & Preparation of Manuscript
Mrs. S. S. Gajbhiye

Cover design
Mr. Dasari Bhoomaiah

Photographs
Mr. D. L. Sawant
Dr. P. P. Srivastava

TECHNICAL REPORT
OF
CONTRACT RESEARCH PROJECT

Title of the Project

Evaluation of cotton seed meal derived from transgenic Bollgard II cotton hybrids seed containing cry-X genes *(Cry 1Ac & Cry 2Ab)* as a feed ingredient for common carp, *Cyprinus carpio* (L.)

28th December, 2002 to 30th October, 2003

Name of the Principal Investigator
Dr. S. C. Mukherjee

Name of the Co-Principal Investigator(s)
Dr. K. K. Jain Dr. P. P. Srivastava

Research Scholar
Dr. Nemi Chand Ujjania

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-2002-03
Experiment : MOU-CR/CIFE-MAHYCOBOLLGARD/ALNUBI/2002/1

Abbreviation

Bt.	:	*Bacillus thuringiensis*
CIFE	:	Central Institute of Fisheries Education
MAHYCO/MAHY	:	Maharashtra Hybrid Seeds Company
MAHYCO BOLLGARD	:	Maharashtra Hybrid Seed Company Bollgard Cotton Se
VM	:	Vitamin Mixture
MM	:	Mineral Mixture
CSM	:	Cotton Seed Meal
$CaHPO_4$	:	Calcium Hydrogen Phosphate
FM	:	Fish meal
SBM	:	Soybean meal
MOC	:	Mustard oil cake
FCR	:	Food conversion ratio
FER	:	Feed Efficiency ratio
g	:	Gram
ND	:	Not detected
CR	:	Contract Research
BG-II/BG	:	Bt-gene (Cry gear) incorporated cotton (Bollgard II)
NBG-II/BG	:	Non Bt-gene incorporated cotton (Bollgard II)
ID	:	Identification
SE	:	Standard Error
ALNUBI	:	Aquafeed Lab. of Nutrition & Biochemistry Div.
MOU	:	Memorandum of Understanding
Bollgard (W)	:	Bollgard II (with *Cry 1Ac & 2Ab genes*)
Bollgard (WO)	:	Bollgard II (without *Cry 1Ac & 2Ab genes*)
mg	:	Milligram
IU	:	International Unit
µg	:	Microgram
PER	:	Protein Efficiency Ratio
DO	:	Dissolved Oxygen
SPSS	:	Statistical Package
RBD	:	Random Block Designing

1.	Title of the Project	:	Evaluation of cotton seed meal derived from transgenic Bollgard II cotton hybrids seed containing cry-X genes *(Cry 1 AC & Cry 2Ab)* as a feed ingredient for common carp, *Cyprinus carpio* (L.)
2.	Name of the Investigator(s)	:	Dr. S. C. Mukherjee, PI Dr. K. K. Jain, Co-PI Dr. P. P. Srivastava, Co-PI
3.	Research Scholar (for 4 months)	:	Dr. Nemi Chand Ujjania (Period 16.4.2003 to 15.8.2003)
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	:	Maharashtra Hybrid Seeds Company Limited, Resham Bhavan, 4th Floor, 78, Veer Nariman Road, Mumbai-400 020, India.
7.	Date of Contract Research undertaken	:	28th December, 2003
8.	Tenure of Contract Research	:	From December, 28, 2002 to October 30, 2003
9.	Date of completion of Contract Research	:	30th October, 2003
10.	Submission of draft report	:	30th November, 2003
11.	Submission of final report	:	20th December, 2003

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.

Names and signature of investigator(s)

04/03/04 4/03/04 04/03/04

(S. C. Mukherjee)	(K. K. Jain)	(P. P. Srivastava)
Principal Investigator	Co-Principal Investigator	Co-Principal Investigator

DIRECTOR 04/03/04

निदेशक / DIRECTOR
केन्द्रीय मत्स्य शिक्षा संस्थान (भा.कृ.अनु.प.)
Central Institute of Fisheries Education
सम्‍मत विश्वविद्यालय / Deemed University (ICAR)
वरसोवा, मुंबई / Versova, Mumbai - 400061

Annexure - I

Objectives :

Objectives as stated in the Contract Research Project proposal from MAHYCO, Mumbai.

1. To evaluate the genetically modified cotton seed (BOLLGARD II) containing *Cry–1Ac and Cry-2Ab genes*, as a feed ingredient for common carp, *Cyprinus carpio.*

2. To study the comparative growth and survival on feeding of given samples of cotton seed as :
   - A : Bt. Cotton variety MRC-6301 *(*BOLLGARD--II with *Cry–1Ac and Cry-2Ab genes)* (Plate-1).
   - B : Bt. Cotton variety MRC-6301 *(*BOLLGARD–II without *Cry–1Ac and Cry-2Ab genes)* (Plate-2).
   - C : Mustard Oil Cake (Laboratory control) (Plate-5).

Purpose :

This contract research was designed to assess whether raw cotton seed meal (Plate-1,3) derived from Bt. Gene *(*BOLLGARD–II with *Cry–1Ac and Cry-2Ab genes)* incorporated cotton plant is as safe and nutritious for growth of the common carp (*Cyprinus carpio*) as the meal derived from the non-Bt. gene *(*BOLLGARD–II without *Cry–1Ac and Cry-2Ab genes)* raw cotton-seed meal (Plate-2,4).

In the present study the assessment was carried out between Bollgard II (W) and Bollgard II (WO) in terms of growth, survival and biochemical composition of common carp (*Cyprinus carpio*). As told by MAHYCO 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.

A. ***Test Material*** : The test material was defined as a cotton meal derived from Bt-gene (BOLLGARD–II with *Cry–1Ac and Cry-2Ab genes)* cotton, ID # as Bt. cotton variety BOLLGARD-II (W), MRC-6301 (Plate-1,3).

B. ***Control Material*** : The control material was defined as the cottonseed meal derived from the control, same germplasm cotton line (BOLLGARD–II with *Cry–1Ac and Cry-2Ab genes)* and ID # as non-Bt. Cotton variety BOLLGARD II (WO) *with Cry–1Ac and Cry-2Ab genes)* MRC-6301 (Plate-2,4).

C. ***Laboratory Control Material*** : The laboratory control material was identified as the mustard oil cake and ID # as MOC (Plate-5).

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 Bollgard II (with *Cry 1Ac and 2Ab genes*) cotton on the growth of carp in comparison to Bollgard II (without *Cry 1Ac and 2Ab genes*) and their use in aquaculture nutritional practices with safety measures.

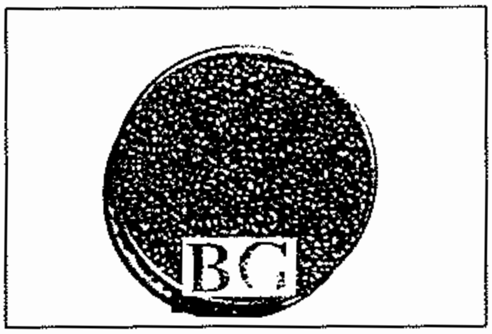


Plate-1 : Boligard II cotton seeds

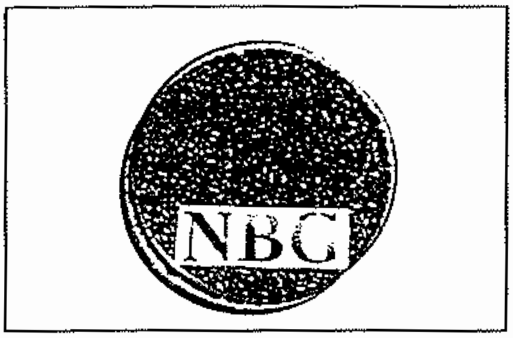


Plate-2 : Non-Boligard II cotton seeds

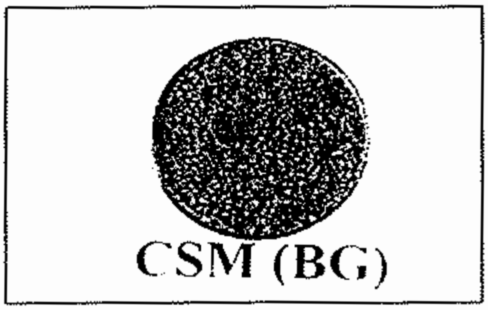


Plate-3 : Raw feed ingredient, cotton seed meal (CSM[BG])

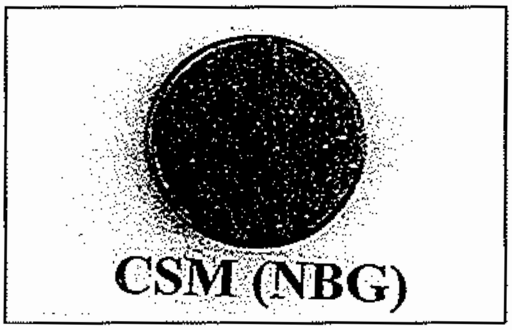


Plate-4 : Raw feed ingredient, cotton seed meal (CSM [NBG])

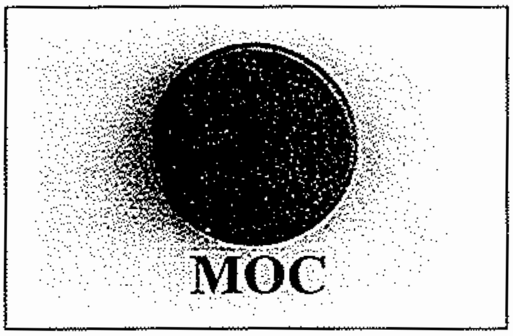


Plate-5 : Raw feed ingredient, Mustard oil cake (Powdered)

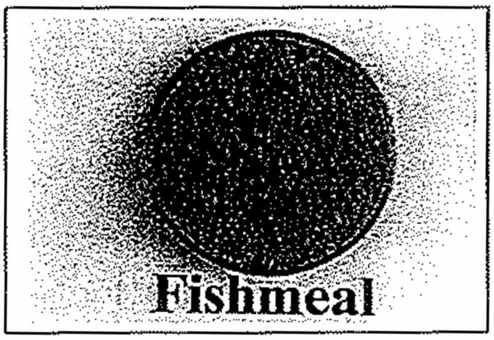


Plate-6 : Raw feed ingredient, fish meal (Powdered)

Annexure - II

Material and Methods :

Experimental diets (Table-1 & 2) were formulated to contain crude protein @ 28-30%. Experimental diets were formulated by substituting cotton seed meals (MRC = 6301 with *Cry 1Ac and Cry 2Ab genes*) @ 5%, 15% and 25% (F_1 contained gossypol 0.063%; F_2 contained gossypol 0.130% and F_3 contained gossypol 0.252%). The other three feeds F_4, F_5 and F_6 contained cotton seed meals (MRC = 6301 without *Cry 1Ac and Cry 2Ab genes*) @ 5%, 15% and 25% (F_4 contained gossypol 0.043%; F_5 contained gossypol 0.144% and F_6 contained gossypol 0.261%). The gossypol contents in cotton seeds and all the six feeds are tabulated in table 3 & 5 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 fish meal (Plate-6), acetes (Plate-7), rice polish (Plate-8), soybean meal (Plate-9), pea hull (Plate-10), maize (Plate-11), $CaHPO_4$ (Plate-12), iron supplement (Plate-13), vitamin and mineral mix. (Plate-14) using Bollgard II three feeds (F_1-F_3) were prepared with graded levels of BGII @ 5%, 15% and 25%(Plate-15, 16, 17 and 18). Similarly, Non-Bollgard II cotton meal was used to prepare three feeds (F_4-F_6) containing NBG @ 5%, 15% and 25% (Plate-19, 20, 21 and 22). The laboratory control feeds (F_7-F_9) were made using mustard oil cake @ 5%, 15%and 25% (Plate-23, 24,25 and 26).

A. ***Survival :***

The survival percentage recorded during the experimentation.

B. ***Water quality :***

The physico-chemical parameters estimated fortnightly and results are shown in Table-8.

C. ***Proximate composition*** :

All the samples of cotton meal, feed (F_1-F_6) and experimental fishes were analysed by AOAC (1989) (Table-3, 4 and 9). 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-17) following AOAC (1989). Further, the proximate composition of fishes after feeding for 56 days, has been estimated using standard methods and the results are recorded in Table-9.

Table-1 : Ingredients composition (%) of isoproteinous feeds for *C. carpio* fingerlings.

S N	Feed Code	CSM (BG)	CSM (NBG)	MOC	Fish meal	Acetes	Rice polish	SBM	Pea hull	Maize powder	Veg. oil	Ca H PO_4	Iron Supplement	VM + MM	Total (g)
1	BG-5	5	-	-	5	5	15	35.000	16.375	10	5	2.5	0.125	1	100
2	BG-15	15	-	-	5	5	15	29.375	12.000	10	5	2.5	0.125	1	100
3	*BG-25*	25	-	-	5	5	15	23.375	8.000	10	5	2.5	0.125	1	100
4	*NBG-5*	-	5	-	5	5	15	35.000	16.375	10	5	2.5	0.125	1	100
5	*NBG-15*	-	15	-	5	5	15	29.375	12.000	10	5	2.5	0.125	1	100
6	*NBG-25*	-	25	-	5	5	15	23.375	8.000	10	5	2.5	0.125	1	100
7	*MOC-5*	-	-	5	5	5	15	34.375	17.000	10	5	2.5	0.125	1	100
8	*MOC-15*	-	-	15	5	5	15	27.375	14.000	10	5	2.5	0.125	1	100
9	*MOC-25*	-	-	25	5	5	15	19.875	11.500	10	5	2.5	0.125	1	100

BG, Bollgard; NBG, Non-Bollgard; MOC, Mustard Oil Cake; CSM, Cotton seed meal; SBM, Soybean meal.

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-19 and 20.

Table-2 : Ingredients composition per kilogram of isoproteinous feeds for *C. carpio* fingerlings.

Sl. No.	Feed Code	CSM (BG)	CSM (NBG)	MOC	Fish meal	Acetes	Rice polish	SBM	Pea hull	Maize powder	Veg. oil	Ca HPO_4	Iron Supple-ment	VM + MM	Total (g)
1.	BG-5	50	-	-	50	50	150	350.00	163.75	100	50	25	1.25	10	1000
2.	BG-15	150	-	-	50	50	150	293.75	120.00	100	50	25	1.25	10	1000
3.	BG-25	250	-	-	50	50	150	233.75	80.00	100	50	25	1.25	10	1000
4.	NBG-5	-	50	-	50	50	150	350.00	163.75	100	50	25	1.25	10	1000
5.	NBG-15	-	150	-	50	50	150	293.75	120.00	100	50	25	1.25	10	1000
6.	NBG-25	-	250	-	50	50	150	233.75	80.00	100	50	25	1.25	10	1000
7.	MOC- 5	-	-	50	50	50	150	343.75	170.00	100	50	25	1.25	10	1000
8.	MOC-15	-	-	150	50	50	150	273.75	140.00	100	50	25	1.25	10	1000
9.	MOC-25	-	-	250	50	50	150	198.75	115.00	100	50	25	1.25	10	1000

BG, Bollgard; NBG, Non-Bollgard; MOC, Mustard Oil Cake; CSM, Cotton seed meal; SBM, Soybean meal.

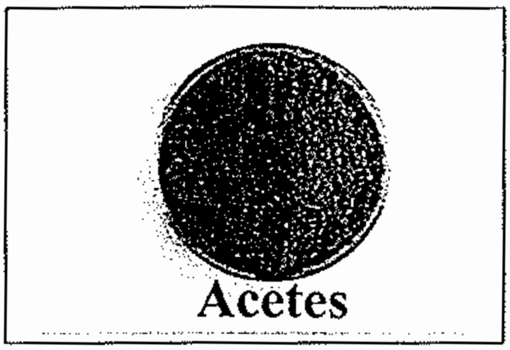


Plate-7 : Raw feed ingredient, *Acetes* (Powdered)

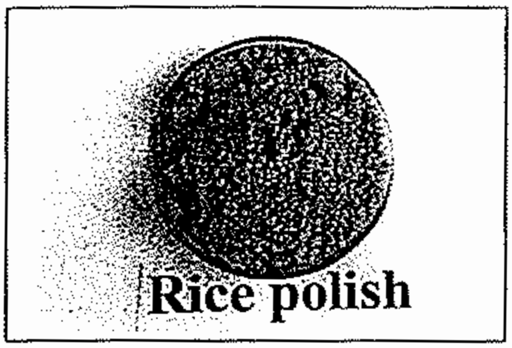


Plate-8 : Raw feed ingredient, Rice Polish (Powdered)

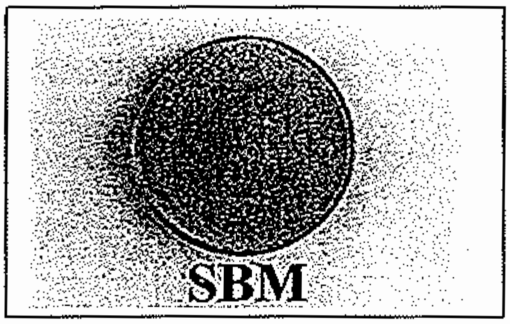


Plate-9 : Raw feed ingredient, soybean meal (Powdered)

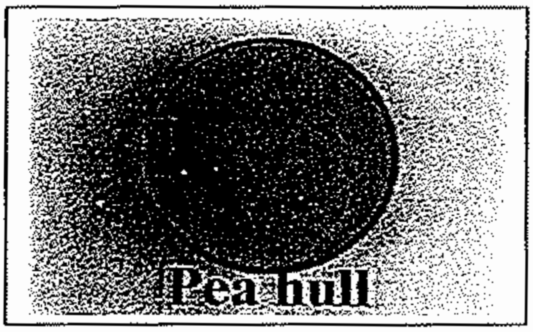


Plate-10 : Raw feed ingredient, pea hull (Powdered)

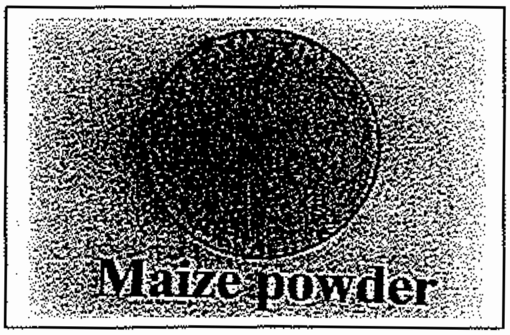


Plate-11 : Raw feed ingredient, maize (Powdered)

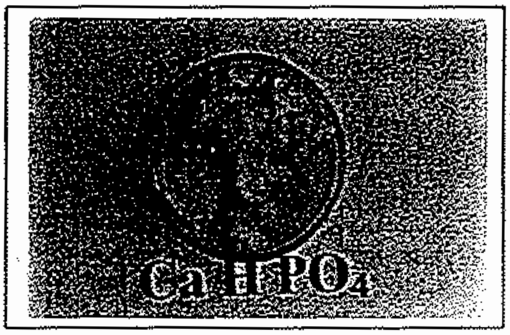


Plate-12 : Calcium hydrogen phosphate (Mineral)

Plate-13 : Iron supplement (Powdered)

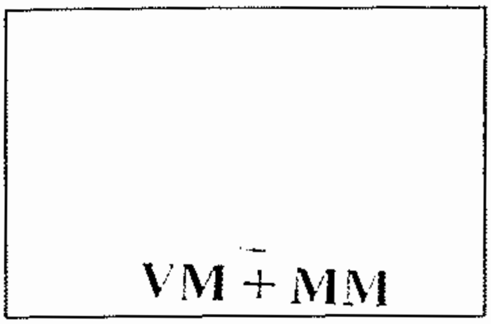


Plate-14 : Vitamin and mineral mixes (VM + MM)

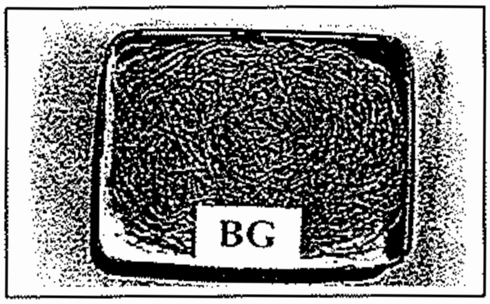


Plate-15 : Extruded feed containing Bollgard II (BG) cotton seed

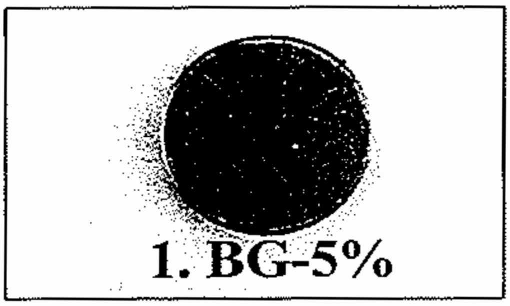


Plate-16 : Prepared feed containing Bollgard-II (BG-5%)

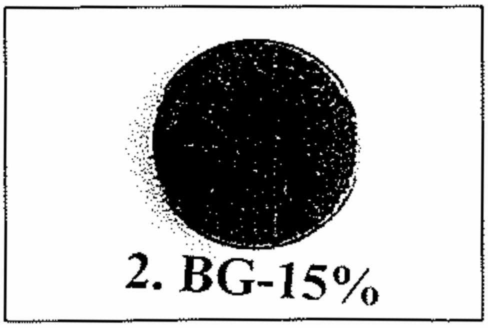


Plate-17 : Prepared feed containing Bollgard-II (BG-15%)

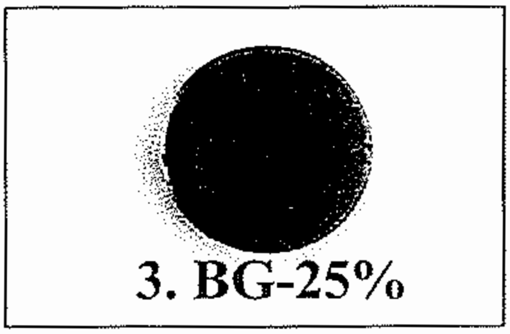


Plate-18 : Prepared feed containing Bollgard-II (BG-25%)

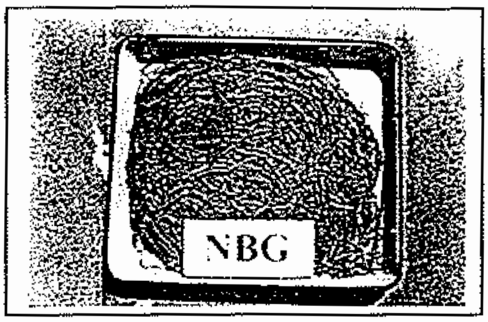


Plate-19 : Extruded feed containing non-Bollgard-II (NBG) cotton seed

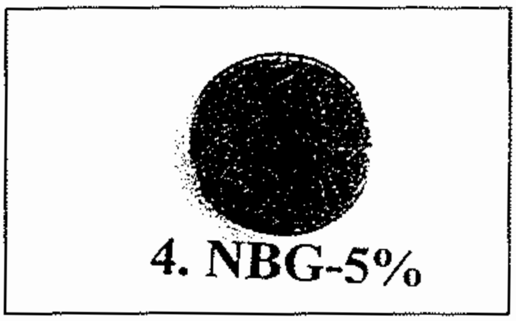


Plate-20 : Prepared feed containing Non-Bollgard II (NBG-5%)

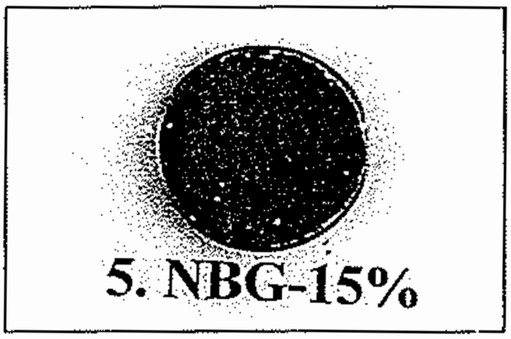


Plate-21 : Prepared feed containing Non-Bollgard-II (NBG-15%)

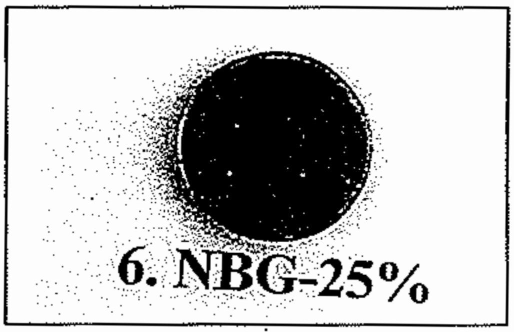


Plate-22 : Prepared feed containing Non-Bollgard-II (NBG-25%)

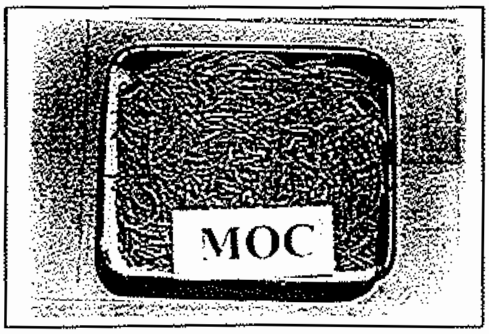


Plate-23 : Extruded feed containing mustard oil cake (MOC)

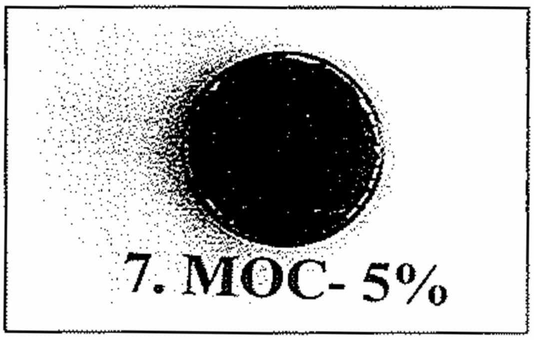


Plate-24 : Prepared feed containing mustard oil cake (MOC-5%)

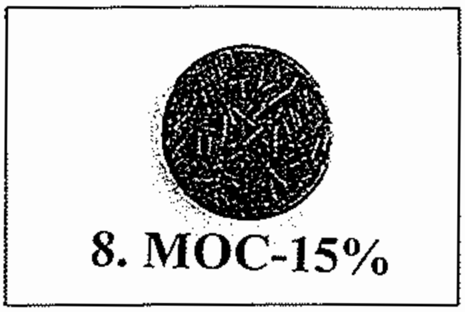


Plate-25 : Prepared feed containing mustard oil cake (MOC-15%)

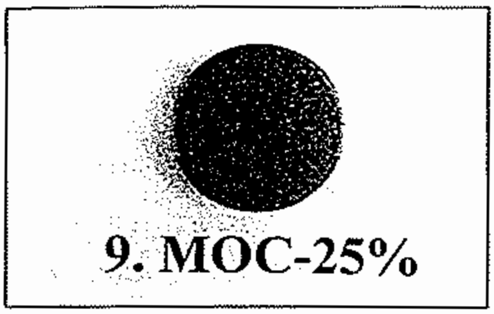


Plate-26 : Prepared feed containing mustard oil cake (MOC-25%)

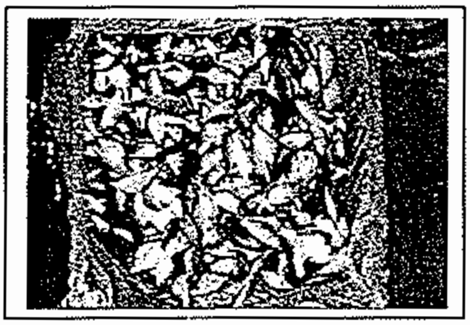


Plate-27 : Stock of the fingerlings of *Cyprinus carpio* fingerling (Before stocking)

Plate-28 : View of plastic pool containing of *Cyprinus carpio*

Plate-29 : Experimental setup

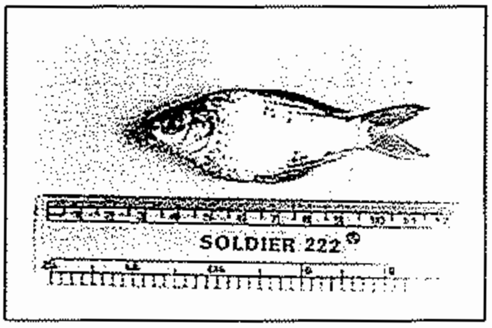


Plate-30 : Fingerling of *Cyprinus carpio* (After rearing of 56 days)

F. ***Histological studies*** :

The gill, liver, intestine and kidney were collected from untreated fishes on day 1 from reserve pool and samples from fishes, fed with 9 feeds, were collected (after 56 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) and standard protocols were followed.

Table 3 : Biochemical composition⁺ of two cotton hybrids*

Lab No.	Cultivar name	Lipid (%)	Protein (%)	Ash (%)	Carbo-hydrate (%)	Gross energy K-cal (per 100g)	Gossypol (%)
5751	MRC-6301 *(BG II containing Cry 1Ac & 2Ab genes)*	22.2	27.0	3.6	50.4	467.6	1.56
5752	MRC-6301 *(BG II without Cry 1Ac & 2Ab genes)*	20.0	26.6	3.9	49.4	444.4	1.53

* All values are mean of two determinations and are on dry weight basis.
⁺ Analysis carried out at MAHYCO Laboratory, MAHYCO, National Highway No.7, Hyderabad-Nagpur Road, Mandal-Toopra, Distt. Medak, KALLAKAL – 502 334 (Andhra Pradesh) alongwith Shri K. Raghunath, Research Scientist (R&D) of MAHYCO.

Table 4 : Composition of feeds (F_1-F_9)

Sr.No.	Feed	Moisture (%)	Lipid (%)	Protein (Nx6.25)	Ash (%)	Carbo-hydrate (%)	Gross Energy (K-cal per 100g)
1.	F_1	7.3	2.5	22.8	9.9	57.5	343.7
2.	F_2	7.4	4.8	24.6	9.9	53.3	354.8
3.	F_3	7.3	9.3	23.1	10.2	50.1	376.5
4.	F_4	8.0	4.8	24.2	11.0	52.0	348.0
5.	F_5	7.9	4.5	23.8	10.2	53.6	350.1
6.	F_6	8.7	8.5	23.8	10.1	48.9	367.3
7.	F_7	8.0	4.9	24.1	10.3	52.7	351.3
8.	F_8	7.3	5.1	24.1	10.7	52.8	353.5
9.	F_9	8.4	5.3	25.1	10.3	50.9	351.7

Table 5 : Total Gossypol contents⁺ in the BG II (W) and BG II (WO) cotton seeds incorporated feeds

Sr.No.	Feed	Containing cotton seed (%)	Total Gossypol* (%)
1	F_1	5	0.063
2	F_2	15	0.130
3	F_3	25	0.252
4	F_4	5	0.043
5	F_5	15	0.144
6	F_6	25	0.261

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

+ Analysis carried out at MAHYCO Laboratory, MAHYCO, National Highway No.7, Hyderabad-Nagpur Road, Mandal-Toopran, Distt. Medak, KALLAKAL – 502 334 (Andhra Pradesh) alongwith Shri K. Raghunath, Research Scientist (R&D) of MAHYCO.

All the six feed containing cotton meal and three control feeds (F_7-F_9) 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 6 and 7 respectively.

Table 6 : Composition of iron supplement (Raricap Forte, Johnson & Johnson, India)

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

Table 7 : Vitamins and Mineral feed supplements

Sr.No.	Components	Quantity per 2.5 kg
1.	Vitamin A	62500 IU
2.	Vitamin D3	62800 IU
3.	Vitamin E	250 mg
4.	Nicotinamide	1 g
5.	Copper	312 mg
6.	Cobalt	45 mg
7.	Magnesium	6 g
8.	Iron	1.5 g
9..	Zinc	2.136 g
10.	Iodine	100 mg
11.	Selenium	10 mg
12.	Manganese	1.2 g
13.	Calcium	247.34 g
14.	Phosphorus	114.68 g
15.	Sulphur	12.20 g
16.	Sodium	5.8 g
17.	Potassium	48.05 mg

Batch No. : S2215-A; Manufactured date : October, 2001; Manufactured by : M/s. Sundar Chemicals Pvt. Ltd., 434, Sideo Ind. Estate, Ambattur, Chennai – 600 098; Marketed by: M/s.Glaxo India Ltd., Agrivet Farm Care Division, Dr. Annibeasant Road, Worli, Mumbai – 400 025.

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 the normal limits. Each pool was stocked with fry of *Cyprinus carpio* (Avg. length, 4.71 ± 0.47 to 4.99 ± 0.041 cm and avg. weight, 1.60 ± 0.48 to 2.06 ± 0.538 g) @ 60 fishes per pool (Plate-27, 28 and 29). Each feed was given in the two replications @ 10% body weight and the quantity of feed was adjusted every fortnight as per the weight gain. The feeding experiment was conducted for four fortnight (56 day, 14 day x 4). After each fortnight the 15 fishes were sampled and were put for various biochemical analysis.

Data Collection and Proximate analysis of fish and feed :

At the end of 56 days feeding experiment, a minimum of five fish from each plastic pool (Plate-30) 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; Kjeltec system (model : 2200 Kjeltec Auto Distillation Extraction Unit, Tecator, Sweden) for protein analysis ; Fibretec system (model : M, 1017, Tecator, Sweden) for fibre 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 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 (Plate-29) with 8 inches of water level (Plate-28). After stabilizing fish samples (Plate-27), the fish was stocked @ 25 fish each species per tank. Sampling/ Data on growth parameters was carried on 2nd week, 4th week, 6th week and at the end of 8th week samples of water, and fish (Plate-30) tissues were preserved quickly to analyse the water quality, tissue histology and biochemical composition beside the growth evaluation. Total 20 plastic pools tanks will be used following RBD system to analyse data statistically.

Experimental design:

(BC+RFI) -	Bollgard II cotton (BC) inclusion along with other Routine feed. ingredients (RFI).	:	5 %, 15%, and 25 %	= 3
(PCC+RFI) -	Parental control cotton (PCC) inclusion along with other Routine feed ingredients (RFI).	:	5 %, 15%, and 25 %	= 3
(MOC+RFI) -	Groundnut oil cake (GOC) inclusion along with other Routine feed ingredients (RFI).	:	5 %, 15%, and 25 %	= 3
	Replication		2	= 2
	Total tubs			= 18

Sampling :

(i) Water sampling : On initial, every week and at the end of 56 days.

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

(A)	POOL		POOL	
	1	BOLLGARD II 5%	4	BOLLGARD II 25 %
(BC+RFI) ≡ BG-II	2	BOLLGARD II 25 %	5	BOLLGARD II 15 %
+ fish @ 60 per pool	3	BOLLGARD II 15 %	6	BOLLGARD II 5%

(B)	POOL		POOL	
	7	Parental Control 5%	10	Parental Control 25 %
(PCC+RFI) ≡ NBG-II	8	Parental Control 25 %	11	Parental Control 15 %
+ fish @ 60 per Pool	9	Parental Control 15 %	12	Parental Control 5%

(C)	POOL		POOL	
	13	Mustard Cake 5%	16	Mustard Cake 25 %
(MOC+RFI)	14	Mustard Cake 25 %	17	Mustard Cake 15 %
+ fish @ 60 per Pool	15	Mustard Cake 15 %	18	Mustard Cake 5%

Sampling days per pool:

Days	POOL NUMBER		
	REPLICATES WITH CONDITION		
0	1-6 (BC + RFI)	7-12 (PCC + RFI)	13-18 (MOC + RFI)
14	1-6 (BC + RFI)	7-12 (PCC + RFI)	13-18 (MOC + RFI)
28	1-6 (BC + RFI)	7-12 (PCC + RFI)	13-18 (MOC + RFI)
42	1-6 (BC + RFI)	7-12 (PCC + RFI)	13-18 (MOC + RFI)
56	1-6 (BC + RFI)	7-12 (PCC + RFI)	13-18 (MOC + RFI)

BC + RFI = Bollgard II (W) + Remaining Feed Ingredient

PCC + RFI = Parental Control Cotton Bollgard II (WO) + Remaining Feed Ingredient

MOC + RFI = Mustard Oil Cake + Remaining Feed Ingredient

All the proximate composition and various growth parameters was carried out along with the Length Weight relationship after completion of the experimentation at CIFE, Mumbai and MAHYCO, Kallakal (Medak).

Annexure-III

Results :

A. Survival :

The data collected from the 56 days feeding study showed no statistically significant differences in the survival of common carp (*Cyprinus carpio*) fed diets containing Bollgard II (W) cotton seed as treatment 'A' compared to fish fed diet containing Bollard II (WO) cotton seed as treatment 'B' and laboratory control 'C' containing Mustard oil Cake alongwith other ingredients. In all the 18 plastic pools all the 60 fishes were healthy and even after sampling of 15 samples from each pool at every fortnight the 60, 45, 30 and 15 fishes survived on day 14, 28, 42 and 56 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-8.

Tabl-8 : Water quality parameters

Parameters / 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	27-28	7.8±0.2	276±8	72±0.5	312±7.2	6.7±0.3	0.21±0.03	0.05±0.007	0.12±0.017	14.7±0.12
F_2	27-28	7.7±0.1	272±6	73±0.4	317±7.0	7.1±0.2	0.20±0.01	0.05±0.005	0.13±0.008	15.4±0.15
F_3	27-28	7.9±0.2	277±6	76±0.2	314±5.3	7.3±0.1	0.22±0.01	0.04±0.002	0.11±0.005	13.8±0.2
F_4	26-28	7.8±0.1	281±5	70±0.1	319±4.2	7.4±0.2	0.21±0.03	0.06±0.004	0.13±0.002	15.3±0.1
F_5	27-28	7.7±0.2	280±4	69±0.2	321±3.1	7.3±0.3	0.22±0.01	0.07±0.002	0.12±0.005	16.8±0.9
F_6	27-29	7.9±0.1	283±2	68±0.7	311±8.8	7.5±0.2	0.20±0.01	0.09±0.008	0.12±0.004	14.5±0.8
F_7	27-28	7.8±0.1	288±7	69±1.0	335±4.7	7.6±0.1	0.24±0.03	0.10±0.010	0.12±0.011	13.7±0.1
F_8	26-28	7.9±0.2	286±2	70±0.8	330±3.6	7.3±0.2	0.21±0.02	0.09±0.005	0.13±0.009	12.7±0.1
F_9	26-27	7.9±0.1	280±1	69±0.5	337±4.5	6.9±0.3	0.22±0.03	0.08±0.005	0.11±0.010	11.8±0.1

Mean ± SE

Table-9 : Proximate composition of experimental fishes (Dry matter basis)

Feed/Day	Moisture* %	Lipid* %	Protein* (Nx6.25) %	Ash* %	Carbohydrate* %	Gross Energy* K.calories Per 100g
Initial	8.9	22.2	39.4	12.6	6.9	425.0
F_1/14	9.2	21.4	47.8	10.4	11.2	428.6
F_2/14	10.2	21.7	44.8	9.6	13.7	429.3
F_3/14	10.0	25.2	44.1	9.3	11.4	448.8
F_4/14	10.8	19.0	42.7	10.6	16.9	456.8
F_5/14	10.6	22.4	46.9	11.1	9.0	425.2
F_6/14	8.9	22.6	45.3	11.4	11.8	431.8
F_7/14	8.1	21.6	47.1	10.6	12.6	433.2
F_8/14	9.4	18.8	48.4	11.4	12.0	410.8
F_9/14	10.4	15.5	47.5	12.8	13.8	384.7
F_1/28	9.2	27.5	42.7	9.0	11.6	464.7
F_2/28	7.8	28.6	44.6	8.9	10.1	476.2
F_3/28	8.7	30.8	41.7	9.5	9.3	481.2
F_4/28	9.4	25.7	45.4	9.7	9.8	452.1
F_5/28	8.1	27.0	42.6	10.4	11.9	461.0
F_6/28	9.8	30.4	41.9	9.5	8.4	474.8
F_7/28	8.8	27.2	41.9	10.2	11.9	460.0
F_8/28	8.8	25.6	42.1	10.4	13.1	451.2
F_9/28	8.4	19.4	44.0	12.3	15.9	414.2
F_1/42	8.5	30.6	44.1	9.7	7.1	480.2
F_2/42	8.5	32.5	41.1	8.9	9.0	492.9
F_3/42	7.5	36.5	38.9	7.6	9.5	522.1
F_4/42	7.3	33.5	40.4	8.6	10.2	503.9
F_5/42	7.6	30.5	42.5	9.0	10.4	478.9
F_6/42	8.5	30.5	40.7	8.3	12.0	485.3
F_7/42	8.4	34.4	37.9	8.5	10.8	504.4
F_8/42	8.0	30.9	41.9	9.5	9.7	484.5
F_9/42	8.3	24.0	46.2	10.8	10.7	443.6
F_1/56	7.5	31.3	39.9	8.8	12.5	491.3
F_2/56	6.9	33.3	43.7	10.5	5.6	496.9
F_3/56	7.4	35.4	38.8	7.9	10.5	515.8
F_4/56	7.2	32.8	43.7	8.3	8.0	502.0
F_5/56	7.5	31.1	42.5	8.4	10.5	491.9
F_6/56	7.3	34.6	40.2	7.8	10.1	512.6
F_7/56	8.2	31.7	41.0	8.4	10.7	492.1
F_8/56	7.3	30.4	43.5	8.9	9.9	487.2
F_9/56	7.1	31.0	43.6	9.2	9.1	489.8

*Average values of 2 replicates.

Table 10 : Average fortnightly weight gain difference of Common carp fed with BG Cotton, Non – BG cotton and control feeds

Tank No.		28/05/03	12/06/03	26/06/03	10/07/03	24/07/03
		W (g)	W (g)	W (g)	W (g)	W (g)
1	I	1.60	0.64	0.10	0.39	0.78
	II	1.60	0.32	0.49	0.27	1.20
2	I	1.70	0.25	0.47	0.15	1.06
	II	1.66	0.75	0.14	0.31	0.82
3	I	1.77	0.60	0.25	0.65	1.38
	II	1.85	0.30	0.12	1.50	1.88
4	I	2.06	0.45	0.59	0.28	1.15
	II	1.89	0.27	0.31	0.41	0.70
5	I	1.93	0.15	0.38	0.44	0.59
	II	1.90	0.21	0.18	0.63	0.77
6	I	1.90	0.10	0.41	0.22	1.15
	II	2.00	0.20	0.21	0.74	1.30
7	I	1.79	0.25	0.63	0.23	1.87
	II	1.68	0.30	0.24	0.15	0.58
8	I	1.68	0.34	0.35	0.47	0.97
	II	1.77	0.29	0.31	0.30	0.49
9	I	1.75	0.31	0.16	0.09	0.13
	II	1.66	0.44	0.11	0.04	0.06

Table 11 : Growth increment in every fortnight in terms of average weight (g) in *Cyprinus carpio* (Common Carp)

Tank No.	28/05/03	12/06/03	26/06/03	10/07/03	24/07/03
	W (g)	W (g)	W (g)	W (g)	W (g)
1	1.60 ± 0.00	0.48± 0.23	0.30 ± 0.28	0.33 ± 0.08	0.99 ± 0.30
2	1.68 ± 0.03	0.50 ± 0.35	0.31 ± 0.23	0.23 ± 0.11	0.94 ± 0.17
3	1.81 ± 0.06	0.45 ± 0.21	0.19 ± 0.09	1.08 ± 0.60	1.63 ± 0.35
4	1.98 ± 0.12	0.36 ± 0.13	0.45 ± 0.20	0.35 ± 0.09	0.93 ± 0.32
5	1.92 ± 0.02	0.18 ± 0.04	0.28 ± 0.14	0.54 ± 0.13	0.68 ± 0.13
6	1.95 ± 0.07	0.15 ± 0.07	0.31 ± 0.14	0.48 ± 0.37	1.23 ± 0.11
7	1.74 ± 0.08	0.28 ± 0.04	0.44 ± 0.28	0.19 ± 0.06	1.23 ± 0.91
8	1.73 ± 0.06	0.32 ± 0.04	0.33 ± 0.03	0.39 ± 0.12	0.73 ± 0.34
9	1.71 ± 0.06	0.38 ± 0.09	0.14 ± 0.04	0.06 ± 0.04	0.10 ± 0.05

Mean ± SE

Table 12: Average fortnightly weight gain of Common carp fed with BG Cotton, Non – BG Cotton and control feeds

Tank No.		28/05/03	12/06/03	26/06/03	10/07/03	24/07/03
		W (g)	W (g)	W (g)	W (g)	W (g)
1	I	1.60	0.64	0.74	1.13	1.91
	II	1.60	0.32	0.81	1.08	2.28
2	I	1.70	0.25	0.72	0.87	1.93
	II	1.66	0.75	0.89	1.20	2.02
3	I	1.77	0.60	0.85	1.50	2.88
	II	1.85	0.30	0.42	1.92	3.80
4	I	2.06	0.45	1.04	1.32	2.47
	II	1.89	0.27	0.58	0.99	1.69
5	I	1.93	0.15	0.53	0.97	1.56
	II	1.90	0.21	0.39	1.02	1.79
6	I	1.90	0.10	0.51	0.73	1.88
	II	2.00	0.20	0.41	1.15	2.45
7	I	1.79	0.25	0.88	1.11	2.98
	II	1.68	0.30	0.54	0.69	1.27
8	I	1.68	0.34	0.69	1.16	2.13
	II	1.77	0.29	0.60	0.90	1.39
9	I	1.75	0.31	0.47	0.56	0.69
	II	1.66	0.44	0.55	0.59	0.65

Table 13 : Growth fortnightly in aggregate increment in terms of average weight (g) in *Cyprinus carpio* (Common carp)

Tank No.	28/05/03	12/06/03	26/06/03	10/07/03	24/07/03
	W (g) (Initial)	W (g)	W (g)	W (g)	W (g)
1	1.60 ± 0.00	0.48 ± 0.23	0.78 ± 0.05	1.11± 0.04	2.10 ± 0.26
2	1.68 ± 0.03	0.50 ± 0.35	0.81 ± 0.12	1.04 ± 0.23	1.98 ± 0.06
3	1.81 ± 0.06	0.45 ± 0.21	0.64± 0.30	1.71± 0.30	3.34 ± 0.65
4	1.98 ± 0.12	0.36 ± 0.13	0.81 ± 0.33	1.16 ± 0.23	2.08 ± 0.55
5	1.92 ± 0.02	0.18 ± 0.04	0.46± 0.10	1.00 ± 0.04	1.68 ± 0.16
6	1.95 ± 0.07	0.15 ± 0.07	0.46 ± 0.07	0.94 ± 0.30	2.17± 0.40
7	1.74 ± 0.08	0.28 ± 0.04	0.71± 0.24	0.90± 0.30	2.13 ± 1.21
8	1.73 ± 0.06	0.32 ± 0.04	0.65± 0.06	1.03 ± 0.18	1.76 ± 0.52
9	1.71 ± 0.06	0.38 ± 0.09	0.51 ± 0.06	0.58 ± 0.02	0.67 ± 0.03

Mean ± SE

Table 14. Growth increment in terms of average length (cm) and average weight (g) in *Cyprinus carpio* (Common carp)

Tank No.		28/05/03		12/06/03		26/06/03		10/07/03		24/07/03	
		L (cm) ± SD	W (g) ± SD	L (cm) ± SD	W (g) ± SD	L (cm) ± SD	W (g) ± SD	L (cm) ± SD	W (g) ± SD	L (cm) ± SD	W (g) ± SD
1	I	4.80± 0.53	1.60± 0.48	5.06±0.51	2.24±0.69	5.14±0.55	2.34± 0.78	5.12± 0.45	2.73± 0.47	5.53±0.51	3.51± 0.94
	II	4.80± 0.51	1.60± 0.52	5.13±0.61	1.92±0.82	5.35± 0.40	2.41± 0.70	5.24± 0.42	2.68± 0.56	5.77± 0.93	3.88± 1.47
2	I	4.80± 0.45	1.70±0. 59	5.15±0.44	1.95±0.59	5.09± 0.28	2.42± 0.42	5.21± 0.42	2.57± 0.51	5.84± 0.51	3.63± 1.14
	II	4.88± 0.40	1.66± 0.42	5.31±0.55	2.41±0.86	5.27± 0.51	2.55± 0.75	5.53± 0.33	2.86± 0.45	5.83± 0.62	3.68± 1.14
3	I	4.87± 0.48	1.77± 0.56	5.21±0.54	2.37±0.81	5.33± 0.65	2.62± 1.05	5.53± 0.40	3.27± 0.71	6.38± 0.91	4.65± 1.05
	II	4.89± 0.51	1.85± 0.61	5.07±0.52	2.15±0.80	5.09± 0.56	2.27± 0.93	4.77± 0.38	3.77± 0.60	6.56± 0.50	5.65± 1.04
4	I	4.99± 0.41	2.06± 0.53	5.21±0.41	2.51±0.64	5.43± 0.43	3.10± 0.70	5.59± 0.31	3.38± 0.51	6.43± 0.62	4.53± 1.52
	II	4.89± 0.45	1.69± 0.52	4.87±0.40	2.16±0.44	5.27± 0.45	2.47± 0.60	5.25± 0.54	2.88± 0.63	5.97± 0.53	3.58± 1.12
5	I	4.95± 0.44	1.93± 0.60	5.09±0.63	2.08±0.99	5.24± 0.37	2.46± 0.47	5.60± 0.31	2.90± 0.49	5.80± 0.74	3.49± 1.11
	II	4.92± 0.45	1.90± 0.54	5.03±0.57	2.11±0.78	5.19± 0.24	2.29± 0.36	5.11± 0.51	2.92± 0.62	6.04± 0.37	3.69± 0.65
6	I	4.91± 0.42	1.90± 0.51	4.96±0.50	2.00±0.64	4.94± 0.38	2.41± 0.41	4.89± 0.60	2.63± 0.75	5.84± 0.47	3.78± 0.91
	II	4.97± 0.60	2.00± 0.88	5.24±0.65	2.20±0.89	4.77± 0.28	2.41± 0.29	5.55± 0.48	3.15± 0.60	6.17± 1.11	4.45± 0.36
7	I	4.84± 0.49	1.79± 0.52	5.07±0.32	2.04±0.35	5.15± 0.53	2.67± 0.76	4.85± 0.52	2.90± 0.77	6.55± 0.65	4.77± 1.28
	II	4.75± 0.39	1.68± 0.41	5.03±0.39	1.98±0.56	4.85± 0.46	2.22± 0.54	5.33± 0.41	2.37± 0.53	5.62± 0.43	2.95± 0.90
8	I	4.71± 0.47	1.68± 0.48	4.84±0.49	2.02±0.56	5.03± 0.40	2.37± 0.55	5.29± 0.43	2.84± 0.65	6.01± 0.58	3.81± 1.22
	II	4.80± 0.40	1.77± 0.47	4.99±0.43	2.06±0.57	5.21± 0.35	2.37± 0.40	5.50± 0.48	2.67± 0.74	5.67± 0.48	3.16± 0.99
9	I	4.81± 0.45	1.75± 0.50	4.99±0.44	2.06±0.51	5.09± 0.62	2.22± 0.79	4.96± 0.36	2.31± 0.46	5.03± 0.68	2.44± 0.93
	II	4.72± 0.41	1.66± 0.42	4.89±0.36	2.10±0.52	4.75± 0.36	2.21± 0.35	4.79± 0.44	2.25± 0.54	5.20± 0.54	2.31± 0.78

Mean ± SE

Table 15 : Average fortnightly weight gain of Common carp fed with BG Cotton, Non–BG cotton and control feeds

Tank No.		28/05/03 W (g)	12/06/03 W (g)	26/06/03 W (g)	10/07/03 W (g)	24/07/03 W (g)
1	I	1.60	2.24	2.34	2.73	3.51
	II	1.60	1.92	2.41	2.68	3.88
2	I	1.70	1.95	2.42	2.57	3.63
	II	1.66	2.41	2.55	2.86	3.68
3	I	1.77	2.37	2.62	3.27	4.65
	II	1.85	2.15	2.27	3.77	5.65
4	I	2.06	2.51	3.10	3.38	4.53
	II	1.89	2.16	2.47	2.88	3.58
5	I	1.93	2.08	2.46	2.90	3.49
	II	1.90	2.11	2.29	2.92	3.69
6	I	1.90	2.00	2.41	2.63	3.78
	II	2.00	2.20	2.41	3.15	4.45
7	I	1.79	2.04	2.67	2.90	4.77
	II	1.68	1.98	2.22	2.37	2.95
8	I	1.68	2.02	2.37	2.84	3.81
	II	1.77	2.06	2.37	2.67	3.16
9	I	1.75	2.06	2.22	2.31	2.44
	II	1.66	2.10	2.21	2.25	2.31

Table 16 : Growth increment in terms of average weight (g) in *Cyprinus carpio* (Common Carp).

Tank No.	28/05/03 W (g)	12/06/03 W (g)	26/06/03 W (g)	10/07/03 W (g)	24/07/03 W (g)
1	1.60 ± 0.00	2.08 ± 0.23	2.38 ± 0.05	2.71 ± 0.04	3.70 ± 0.26
2	1.68 ± 0.03	2.18 ± 0.33	2.49 ± 0.09	2.72 ± 0.21	3.66 ± 0.04
3	1.81 ± 0.06	2.26 ± 0.16	2.45 ± 0.25	3.52 ± 0.35	5.15 ± 0.71
4	1.98 ± 0.12	2.34 ± 0.25	2.79 ± 0.45	3.13 ± 0.35	4.06 ± 0.67
5	1.92 ± 0.02	2.10 ± 0.02	2.38 ± 0.12	2.91 ± 0.01	3.59 ± 0.14
6	1.95 ± 0.07	2.10 ± 0.14	2.41 ± 0.00	2.89 ± 0.37	4.12 ± 0.47
7	1.74 ± 0.08	2.01 ± 0.04	2.45 ± 0.32	2.64 ± 0.37	3.86 ± 1.29
8	1.73 ± 0.06	2.04 ± 0.03	2.37 ± 0.00	2.76 ± 0.12	3.49 ± 0.46
9	1.71 ± 0.06	2.08 ± 0.03	2.22 ± 0.01	2.28 ± 0.04	2.38 ± 0.09

Mean ± SE

Table-17 : Growth performance of *Cyprinus carpio* fed varying levels of BG-II cotton, NBG-II cotton and mustard oil cake for 56 days

Parameters	Initial	BG-II			NBG-II			MOC		
		5%	15%	25%	5%	15%	25%	5%	15%	25%
Initial body weight (g)	-	1.60 ± 0.00	1.68 ± 0.03	1.81 ± 0.06	1.98 ± 0.12	1.92 ± 0.02	1.95 ± 0.07	1.74 + 0.08	1.73 + 0.06	1.71 + 0.06
Final body weight (g)	-	3.70 + 0.26	3.66 + 0.04	5.15 + 0.71	4.06 + 0.67	3.59 + 0.14	4.12 + 0.47	3.89 + 0.29	3.49 + 0.46	2.38 + 0.09
Specific growth rate (SGR) %	-	0.65 ± 0.03^{a}	0.64 ± 0.02^{a}	0.81 ± 0.05^{b}	0.55 ± 0.01^{c}	0.49 ± 0.03^{c}	0.58 ± 0.03^{c}	0.62 ± 0.02^{a}	0.54 ± 0.04^{c}	0.26 ± 0.01^{a}
Feed conversion ratio (FCR)	-	2.71 + 0.12^{a}	2.83 + 0.09^{a}	2.80 + 0.06^{a}	2.69 + 0.03^{a}	2.63 + 0.10^{a}	2.72 + 0.11^{a}	2.56 + 0.13^{c}	2.62 + 0.15^{a}	2.72 ± 0.16^{a}
Feed efficiency ratio (FER)	-	0.37 + 0.02^{a}	0.35 + 0.01^{a}	0.36 + 0.03^{a}	0.37 + 0.01^{a}	0.38 + 0.03^{b}	0.37 + 0.01^{a}	0.39 + 0.02^{b}	0.38 ± 0.01^{b}	0.37 ± 0.02^{a}
Protein efficiency ratio (PER)	-	1.13 ± 0.03^{a}	1.14 ± 0.02^{a}	1.12 ± 0.04^{a}	1.16 ± 0.03^{a}	1.17 ± 0.05^{a}	1.18 ± 0.03^{a}	1.21 ± 0.02^{b}	1.23 ± 0.02^{b}	1.25 ± 0.06^{b}
Feed Intake (g)	-	5.69+0.21^{a}	5.60+0.33^{a}	9.35+0.65^{b}	5.59+0.17^{a}	4.39+0.14^{c}	5.90+0.32^{a}	5.50+0.46^{a}	4.61+0.09^{c}	1.82+0.07^{d}
Final carcass composition (Dry matter basis)										
1. Moisture (%)	8.9	7.5	6.9	7.4	7.2	7.5	7.3	8.2	7.3	7.1
2. Dry matter (%) DM	91.1	92.5	93.1	92.6	92.8	92.5	92.7	91.8	92.7	92.9
3. Lipid (%)	22.2	31.3	33.3	35.4	32.8	31.1	34.6	31.7	30.4	31.0
4. Crude-protein (%)	39.4	39.9	43.7	38.8	43.7	42.5	40.2	41.0	43.5	43.6
5. Ash(%)	12.6	8.8	10.5	7.9	8.3	8.4	7.8	8.4	8.9	9.2
6. Carbohydrate (%)	6.9	12.5	5.6	10.5	8.0	10.5	10.1	10.7	9.9	9.1
7. DM Digestibility (%)	50.7	51.3	52.4	50.6	51.5	53.2	52.1	55.1	53.8	54.9
8. CP Digestibility (%)	76.5	74.5	73.7	75.5	76.1	74.8	74.3	76.8	76.9	73.5
9. Gross energy (K.cal/100 g)	425.0	491.3	496.9	515.8	502.0	491.9	512.6	492.1	487.2	489.8

- 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-c, c-d and $P<0.01$ in a to c, a to d)
- Mean ± SE

Table 18 : Regression equation of total length on weight of *Cyprinus carpio* during different fortnightly intervals

Tank No.		28/05/03			12/06/03			26/06/03			10/07/03			24/07/03		
		a ± SE	b ± SE	r (n)	a ± SE	b ± SE	r (n)	a ± SE	b ± SE	r (n)	a ± SE	b ± SE	r (n)	a ± SE	b ± SE	r (n)
1	I	-1.583 ± 0.088	2.599 ± 0.128	0.936 (60)	-1.811 ± 0.076	3.056 ± 0.108	0.992 (60)	-1.701 ± 0.062	2.894 ± 0.088	0.994 (45)	-1.775 ± 0.134	2.894 ± 0.189	0.977 (30)	-2.086 ± 0.265	3.372 ± 0.357	0.948 (15)
	II	-1.868 ± 0.100	3.016 ± 0.147	0.937 (60)	-1.832 ± 0.126	3.021 ± 0.177	0.978 (60)	-1.740 ± 0.215	2.985 ± 0.295	0.942 (45)	-1.856 ± 0.160	3.065 ± 0.223	0.970 (30)	-2.078 ± 0.102	3.363 ± 0.135	0.991 (15)
2	I	-1.988 ± 0.117	3.229 ± 0.172	0.927 (60)	-1.955 ± 0.164	3.211 ± 0.230	0.968 (60)	-1.761 ± 0.201	3.010 ± 0.284	0.947 (45)	-1.545 ± 0.158	2.666 ± 0.221	0.958 (30)	-2.032 ± 0.186	3.321 ± 0.242	0.969 (15)
	II	-1.804 ± 0.086	2.924 ± 0.125	0.951 (60)	-1.918 ± 0.166	3.210 ± 0.229	0.968 (60)	-1.604 ± 0.274	2.775 ± 0.380	0.897 (45)	-1.654 ± 0.139	2.776 ± 0.187	0.972 (30)	-1.862 ± 0.114	3.097 ± 0.149	0.986 (15)
3	I	-1.652 ± 0.138	2.742 ± 0.201	0.873 (60)	-1.848 ± 0.138	3.159 ± 0.193	0.977 (60)	-1.748 ± 0.111	2.978 ± 0.153	0.983 (45)	-2.178 ± 0.150	3.505 ± 0.202	0.979 (30)	-1.544 ± 0.207	2.727 ± 0.254	0.955 (15)
	II	-1.798 ± 0.079	2.973 ± 0.115	0.959 (60)	-1.755 ± 0.155	3.001 ± 0.220	0.967 (60)	-2.030 ± 0.113	3.376 ± 0.161	0.986 (45)	-1.864 ± 0.158	3.096 ± 0.214	0.975 (30)	-2.086 ± 0.265	3.372 ± 0.357	0.948 (15)
4	I	-1.529 ± 0.122	2.629 ± 0.175	0.892 (60)	-1.903 ± 0.173	3.179 ± 0.242	0.964 (60)	-1.128 ± 0.420	2.160 ± 0.572	0.723 (45)	-1.504 ± 0.149	2.586 ± 0.207	0.961 (30)	-1.875 ± 0.146	3.113 ± 0.181	0.979 (15)
	II	-1.729 ± 0.070	2.893 ± 0.102	0.966 (60)	-1.693 ± 0.087	2.860 ± 0.126	0.988 (60)	-1.764 ± 0.110	2.979 ± 0.153	0.983 (45)	-1.775 ± 0.134	2.894 ± 0.189	0.977 (30)	-1.832 ± 0.093	3.060 ± 0.123	0.987 (15)
5	I	-1.831 ± 0.061	3.031 ± 0.088	0.976 (60)	-1.901 ± 0.095	3.125 ± 0.134	0.992 (60)	-1.440 ± 0.186	2.529 ± 0.259	0.938 (45)	-1.873 ± 0.163	3.074 ± 0.218	0.969 (30)	-1.375 ± 0.252	2.491 ± 0.331	0.902 (15)
	II	-1.765 ± 0.071	2.939 ± 0.102	0.967 (60)	-1.727 ± 0.141	2.918 ± 0.202	0.970 (60)	-1.815 ± 0.320	3.029 ± 0.448	0.882 (45)	-1.881 ± 0.164	3.085 ± 0.323	0.965 (30)	-1.519 ± 0.221	2.664 ± 0.283	0.934 (15)
6	I	-1.637 ± 0.100	2.755 ± 0.145	0.928 (60)	-1.760 ± 0.122	2.944 ± 0.176	0.978 (60)	-1.581 ± 0.175	2.698 ± 0.252	0.952 (45)	-1.796 ± 0.112	2.981 ± 0.156	0.984 (30)	-1.796 ± 0.152	3.046 ± 0.198	0.976 (15)
	II	-1.887 ± 0.055	3.109 ± 0.079	0.982 (60)	-1.730 ± 0.107	2.900 ± 0.148	0.983 (60)	-1.453 ± 0.210	2.2.516 ± 0.310	0.913 (45)	-1.437 ± 0.172	2.487 ± 0.233	0.947 (30)	-1.856 ± 0.099	3.118 ± 0.126	0.990 (15)
7	I	-1.755 ± 0.051	2.913 ± 0.074	0.982 (60)	-1.372 ± 0.152	2.403 ± 0.215	0.952 (60)	-1.728 ± 0.142	2.963 ± 0.199	0.972 (45)	-2.058 ± 0.202	3.324 ± 0.277	0.958 (30)	-1.502 ± 0.160	2.659 ± 0.197	0.966 (15)
	II	-1.797 ± 0.073	2.960 ± 0.107	0.964 (60)	-1.829 ± 0.144	3.060 ± 0.205	0.972 (60)	-1.899 ± 0.113	3.167 ± 0.165	0.983 (45)	-1.793 ± 0.224	2.969 ± 0.310	0.934 (30)	-2.168 ± 0.236	3.502 ± 0.315	0.951 (15)
8	I	-1.609 ± 0.062	2.708 ± 0.092	0.968 (60)	-1.658 ± 0.199	2.810 ± 0.174	0.976 (60)	-1.688 ± 0.157	2.876 ± 0.224	0.963 (45)	-1.782 ± 0.137	2.975 ± 0.189	0.975 (30)	-2.806 ± 0.143	3.048 ± 0.184	0.977 (15)
	II	-1.875 ± 0.063	3.099 ± 0.093	0.975 (60)	-1.897 ± 0.156	3.161 ± 0.223	0.969 (60)	-1.395 ± 0.184	2.461 ± 0.256	0.936 (45)	-1.724 ± 0.164	2.878 ± 0.222	0.964 (30)	-2.164 ± 0.246	3.512 ± 0.362	0.948 (15)
9	I	-1.717 ± 0.064	2.858 ± 0.094	0.970 (60)	-1.785 ± 0.143	2.966 ± 0.205	0.970 (60)	-1.459 ± 0.237	2.550 ± 0.336	0.903 (45)	-1.851 ± 0.200	3.043 ± 0.277	0.950 (30)	-1.344 ± 0.324	2.379 ± 0.463	0.809 (15)
	II	-1.735 ± 0.066	2.886 ± 0.098	0.968 (60)	-2.114 ± 0.270	3.455 ± 0.392	0.925 (60)	-1.511 ± 0.189	2.556 ± 0.279	0.931 (45)	-2.179 ± 0.170	3.493 ± 0.250	0.966 (30)	-1.423 ± 0.313	2.473 ± 0.438	0.843 (15)

Mean ± SE

C. **Proximate Composition :**

The proximate composition of cotton meal feeds (F_1-F_6),mustard oil cake incorporated feeds (F_7-F_9) and experimental fishes analysed by AOAC (1989) are shown in Table-3, 4, 5, 9 and 17.

The cotton seeds contains Gossypol contents as 1.56% and 1.53% in Bollgard-II and Non-bollgard-II samples (Table-3). The gross energy of prepared feeds (F_1-F_9) ranged between 343.7 K.cal/100 g to 376.5 K.cal/100 g (Table-4). After mixing the cotton seed (BG-II and Non-BG-II) in feed F_1-F_6 @ 5%, 15% and 25% of both the samples the gossypol contents recorded as 0.063%, 0.130%, 0.252%, 0.043%, 0.144%and 0.261% in F_1, F_2, F_3,F_4, F_5 and F_6 respectively (Table-5).

The proximate composition of experimental fishes (of every fortnight) are shown in Table-9 and 17. The moisture contents ranged, in dried samples, from 8.1% to 10.8% in first fortnight, 7.8% to 9.8% second fortnight samples, 7.3% to 8.5% in third fortnight samples and 6.9% to 8.2% in fourth fortnight i.e. last samples. The protein contents (on dry weight basis) ranged between 38.8% to 48.4% during the experimental sampling (Table-9). The ash and carbohydrate contents ranged from 7.6% to 12.3% and 5.6% to 16.9% respectively (on dry matter basis). The gross energy contents of the fishes ranged from 384.7 K.cal/100g to 522.1 K.cal/100g (Table-9).

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-10, 11,12, 13, 14, 15, 16 and 17 respectively. The regression equation of total length and weight are tabulated in Table-18.

Specific growth rate (SGR) of fishes fed with BG-II cotton supplemented feeds (F_1, F_2 and F_3) ranged between 0.64 $\pm$ 0.02% to 0.81 $\pm$ 0.05% (Table-17). Similarly, SGR of Non-BG-II fed fishes recorded as 0.49 $\pm$ 0.03% to 0.58 $\pm$ 0.03%, however, SGR of mustard oil cake fed fishes recorded from 0.26 $\pm$ 0.01% to 0.62 $\pm$ 0.02%. The feed efficiency ratio of F_1 to F_9 recorded as 0.37 $\pm$ 0.02, 0.35 $\pm$ 0.01, 0.36 $\pm$ 0.03, 0.37 $\pm$ 0.01, 0.38 $\pm$ 0.03, 0.37 $\pm$ 0.01, 0.39 $\pm$ 0.02, 0.38 $\pm$ 0.01 and 0.37 $\pm$ 0.02 respectively (Table-17). The protein efficiency ratio (PER) of feed F_1 to F_9 ranged between 1.12 $\pm$ 0.04 to 1.25 $\pm$ 0.06. The highest protein efficiency recorded in MOC fed fishes followed by Non-BG-II and BG-II fed fishes (Table-17).

E. Mineral Analysis :

The metal contents of feeds (F1-F9) and fish carcass recorded and results are shown in Table-19 and 20. In feed Cd, Co, Cu, Fe, Mn, Zn and Mg ranged between 1.239 $\mu g.g^{-1}$ to 3.133 $\mu g.g^{-1}$, 1.016 $\mu g.g^{-1}$ to 16.401 $\mu g.g^{-1}$, 0.816 $\mu g.g^{-1}$ to 1.172 $\mu g.g^{-1}$, 86.111 $\mu g.g^{-1}$ to 2189.006 $\mu g.g^{-1}$, 68.135 $\mu g.g^{-1}$ to 106.474 $\mu g.g^{-1}$, 68.229 $\mu g.g^{-1}$ to 105.140 $\mu g.g^{-1}$ and 3054.217 $\mu g.g^{-1}$ to 6059.028 $\mu g.g^{-1}$ respectively (Table-19).

Similarly, metal contents of the carcass recorded as 1.855 $\mu g.g^{-1}$ to 3.236 $\mu g.g^{-1}$, 2.790 $\mu g.g^{-1}$ to 16.519 $\mu g.g^{-1}$, 0.269 $\mu g.g^{-1}$ to 2.187 $\mu g.g^{-1}$, 294.817 $\mu g.g^{-1}$ to 917.945 $\mu g.g^{-1}$, 9.299 $\mu g.g^{-1}$ to 38.039 $\mu g.g^{-1}$, 257.669 $\mu g.g^{-1}$ to 410.231 $\mu g.g^{-1}$ and 837.423 $\mu g.g^{-1}$ to 2637.340 $\mu g.g^{-1}$ for Cd, Co, Cu, Fe, Mn, Zn and Mg respectively (Table-20).

Table 19 : Metal content of the F_1 to F_9

Sr.No.	Feed	Cadmium (µg.g^{-1})	Cobalt (µg.g^{-1})	Copper (µg.g^{-1})	Iron (µg.g^{-1})	Manganese (µg.g^{-1})	Zinc (µg.g^{-1})	Magnesium (µg.g^{-1})
1	F_1	3.133	16.401	1.054	2189.006	71.807	70.934	3054.217
2	F_2	1.833	7.288	1.167	1807.576	70.303	72.727	3584.848
3	F_3	1.489	15.638	1.003	898.176	106.474	78.419	3677.812
4	F_4	2.040	6.776	1.012	1957.165	80.312	105.140	3320.872
5	F_5	1.741	10.575	1.118	1388.978	76.374	92.013	3683.706
6	F_6	1.782	14.406	1.172	1993.399	68.135	88.614	5534.653
7	F_7	2.205	8.507	0.920	1656.250	71.771	68.229	6059.028
8	F_8	1.239	2.749	0.816	589.350	80.755	90.181	5697.885
9	F_9	2.238	1.016	0.841	86.111	82.857	80.476	4860.317

Table-20 : Metal content of the carcass of the fishes fed with F_1 to F_9 for 56 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	2.152	4.837	0.275	444.141	12.685	327.645	1472.380
2	F_1	3.236	16.519	0.599	507.486	38.039	333.955	2637.340
3	F_2	1.855	2.790	0.269	345.008	10.592	259.553	1175.280
4	F_3	2.167	11.233	0.380	392.178	9.302	395.816	1173.844
5	F_4	2.961	9.419	2.187	382.937	11.502	339.710	1160.640
6	F_5	3.079	7.683	0.381	252.222	12.444	384.762	1212.698
7	F_6	2.914	8.880	0.322	917.945	11.135	257.669	837.423
8	F_7	2.767	7.046	0.403	355.980	15.086	410.231	1440.922
9	F_8	2.363	9.817	0.335	294.817	9.299	262.500	1204.268
10	F_9	3.213	13.591	0.378	366.409	17.904	329.381	1384.880

F. **Histopathological studies :**

After feeding BG-II, NBG-II and MOC for 56 days the histological studies have been carried out and results are shown in Plate-31 to 70.

Gills :

Changes in Gill Histopathological studies has been taken up and the results are shown in Plate 31 to 40.

The control (Plate-31) gill of common carp, *Cyprinus carpio* showing primary gill lamellae, secondary gill lamellae, pilaster cells, blood cells, chloride cells, epithelial cells and prominent nucleus.

Fishes fed with Bollgard II incorporated feed (F_1, F_2 and F_3) for 56 days showing some alterations in the general structure of Gill. In F_1 (5% BG cotton seed) fed fishes showing fusion of secondary gill lamellae and enlargement of cartilaginous cells (Plate-32). Fishes fed with F_2 (75% BG cotton seed) demonstrating some damages in primary and secondary gill lamellae (Plate-33). Furthermore, fishes fed with F_3 (25% BG cotton seed) shows some damages to secondary gill lamellae (Plate-34).

Fishes fed with non-bollgard II incorporated feeds for 56 days (F_4, F_5 and F_7) also showing some changes in the general structure of gill. In F_4 (5% non-BG cotton seed) fed fishes showing fusion of secondary gill lamellae and enlargement of cartilaginous cells. Fishes fed with F_5 (15% NBG cotton seed) showing (Plate-35) furthermore, fusion of gill lamellae and enlarged cartilaginous cells (Plate-36). On feeding F_6 (25% NBG cotton seeds) the gill lamellae of *C. carpio* is showing degenerative conditions as damaged primary gill lamellae (Plate-37).

Fishes fed with mustard oil cake incorporated feeds (F_7, F_8 and F_9) for 56 days demonstrate some alterations. In F_7 (5% MOC) showing normal structure of gill (Plate-38) fishes fed with F_8 (15% MOC) demonstrate enlargement of cartilaginous cells and degeneration of secondary gill lamellae (Plate-39). Fishes, fed with feed F_9 (25% MOC) showing fusion of secondary lamellae, enlargement of cartilaginous cells and damage of primary gill lamellae.

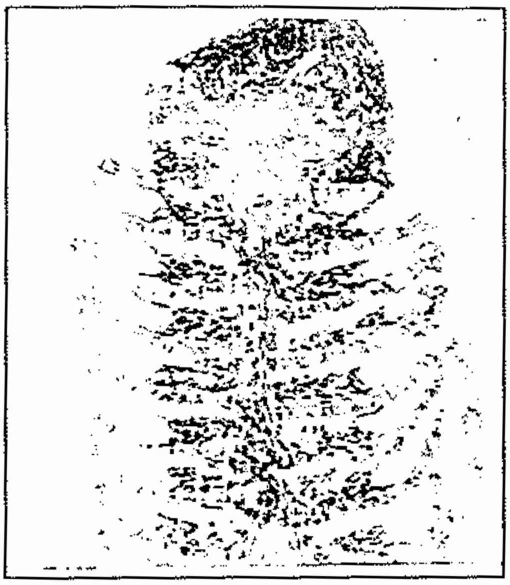


Plate-31 : Control gill of *Cyprinus carpio* **160X (H/E)**

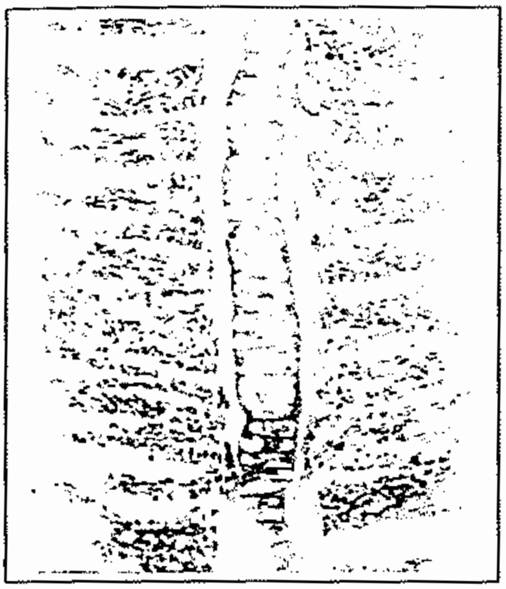


Plate-32 : Gill of *Cyprinus carpio* fed with Bollgard-II (5%) (Feed-1)**160X (H/E)**

Plate-33 : Gill of *Cyprinus carpio* fed with Bollgard-II (15%) (Feed-2) **160X (H/E)**

Plate-34 : Gill of *Cyprinus carpio* fed with Bollgard-II (25%) (Feed-3) **160X (H/E)**

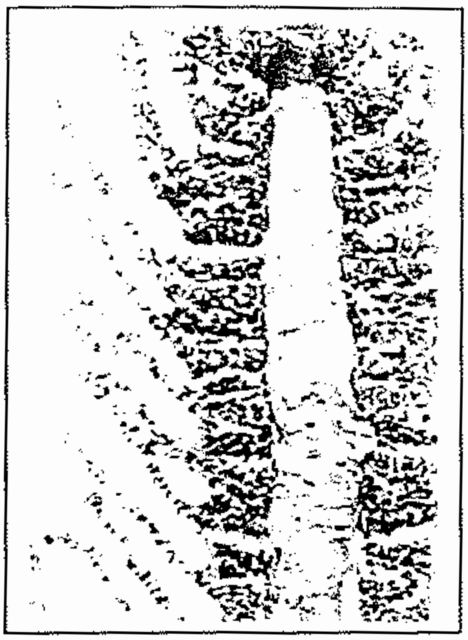


Plate-35 : Gill of *Cyprinus carpio* fed with Non-Bollgard-II (5%) (Feed-4) **160X (H/E)**

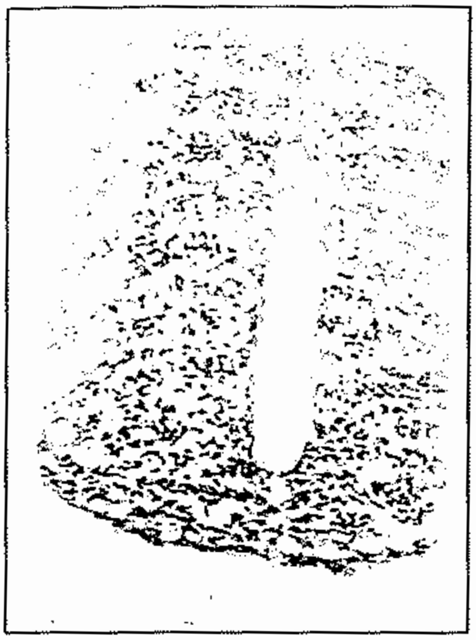


Plate-34 : Gill of *Cyprinus carpio* fed with Non-Bollgard-II (15%) (Feed-5) **160X (H/E)**

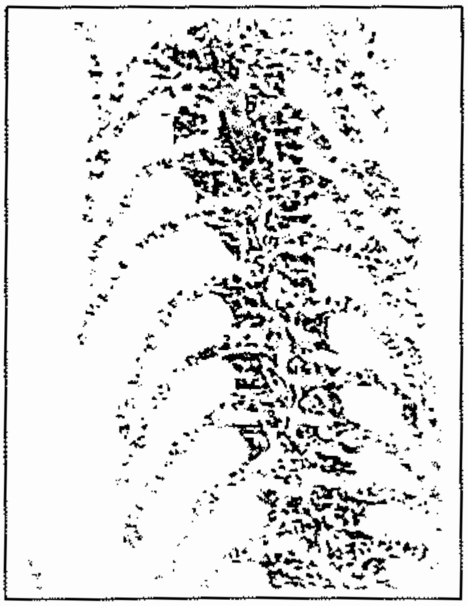


Plate-37 : Gill of *Cyprinus carpio* fed with Non-Bollgard-II (25%) (Feed-6) **160X (H/E)**

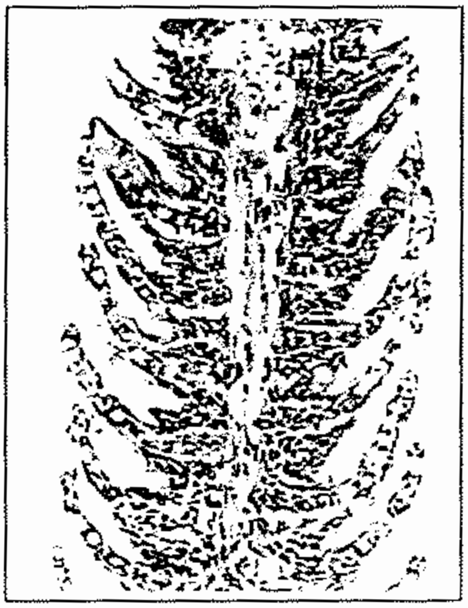


Plate-38 : Gill of *Cyprinus carpio* fed with MOC (5%) (Feed-7) **160X(H/E)**

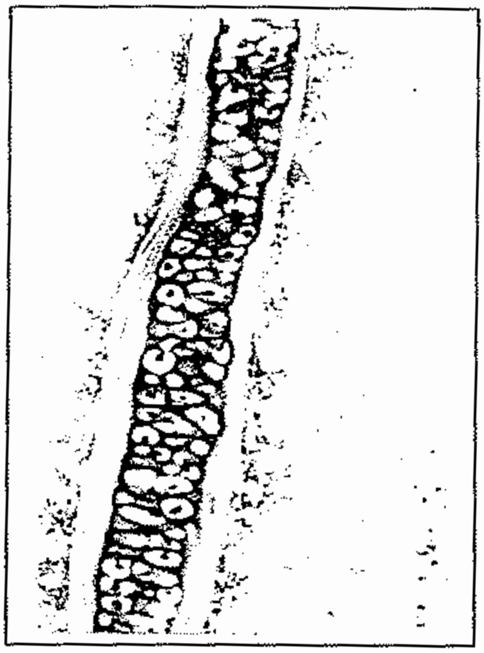


Plate-39 : Gill of *Cyprinus carpio* fed with MOC (15%) (Feed-8) **160X (H/E)**

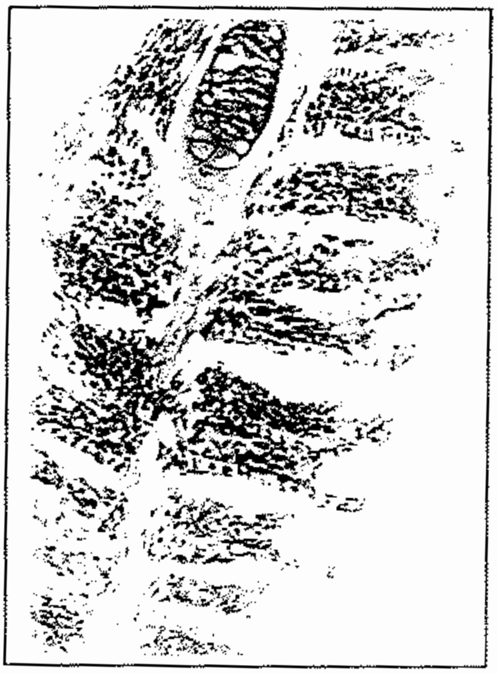


Plate-40 : Gill of *Cyprinus carpio* fed with MOC (25%) (Feed-9) **160X (H/E)**

Liver :

Changes in Liver Histopathological studies has been under taken and the results are shown in Plate 41 to 50.

The control (Plate-41) liver of common carp, *Cyprinus carpio* showing normal hepatocytes with this cytoplasm and prominent nucleus.

Fishes fed with Bollgard II feeds (F_1, F_2 and F_3), non-bollgard II feeds (F_4, F_5 and F_6) and mustard oil cake (F_7, F_8 and F_9) showed no cellular changes or degeneration of boundary wall or vacuolation. No necrosis is recorded and nucleus are prominent (Plate-42 to 50).

Intestine :

Changes in intestine histopathological studies has been under taken and the results are shown in Plate 51 to 60.

The control (Plate-51) fish intestine showing normal appearance of villi.

Fishes fed with Bollgard II incorporated feeds (F_1, F_2 and F_3) and non-bollgard II incorporated diets (F_4, F_5 and F_6) showing no change in general histopathological structures of villi, circular muscle, serosa and longitudinal muscles (Plate-52 to 57).

Fishes fed with mustard oil cake feeds (F_7 and F_8) showed fusion of villi (Plate-58 and 59), whereas in fishes fed with F_9 (25% MOC) shows elongation of lumen in villi (Plate-60).

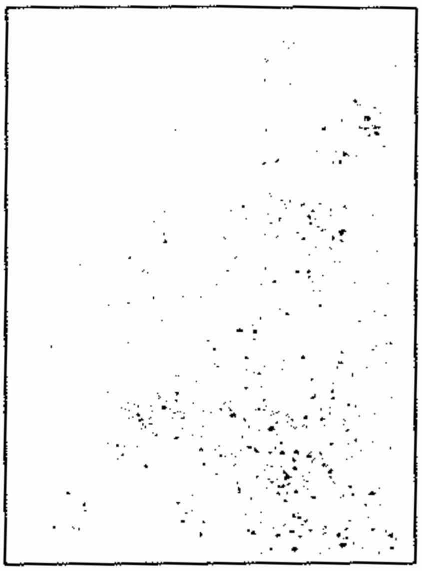


Plate-41 : Control liver of *Cyprinus carpio* **160X (H/E)**

Plate-42 : Liver of *Cyprinus carpio* fed with Bollgard-II(5%)(Feed-1)**160X (H/E)**

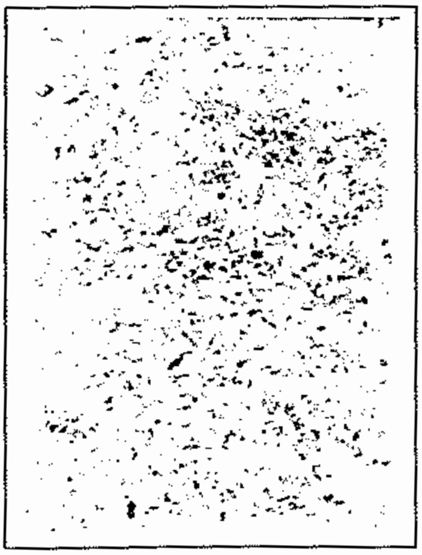


Plate-43 : Liver of *Cyprinus carpio* fed with Bollgard-II (15%) (Feed-2) **160X (H/E)**

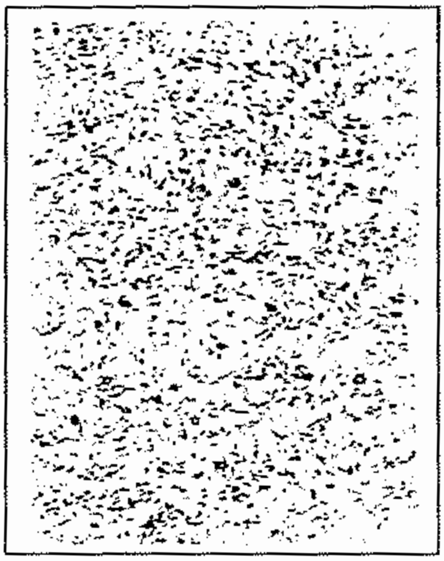


Plate-44 : Liver of *Cyprinus carpio* fed with Bollgard-II (25%) (Feed-3) **160X (H/E)**

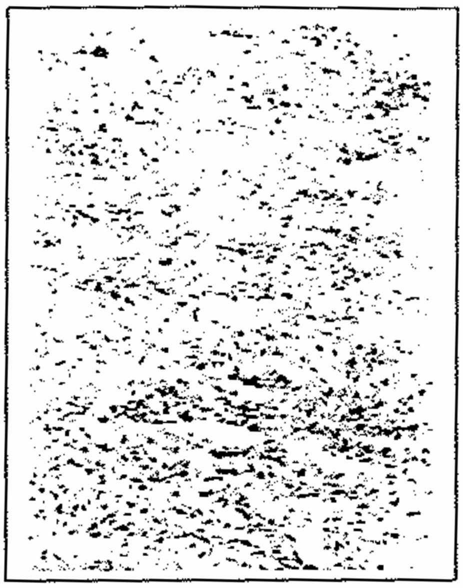


Plate-45 : Liver of *Cyprinus carpio* fed with Non- Bollgard-II(5%) (Feed-4)
160X (H/E)

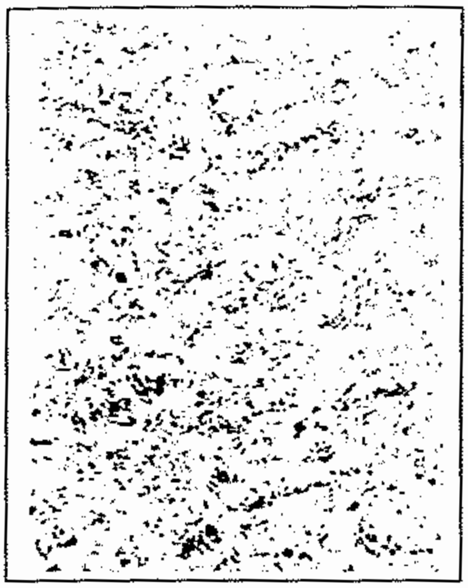


Plate-46 : Liver of *Cyprinus carpio* fed with Non-Bollgard-II (15%) (Feed-5)
160X (H/E)

Plate-47 : Liver of *Cyprinus carpio* fed with Non- Bollgard-II (25%) (Feed-6)
160X (H/E)

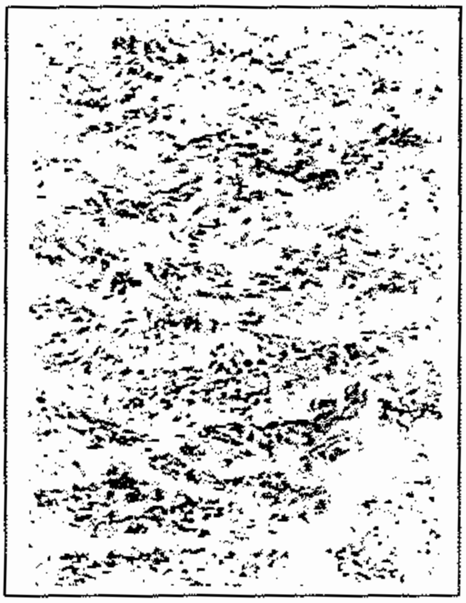


Plate-48 : Liver of *Cyprinus carpio* fed with MOC (5%) (Feed-7)
160X (H/E)

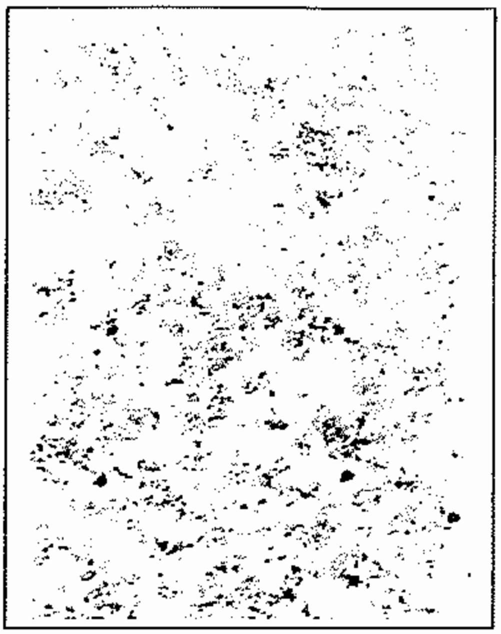


Plate-49 : Liver of *Cyprinus carpio* fed with MOC (15%) (Feed-8) **160X (H/E)**

Plate-50 : Liver of *Cyprinus carpio* fed with MOC (25%) (Feed-9) **160X (H/E)**

Plate-51 : Control intestine of *Cyprinus carpio* **160X (H/E)**

Plate-52 : Intestine of *Cyprinus carpio* fed with Bollgard-II (5%) (Feed-1) **160X (H/E)**

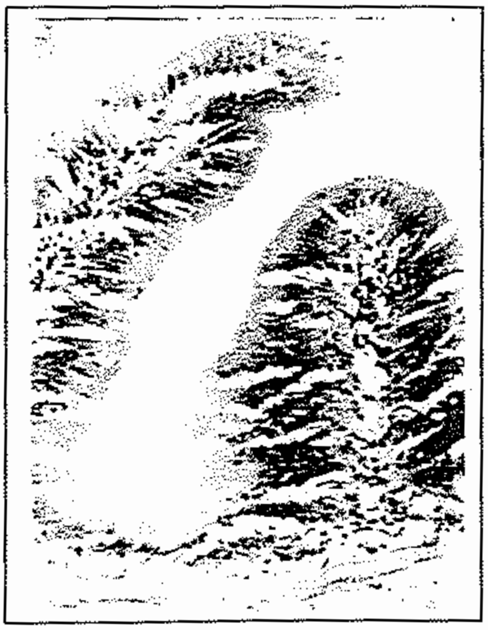


Plate-53 : Intestine of *Cyprinus carpio* fed with Bollgard-II (15%) (Feed-2) **160X (H/E)**

Plate-54 : Intestine of *Cyprinus carpio* fed with Bollgard-II (25%) (Feed-3) **160X (H/E)**

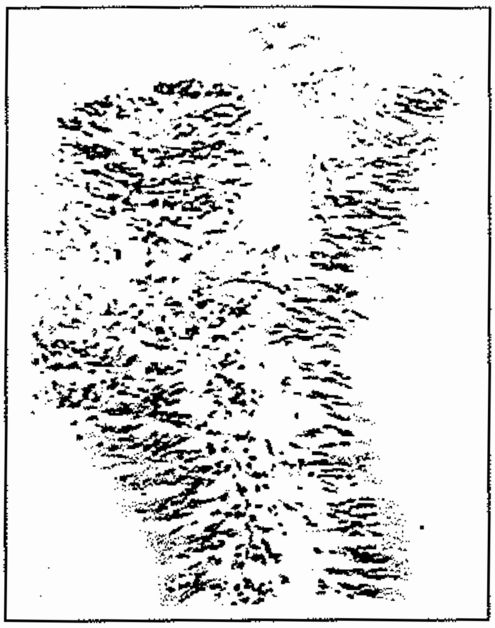


Plate-55 : Intestine of *Cyprinus carpio* fed with Non-Bollgard-II (5%) (Feed-4) **160X (H/E)**

Plate-56 : Intestine of *Cyprinus carpio* fed with Non-Bollgard-II (15%) (Feed-5) **160X (H/E)**

Plate-57 : Intestine of *Cyprinus carpio* fed with Non-Bollgard-II (25%)(Feed-6) **160X(H/E)**

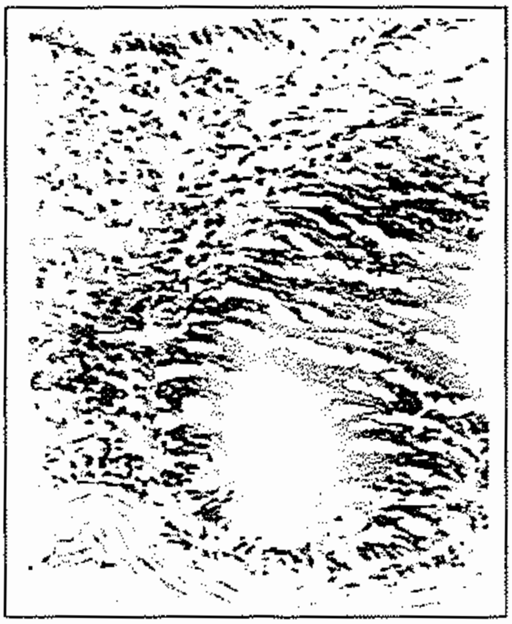


Plate-58 : Intestine of *Cyprinus carpio* fed with MOC (5%) (Feed-7) **160X (H/E)**

Plate-59 : Intestine of *Cyprinus carpio* fed with MOC (15%) (Feed-8) **160X (H/E)**

Plate-60 : Intestine of *Cyprinus carpio* fed with MOC (25%) (Feed-9) **160X (H/E)**

Kidney :

The histopathological changes in kidney has been taken up for studies after feeding for 56 days with Bollgard II feeds (F_1, F_2 and F_3), non-bollgard II feeds (F_4, F_5 and F_6) and mustard oil cake feeds (F_7, F_8 and F_9). Results are shown in Plate-61 to 70.

The control kidney showing well vascularized glomerular capsule and rural lobules (Plate-61).

Fishes fed with Bollgard II feeds (F_1, F_2 and F_3) showing some alterations in the kidney tissue. F_1 (BG 5% cotton seed) fed fishes have almost normal structure except space in Bowman's capsule Plate (62). The space in F_2 (BG 15% cotton seed) showing more spaces in Bowman capsule (Plate-63).

In fishes fed with F_3 (BG 25% cotton seed) showing some necrotic patches and structure glomarulus (Plate-64).

Feeding the fishes with Non-Bollgard II feeds (F_4, F_5 and F_6) there are some a\changes at cellular levels. On feeding F_4 (Non-BG 5% cotton seed), the space is observed in Bowman's capsule (Plate-65). The rupture of renal tubule and some necrotic patches are seen (Plate-66) on feeding F_5 (Non-BG 15% cotton seed). The necrotic patches are more in fishes kidney tissue on feeding F_6 (Non-BG 25% cotton seeds) (Plate-67).

Feeding with mustard oil cake diet (F_7 F_8 and F_9) the alterations in general structure is noticed and shown in Plate-68, 69 and 70.

Feeding with F_7 (5% MOC) there is not much difference recorded and structure are similar to the normal (Plate-68). However, feeding with F_8 (15% MOC) the renal tubules are showing rupture sides (Plate-69). And in the F_9 (25% MOC) the outer wall of many tubules are observed and necrotic patches are seen (Plate-70).

Plate-61 : Control kidney of *Cyprinus carpio* **160X (H/E)**

Plate-62 : Kidney of *Cyprinus carpio* fed with Bollgard-II (5%) (Feed-1) **160X (H/E)**

Plate-63 : Kidney of *Cyprinus carpio* fed with Bollgard-II (15%) (Feed-2) **160X (H/E)**

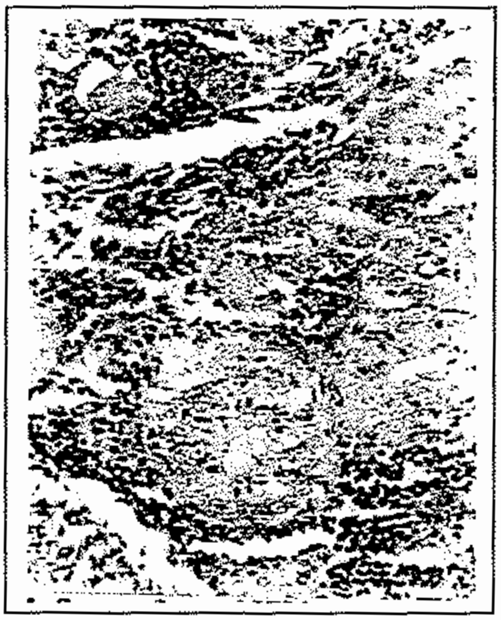


Plate-64 : Kidney of *Cyprinus carpio* fed with Bollgard-II (25%) (Feed-3) **160X (H/E)**

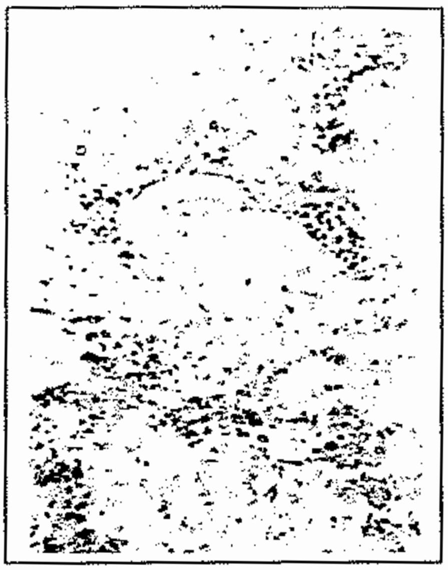


Plate-65 : Kidney of *Cyprinus carpio* fed with Non-Bollgard-II (5%) (Feed-4) **160X (H/E)**

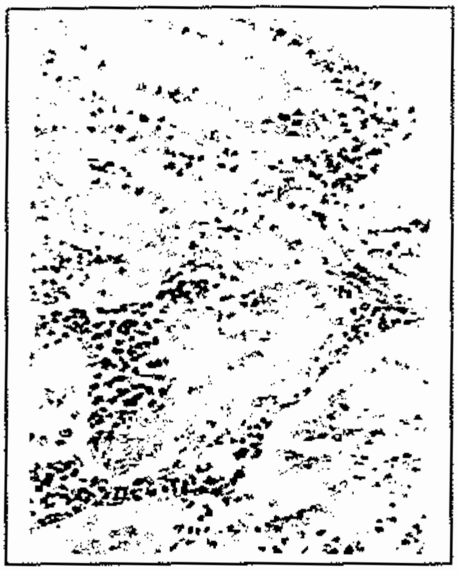


Plate-66 : Kidney of *Cyprinus carpio* fed with **Non-Bollgard-II (15%) (Feed-5) 160X (H/E)**

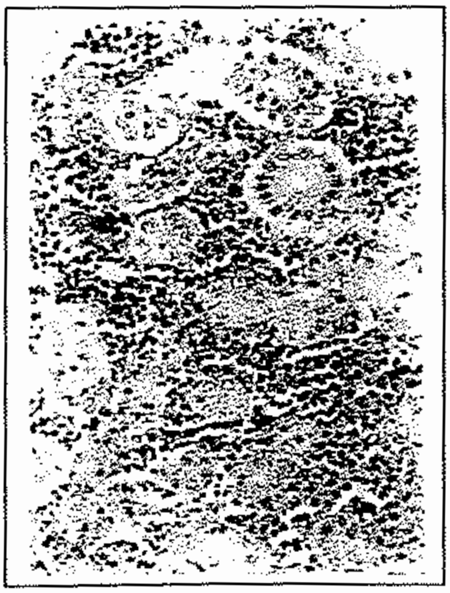


Plate-67 : Kidney of *Cyprinus carpio* fed with Non-Bollgard-II (25%) (Feed-6) **160X (H/E)**

Plate-68 : Kidney of *Cyprinus carpio* fed with MOC (5%) (Feed-7) **160X (H/E)**

Plate-69 : Kidney of *Cyprinus carpio* fed with MOC (15%) (Feed-8) **160X (H/E)**

Plate-70 : Kidney of *Cyprinus carpio* fed with MOC (25%) (Feed-9) **160X (H/E)**

Statistical Analysis :

Statistical analysis and ANOVA results of each fortnight and Tank (pool) wise including replicates (T1/I, 1/II, 2/I, 2/II, 3/I, 3/II, 4/I, 4/II, 5/I, 5/II, 6/I.6/II, 7/I, 7/II, 8/I, 8/II,9/I and 9/II) are shown in APPENDIX.

ANNEXURE-IV

Conclusion Summarizing the Results

1. Composition of the isoproteinaceous feeds (F_1-F_9) are summarized in Table-1&2 and ingredients are shown in Plate-1 to 26.
2. Proximate composition of two cotton hybrids BG-II and NBG-II shown in Table-3. Both seeds have gossipol @ 1.56% and 1.53% respectively. And the gross energy estimated as 467.6 K.cal/100 g and 444.4 K.cal/100 g. Lipid content of BG-II (MRC-6301) has more lipid content (22.2%) than Non-BG-II (MRC-6301) (20.0%).
3. All the feeds (F_1-F_9) contain protein between 22.8-25.1% (Nx6.25) and gross energy ranged from 343.7 K.cal/100 g to 376.5 K.cal/100 g (Table-3).
4. The gossypol content of feeds (F_1-F_6) recorded as 0.063%,0.130%, 0.252%, 0.043%, 0.144% and 0.261% after incorporating BG-II @ 5%, 15% and 25% (F_1-F_3) and Non-BG-II @ 5%, 15% and 25% (F_4-F_6) in other basal ingredients (Table-4 & 5).
5. The water quality parameters are exhibited in Table-8.
6. The proximate composition of experimental fishes are presented in Table-9 and 17. There is no statistical significant difference ($P<0.05$) in gross energy contents and protein contents ($P<0.01$).
7. Growth parameters are shown in Table-10 to 17. Feed efficiency ranged between 0.35 $\pm$ 0.01 to 0.39 $\pm$ 0.02; protein efficiency ranged between 1.12 $\pm$ 0.04 to 1.25 $\pm$ 0.06. However, lowest protein efficiency (1.12 $\pm$ 0.04) recorded in 25% BG-II feed (F_3) and best (1.25 $\pm$ 0.06) recorded in 25% MOC (F_9). The growth results are significant of 1% ($P<0.01$) in comparison to control feed.
8. Gross energy of fish ranged between 487.2 K.cal/100 g to 515.8 K.cal/100 g. Lowest GE recorded in MOC - 15% and highest GE recorded in BG-II – 25%. Overall no reduced gross energy is recorded in Bollgard-II fed fishes in comparison to MOC fed fishes (Table-17).

9. Specific growth rate (SGR) ranged from 0.26 ± 0.01% to 0.81 ± 0.05%. Lowest recorded in MOC – 25% (0.26 ± 0.01%) and highest recorded in BG-II (0.81 ± 0.05%). Among the three groups BG-II showed significant higher ($P<0.05$) SGR followed by Non-BG-II group in comparison to MOC fed fishes (Table-17).
10. The dry matter digestibility ranged from 50.6% to 55.1% and crude protein digestibility ranged from 73.5% to 76.9%. No significant difference recorded among the groups ($P>0.05$).
11. Regression analysis of length on weight suggests that BG-II fed fishes (F_1, F_2 and F_3) have value of 'b' as 3.000 or near 3.000 which means growth is good (Table-18). In case of F_4, F_5 and F_6 (Non-BG-II) feed F_5 shows value of 'b' less than 3.000 i.e. 2.491 ± 0.0331 and 2.664 ± 0.283 which signs poor growth performances. Similarly, feed-9 out of three MOC incorporated feeds demonstrated lesser values in both the replicates (2.379 ± 0.463 and 2.473 ± 0.438). Thus, showing poor growth pattern (Table-18).
12. The mineral contents of feed as well as fish carcass demonstrate arbitrary concentrations and not shows any pattern (Table-19 and 20).
13. Histopathological alterations have been recorded in all the three groups (BG-II, Non-BG-II and MOC).Minor changes in gill, liver, intestine and kidney have been shown in Plate-31 to 70.

CONCISE SUMMARY

The genetically modified cotton seed (Bollgard-II) containing *Cry-IAC & Cry-2Ab* genes, in comparison to Bt.cotton variety without *Cry-IAc & Cry-2Ab* (Non-BG-II) genes shows similar growth pattern and there was no significant difference ($P<0.05$) among FCR, FER and PER of these two varieties on feeding to common carp (*Cyprinus carpio*) for 56 days. However, histopathological alterations in gill, liver, intestine and kidney have been recorded in all the three groups (BG-II, NBG-II and laboratory control feed MOC) during the experimentation. The BG-II and Non-BG-II feeds (F_1-F_3 and F_4-F_5) are compared with MOC incorporated feed (F_7-F_9) on the basis of isocaloric and isoproteinaceous feeds in terms of growth studies.

Tank 1/I Date 28/05/03

Regression Statistics	
Multiple R	0.936
R Square	0.876
Adjusted R Square	0.874
Standard Error	0.047
Observations	60.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.900	0.900	411.486	0.000
Residual	58	0.127	0.002		
Total	59	1.027			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.583	0.088	-18.060	0.000	-1.758	-1.407	-1.758	-1.407
X Variable 1	2.599	0.128	20.285	0.000	2.342	2.855	2.342	2.855

Tank 1/II Date 28/05/03

Regression Statistics	
Multiple R	0.937
R Square	0.878
Adjusted R Square	0.876
Standard Error	0.053
Observations	60.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	1.162440	1.162440	419.241	0.000000
Residual	58	0.160818	0.002773		
Total	59	1.323258			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.868	0.100	-18.692	0.000	-2.068	-1.668	-2.068	-1.668
X Variable 1	3.016	0.147	20.475	0.000	2.721	3.311	2.721	3.311

Tank 1/I Date 12/06/03

Regression Statistics	
Multiple R	0.992
R Square	0.984
Adjusted R Square	0.983
Standard Error	0.018
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.256	0.256	803.779	0.000
Residual	13	0.004	0.000		
Total	14	0.260			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.811	0.076	-23.885	0.000	-1.974	-1.647	-1.974	-1.647
X Variable 1	3.056	0.108	28.351	0.000	2.823	3.289	2.823	3.289

Tank 1/II Date 12/06/03

Regression Statistics	
Multiple R	0.978
R Square	0.957
Adjusted R Square	0.954
Standard Error	0.033
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.315	0.315	290.856	0.000
Residual	13	0.014	0.001		
Total	14	0.330			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.832	0.126	-14.581	0.000	-2.103	-1.560	-2.103	-1.560
X Variable 1	3.021	0.177	17.055	0.000	2.638	3.403	2.638	3.403

Tank 1/I Date 26/06/03

Regression Statistics	
Multiple R	0.994
R Square	0.988
Adjusted R Square	0.987
Standard Error	0.015
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.236	0.236	1089.060	0.000
Residual	13	0.003	0.000		
Total	14	0.239			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.701	0.062	-27.324	0.000	-1.836	-1.567	-1.836	-1.567
X Variable 1	2.894	0.088	33.001	0.000	2.704	3.083	2.704	3.083

Tank 1/II Date 26/06/03

Regression Statistics	
Multiple R	0.942
R Square	0.887
Adjusted R Square	0.879
Standard Error	0.036
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.135	0.135	102.481	0.000
Residual	13	0.017	0.001		
Total	14	0.152			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.740	0.215	-8.106	0.000	-2.204	-1.277	-2.204	-1.277
X Variable 1	2.985	0.295	10.123	0.000	2.348	3.622	2.348	3.622

Tank 1/I Date 10/07/03

Regression Statistics	
Multiple R	0.977
R Square	0.955
Adjusted R Square	0.951
Standard Error	0.026
Observations	13.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.154	0.154	234.350	0.000
Residual	11	0.007	0.001		
Total	12	0.161			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.757	0.134	-13.123	0.000	-2.052	-1.463	-2.052	-1.463
X Variable 1	2.894	0.189	15.308	0.000	2.478	3.310	2.478	3.310

Tank 1/II Date 10/07/03

Regression Statistics	
Multiple R	0.970
R Square	0.940
Adjusted R Square	0.935
Standard Error	0.028
Observations	14.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.153	0.153	189.544	0.000
Residual	12	0.010	0.001		
Total	13	0.163			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.856	0.160	-11.598	0.000	-2.205	-1.508	-2.205	-1.508
X Variable 1	3.065	0.223	13.767	0.000	2.580	3.550	2.580	3.550

Tank 1/I Date 24/07/03

Regression Statistics	
Multiple R	0.948
R Square	0.899
Adjusted R Square	0.889
Standard Error	0.046
Observations	12.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.192	0.192	89.171	0.000
Residual	10	0.021	0.002		
Total	11	0.213			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-2.086	0.265	-7.869	0.000	-2.677	-1.495	-2.677	-1.495
X Variable 1	3.372	0.357	9.443	0.000	2.577	4.168	2.577	4.168

Tank 1/II Date 24/07/03

Regression Statistics	
Multiple R	0.991
R Square	0.983
Adjusted R Square	0.981
Standard Error	0.036
Observations	13.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.785	0.785	622.591	0.000
Residual	11	0.014	0.001		
Total	12	0.799			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-2.078	0.102	-20.322	0.000	-2.303	-1.853	-2.303	-1.853
X Variable 1	3.363	0.135	24.952	0.000	3.066	3.659	3.066	3.659

Tank 2/I Date 28/05/03

Regression Statistics	
Multiple R	0.927
R Square	0.859
Adjusted R S	0.856
Standard Erro	0.054
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.048	1.048	353.132	0.000
Residual	58	0.172	0.003		
Total	59	1.221			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.988	0.117	-16.996	0.000	-2.222	-1.754	-2.222	-1.754
X Variable 1	3.229	0.172	18.792	0.000	2.885	3.573	2.885	3.573

Tank 2/II Date 28/05/03

Regression Statistics	
Multiple R	0.951
R Square	0.904
Adjusted R S	0.903
Standard Erro	0.034
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.634	0.634	547.519	0.000
Residual	58	0.067	0.001		
Total	59	0.702			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.804	0.086	-20.976	0.000	-1.976	-1.632	-1.976	-1.632
X Variable 1	2.924	0.125	23.399	0.000	2.674	3.175	2.674	3.175

Tank 2/I Date 12/06/03

Regression Statistics	
Multiple R	0.968
R Square	0.937
Adjusted R S	0.932
Standard Erro	0.033
Observations	15

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.207	0.207	194.147	0.000
Residual	13	0.014	0.001		
Total	14	0.221			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.955	0.164	-11.925	0.000	-2.309	-1.601	-2.309	-1.601
X Variable 1	3.211	0.230	13.934	0.000	2.713	3.708	2.713	3.708

Tank 2/II Date 12/06/03

Regression Statistics	
Multiple R	0.968
R Square	0.938
Adjusted R S	0.933
Standard Erro	0.039
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.295	0.295	196.425	0.000
Residual	13	0.020	0.002		
Total	14	0.315			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.918	0.166	-11.565	0.000	-2.276	-1.559	-2.276	-1.559
X Variable 1	3.210	0.229	14.015	0.000	2.715	3.705	2.715	3.705

Tank 2/I Date 26/06/03

Regression Statistics	
Multiple R	0.947
R Square	0.896
Adjusted R S	0.888
Standard Erro	0.026
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.077	0.077	111.968	0.000
Residual	13	0.009	0.001		
Total	14	0.085			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.761	0.201	-8.759	0.000	-2.195	-1.327	-2.195	-1.327
X Variable 1	3.010	0.284	10.582	0.000	2.395	3.624	2.395	3.624

Tank 2/II Date 26/06/03

Regression Statistics	
Multiple R	0.897
R Square	0.804
Adjusted R S	0.789
Standard Erro	0.060
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.190	0.190	53.398	0.000
Residual	13	0.046	0.004		
Total	14	0.236			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.604	0.274	-5.861	0.000	-2.195	-1.013	-2.195	-1.013
X Variable 1	2.775	0.380	7.307	0.000	1.954	3.595	1.954	3.595

Tank 2/I Date 10/07/03

Regression Statistics	
Multiple R	0.958
R Square	0.918
Adjusted R S	0.912
Standard Erro	0.030
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.129	0.129	145.681	0.000
Residual	13	0.011	0.001		
Total	14	0.140			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.545	0.158	-9.771	0.000	-1.887	-1.204	-1.887	-1.204
X Variable 1	2.666	0.221	12.070	0.000	2.189	3.143	2.189	3.143

Tank 2/II Date 10/07/03

Regression Statistics	
Multiple R	0.972
R Square	0.944
Adjusted R S	0.940
Standard Erro	0.019
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.077	0.077	221.214	0.000
Residual	13	0.005	0.000		
Total	14	0.082			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.654	0.139	-11.945	0.000	-1.954	-1.355	-1.954	-1.355
X Variable 1	2.776	0.187	14.873	0.000	2.373	3.179	2.373	3.179

Tank 2/I	Date 24/07/03

Regression Statistics	
Multiple R	0.969
R Square	0.940
Adjusted R S	0.935
Standard Erro	0.037
Observations	14.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.263	0.263	187.545	0.000
Residual	12	0.017	0.001		
Total	13	0.280			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-2.032	0.186	-10.948	0.000	-2.436	-1.627	-2.436	-1.627
X Variable 1	3.321	0.242	13.695	0.000	2.792	3.849	2.792	3.849

Tank 2/II	Date 24/07/03

Regression Statistics	
Multiple R	0.986
R Square	0.973
Adjusted R S	0.971
Standard Erro	0.024
Observations	14.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.261	0.261	434.941	0.000
Residual	12	0.007	0.001		
Total	13	0.268			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.862	0.114	-16.394	0.000	-2.109	-1.614	-2.109	-1.614
X Variable 1	3.097	0.149	20.855	0.000	2.774	3.421	2.774	3.421

Tank No. 3/I Date 28/05/03

Regression Statistics	
Multiple R	0.873
R Square	0.763
Adjusted R Square	0.759
Standard Error	0.065
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.787	0.787	186.514	0.000
Residual	58	0.245	0.004		
Total	59	1.031			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.652	0.138	-11.988	0.000	-1.928	-1.376	-1.928	-1.376
X Variable 1	2.742	0.201	13.657	0.000	2.340	3.144	2.340	3.144

Tank No. 3/II Date 28/05/03

Regression Statistics	
Multiple R	0.959
R Square	0.920
Adjusted R Square	0.918
Standard Error	0.040
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.070965601	1.070965601	664.3436173	1.86956E-33
Residual	58	0.093499814	0.001612066		
Total	59	1.164465415			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.798	0.079	-22.654	0.000	-1.957	-1.640	-1.957	-1.640
X Variable 1	2.973	0.115	25.775	0.000	2.743	3.204	2.743	3.204

Tank No. 3/I **Date 12/06/03**

Regression Statistics	
Multiple R	0.977
R Square	0.954
Adjusted R Square	0.950
Standard Error	0.034
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.316	0.316	267.874	0.000
Residual	13	0.015	0.001		
Total	14	0.331			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.848	0.138	-13.367	0.000	-2.147	-1.549	-2.147	-1.549
X Variable 1	3.159	0.193	16.367	0.000	2.742	3.576	2.742	3.576

Tank No. 3/II Date 12/06/03

Regression Statistics	
Multiple R	0.967
R Square	0.935
Adjusted R Square	0.930
Standard Error	0.036
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.241	0.241	186.724	0.000
Residual	13	0.017	0.001		
Total	14	0.258			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.755	0.155	-11.354	0.000	-2.089	-1.421	-2.089	-1.421
X Variable 1	3.001	0.220	13.665	0.000	2.526	3.475	2.526	3.475

Tank No. 3/I Date 26/06/03

Regression Statistics	
Multiple R	0.983
R Square	0.967
Adjusted R Square	0.964
Standard Error	0.030
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.345	0.345	377.877	0.000
Residual	13	0.012	0.001		
Total	14	0.357			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.748	0.111	-15.717	0.000	-1.988	-1.507	-1.988	-1.507
X Variable 1	2.978	0.153	19.439	0.000	2.647	3.309	2.647	3.309

Tank No. 3/II Date 26/06/03

Regression Statistics	
Multiple R	0.986
R Square	0.971
Adjusted R Square	0.969
Standard Error	0.029
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.364	0.364	440.623	0.000
Residual	13	0.011	0.001		
Total	14	0.375			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-2.030	0.113	-17.887	0.000	-2.275	-1.785	-2.275	-1.785
X Variable 1	3.376	0.161	20.991	0.000	3.029	3.724	3.029	3.724

Tank No. 3/I Date 10/07/03

Regression Statistics	
Multiple R	0.979
R Square	0.959
Adjusted R Square	0.956
Standard Error	0.024
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.168	0.168	301.630	0.000
Residual	13	0.007	0.001		
Total	14	0.175			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-2.178	0.150	-14.542	0.000	-2.501	-1.854	-2.501	-1.854
X Variable 1	3.505	0.202	17.368	0.000	3.069	3.941	3.069	3.941

Tank No. 3/II Date 10/07/03

Regression Statistics	
Multiple R	0.975
R Square	0.950
Adjusted R Square	0.946
Standard Error	0.023
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.112	0.112	209.382	0.000
Residual	13	0.006	0.001		
Total	14	0.118			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.864	0.158	-11.775	0.000	-2.213	-1.516	-2.213	-1.516
X Variable 1	3.096	0.214	14.470	0.000	2.625	3.567	2.625	3.567

Tank No. 3/I **Date 24/07/03**

Regression Statistics	
Multiple R	0.980
R Square	0.960
Adjusted R Square	0.957
Standard Error	0.039
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.490	0.490	314.657	0.000
Residual	13	0.020	0.002		
Total	14	0.510			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.789	0.137	-13.086	0.000	-2.085	-1.494	-2.085	-1.494
X Variable 1	3.021	0.170	17.739	0.000	2.653	3.388	2.653	3.388

Tank No. 3/II Date 24/07/03

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Regression Statistics	
Multiple R	0.955
R Square	0.913
Adjusted R Square	0.905
Standard Error	0.029
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.098	0.098	115.297	0.000
Residual	13	0.009	0.001		
Total	14	0.107			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.544	0.207	-7.446	0.000	-2.000	-1.087	-2.000	-1.087
X Variable 1	2.727	0.254	10.738	0.000	2.168	3.286	2.168	3.286

Tank 4/I Date 28/05/03

Regression Statistics	
Multiple R	0.892
R Square	0.796
Adjusted R Square	0.792
Standard Error	0.047
Observations	60.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.503	0.503	226.250	0.000
Residual	58	0.129	0.002		
Total	59	0.632			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	ower 95.0	pper 95.0%
Intercept	-1.529	0.122	-12.547	0.000	-1.772	-1.285	-1.772	-1.285
X Variable 1	2.629	0.175	15.042	0.000	2.279	2.978	2.279	2.978

Tank 4/II Date 28/05/03

Regression Statistics	
Multiple R	0.966
R Square	0.933
Adjusted R Square	0.932
Standard Error	0.032
Observations	60.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.816	0.816	812.084	0.000
Residual	58	0.058	0.001		
Total	59	0.874			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	ower 95.0	pper 95.0%
Intercept	-1.729	0.070	-24.721	0.000	-1.869	-1.589	-1.869	-1.589
X Variable 1	2.893	0.102	28.497	0.000	2.690	3.096	2.690	3.096

Tank 4/I Date 12/06/03

Regression Statistics	
Multiple R	0.964
R Square	0.930
Adjusted R Square	0.925
Standard Error	0.031
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.166	0.166	172.774	0.000
Residual	13	0.012	0.001		
Total	14	0.178			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0*	*pper 95.0%*
Intercept	-1.903	0.173	-10.982	0.000	-2.278	-1.529	-2.278	-1.529
X Variable 1	3.179	0.242	13.144	0.000	2.656	3.701	2.656	3.701

Tank 4/II Date 12/06/03

Regression Statistics	
Multiple R	0.988
R Square	0.975
Adjusted R Square	0.973
Standard Error	0.017
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.154	0.154	513.235	0.000
Residual	13	0.004	0.000		
Total	14	0.158			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0*	*pper 95.0%*
Intercept	-1.693	0.087	-19.526	0.000	-1.880	-1.506	-1.880	-1.506
X Variable 1	2.860	0.126	22.655	0.000	2.587	3.133	2.587	3.133

Tank 4/I Date 26/06/03

Regression Statistics	
Multiple R	0.723
R Square	0.523
Adjusted R Square	0.487
Standard Error	0.075
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.080	0.080	14.277	0.002
Residual	13	0.073	0.006		
Total	14	0.152			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0*	*pper 95.0%*
Intercept	-1.128	0.420	-2.687	0.019	-2.035	-0.221	-2.035	-0.221
X Variable 1	2.160	0.572	3.779	0.002	0.925	3.395	0.925	3.395

Tank 4/II Date 26/06/03

Regression Statistics	
Multiple R	0.983
R Square	0.967
Adjusted R Square	0.964
Standard Error	0.022
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.193	0.193	381.086	0.000
Residual	13	0.007	0.001		
Total	14	0.199			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0*	*pper 95.0%*
Intercept	-1.764	0.110	-16.035	0.000	-2.002	-1.526	-2.002	-1.526
X Variable 1	2.979	0.153	19.521	0.000	2.649	3.308	2.649	3.308

Tank 4/I Date 10/07/03

Regression Statistics	
Multiple R	0.977
R Square	0.954
Adjusted R Square	0.951
Standard Error	0.018
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.084	0.084	270.068	0.000
Residual	13	0.004	0.000		
Total	14	0.088			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0*	*pper 95.0%*
Intercept	-1.938	0.145	-13.343	0.000	-2.251	-1.624	-2.251	-1.624
X Variable 1	3.193	0.194	16.434	0.000	2.773	3.612	2.773	3.612

Tank 4/II Date 10/07/03

Regression Statistics	
Multiple R	0.961
R Square	0.923
Adjusted R Square	0.917
Standard Error	0.035
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.197	0.197	156.641	0.000
Residual	13	0.016	0.001		
Total	14	0.213			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0*	*pper 95.0%*
Intercept	-1.504	0.149	-10.115	0.000	-1.825	-1.183	-1.825	-1.183
X Variable 1	2.586	0.207	12.516	0.000	2.140	3.033	2.140	3.033

Tank 4/I Date 24/07/03

Regression Statistics	
Multiple R	0.979
R Square	0.958
Adjusted R Square	0.955
Standard Error	0.027
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.224	0.224	296.538	0.000
Residual	13	0.010	0.001		
Total	14	0.234			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	ower 95.0	pper 95.0%
Intercept	-1.875	0.146	-12.841	0.000	-2.190	-1.559	-2.190	-1.559
X Variable 1	3.113	0.181	17.220	0.000	2.723	3.504	2.723	3.504

Tank 4/II Date 24/07/03

Regression Statistics	
Multiple R	0.987
R Square	0.975
Adjusted R Square	0.973
Standard Error	0.028
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.485	0.485	620.036	0.000
Residual	13	0.013	0.001		
Total	14	0.497			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	ower 95.0	pper 95.0%
Intercept	-1.832	0.093	-19.674	0.000	-2.029	-1.634	-2.029	-1.634
X Variable 1	3.060	0.123	24.901	0.000	2.800	3.321	2.800	3.321

Tank 5/I Date 28/05/03

Regression Statistics	
Multiple R	0.976
R Square	0.953
Adjusted R Square	0.952
Standard Error	0.025
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.759	0.759	1179.252	0.000
Residual	58	0.037	0.001		
Total	59	0.796			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.831	0.061	-29.904	0.000	-1.953	-1.708	-1.953	-1.708
X Variable 1	3.031	0.088	34.340	0.000	2.854	3.208	2.854	3.208

Tank 5/II Date 28/05/03

Regression Statistics	
Multiple R	0.967
R Square	0.934
Adjusted R Square	0.933
Standard Error	0.031
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.817	0.817	826.399	0.000
Residual	58	0.057	0.001		
Total	59	0.874			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.765	0.071	-24.980	0.000	-1.906	-1.623	-1.906	-1.623
X Variable 1	2.939	0.102	28.747	0.000	2.734	3.144	2.734	3.144

Tank 5/I Date 12/06/03

Regression Statistics	
Multiple R	0.988
R Square	0.977
Adjusted R Square	0.975
Standard Error	0.025
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.344	0.344	540.648	0.000
Residual	13	0.008	0.001		
Total	14	0.352			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.901	0.095	-20.056	0.000	-2.106	-1.696	-2.106	-1.696
X Variable 1	3.125	0.134	23.252	0.000	2.835	3.416	2.835	3.416

Tank 5/II Date 12/06/03

Regression Statistics	
Multiple R	0.970
R Square	0.941
Adjusted R Square	0.937
Standard Error	0.037
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.285	0.285	208.958	0.000
Residual	13	0.018	0.001		
Total	14	0.303			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.727	0.141	-12.210	0.000	-2.033	-1.422	-2.033	-1.422
X Variable 1	2.918	0.202	14.455	0.000	2.482	3.354	2.482	3.354

Tank 5/I Date 26/06/03

Regression Statistics	
Multiple R	0.938
R Square	0.880
Adjusted R Square	0.871
Standard Error	0.030
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.085	0.085	95.601	0.000
Residual	13	0.012	0.001		
Total	14	0.096			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.440	0.186	-7.746	0.000	-1.842	-1.039	-1.842	-1.039
X Variable 1	2.529	0.259	9.778	0.000	1.970	3.088	1.970	3.088

Tank 5/II Date 26/06/03

Regression Statistics	
Multiple R	0.882
R Square	0.779
Adjusted R Square	0.762
Standard Error	0.035
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.057	0.057	45.715	0.000
Residual	13	0.016	0.001		
Total	14	0.073			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.815	0.320	-5.663	0.000	-2.507	-1.122	-2.507	-1.122
X Variable 1	3.029	0.448	6.761	0.000	2.061	3.997	2.061	3.997

Tank 5/I Date 10/07/03

Regression Statistics	
Multiple R	0.969
R Square	0.938
Adjusted R Square	0.934
Standard Error	0.020
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.080	0.080	197.960	0.000
Residual	13	0.005	0.000		
Total	14	0.086			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.873	0.163	-11.464	0.000	-2.226	-1.520	-2.226	-1.520
X Variable 1	3.074	0.218	14.070	0.000	2.602	3.546	2.602	3.546

Tank 5/II Date 10/07/03

Regression Statistics	
Multiple R	0.965
R Square	0.932
Adjusted R Square	0.926
Standard Error	0.039
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.274	0.274	176.936	0.000
Residual	13	0.020	0.002		
Total	14	0.294			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.881	0.164	-11.465	0.000	-2.235	-1.526	-2.235	-1.526
X Variable 1	3.085	0.232	13.302	0.000	2.584	3.586	2.584	3.586

Tank 5/I Date 24/07/03

Regression Statistics	
Multiple R	0.902
R Square	0.813
Adjusted R Square	0.799
Standard Error	0.072
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.289	0.289	56.526	0.000
Residual	13	0.067	0.005		
Total	14	0.356			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.375	0.252	-5.447	0.000	-1.921	-0.830	-1.921	-0.830
X Variable 1	2.491	0.331	7.518	0.000	1.775	3.207	1.775	3.207

Tank 5/II Date 24/07/03

Regression Statistics	
Multiple R	0.934
R Square	0.872
Adjusted R Square	0.862
Standard Error	0.028
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.069	0.069	88.708	0.000
Residual	13	0.010	0.001		
Total	14	0.080			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.519	0.221	-6.877	0.000	-1.997	-1.042	-1.997	-1.042
X Variable 1	2.664	0.283	9.418	0.000	2.053	3.275	2.053	3.275

Tank 6/I	Date 28/05/03

Regression Statistics	
Multiple R	0.928
R Square	0.862
Adjusted R Square	0.859
Standard Error	0.042
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.634	0.634	361.317	0.000
Residual	58	0.102	0.002		
Total	59	0.736			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.637	0.100	-16.348	0.000	-1.837	-1.436	-1.837	-1.436
X Variable 1	2.755	0.145	19.008	0.000	2.465	3.045	2.465	3.045

Tank 6/II	Date 28/05/03

Regression Statistics	
Multiple R	0.982
R Square	0.964
Adjusted R Square	0.963
Standard Error	0.030
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.392	1.392	1556.583	0.000
Residual	58	0.052	0.001		
Total	59	1.444			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.887	0.055	-34.427	0.000	-1.996	-1.777	-1.996	-1.777
X Variable 1	3.109	0.079	39.454	0.000	2.951	3.267	2.951	3.267

Tank 6/I	Date 12/06/03

Regression Statistics	
Multiple R	0.978
R Square	0.956
Adjusted R Square	0.952
Standard Error	0.029
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.239	0.239	281.002	0.000
Residual	13	0.011	0.001		
Total	14	0.250			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.760	0.122	-14.423	0.000	-2.023	-1.496	-2.023	-1.496
X Variable 1	2.944	0.176	16.763	0.000	2.565	3.324	2.565	3.324

Tank 6/II	Date 12/06/03

Regression Statistics	
Multiple R	0.983410106
R Square	0.967095437
Adjusted R Square	0.964564317
Standard Error	0.029228679
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.326	0.326	382.082	0.000
Residual	13	0.011	0.001		
Total	14	0.338			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.730	0.107	-16.237	0.000	-1.960	-1.500	-1.960	-1.500
X Variable 1	2.900	0.148	19.547	0.000	2.580	3.221	2.580	3.221

Tank 6/I	Date 26/06/03

Regression Statistics	
Multiple R	0.951515469
R Square	0.905381689
Adjusted R Square	0.897496829
Standard Error	0.027261242
Observations	14

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.085	0.085	114.825	0.000
Residual	12	0.009	0.001		
Total	13	0.094			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.581	0.175	-9.056	0.000	-1.962	-1.201	-1.962	-1.201
X Variable 1	2.698	0.252	10.716	0.000	2.149	3.247	2.149	3.247

Tank 6/II	Date 26/06/03

Regression Statistics	
Multiple R	0.913900381
R Square	0.835213907
Adjusted R Square	0.822538053
Standard Error	0.029514471
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.057	0.057	65.890	0.000
Residual	13	0.011	0.001		
Total	14	0.069			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.45318725	0.21054681	-6.90197	1.082E-05	-1.908045892	-0.998328608	-1.908045892	-0.998328608
X Variable 1	2.51639702	0.310005148	8.11727	1.906E-06	1.846671743	3.186122297	1.846671743	3.186122297

Tank 6/I	Date 10/07/03

Regression Statistics	
Multiple R	0.984
R Square	0.968
Adjusted R Square	0.966
Standard Error	0.028
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.296	0.296	365.228	0.000
Residual	13	0.010	0.001		
Total	14	0.305			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.796	0.112	-16.036	0.000	-2.040	-1.552	-2.040	-1.552
X Variable 1	2.981	0.156	19.111	0.000	2.641	3.320	2.641	3.320

Tank 6/II	Date 10/07/03

Regression Statistics	
Multiple R	0.947356742
R Square	0.897484796
Adjusted R Square	0.889599011
Standard Error	0.036011895
Observations	15

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.147595838	0.1476	113.81046	8.45241E-08
Residual	13	0.016859135	0.0013		
Total	14	0.164454973			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.437128817	0.171568806	-8.3764	1.347E-06	-1.807780616	-1.066477019	-1.807780616	-1.066477019
X Variable 1	2.486867074	0.233110313	10.6682	8.452E-08	1.983262958	2.99047119	1.983262958	2.99047119

Tank 6/I	Date 24/07/03

Regression Statistics	
Multiple R	0.976
R Square	0.952
Adjusted R Square	0.948
Standard Error	0.025
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.145142484	0.14514	236.3978	2.92889E-09
Residual	13	0.007367707	0.00061		
Total	14	0.152510191			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.796	0.152	-11.836	0.000	-2.127	-1.466	-2.127	-1.466
X Variable 1	3.046	0.198	15.375	0.000	2.614	3.478	2.614	3.478

Tank 6/II	Date 24/07/03

Regression Statistics	
Multiple R	0.990
R Square	0.979
Adjusted R Square	0.978
Standard Error	0.038
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.883	0.883	611.093	0.000
Residual	13	0.019	0.001		
Total	14	0.902			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.856	0.099	-18.693	0.000	-2.070	-1.641	-2.070	-1.641
X Variable 1	3.118	0.126	24.720	0.000	2.846	3.391	2.846	3.391

Tank 7/I Date 28/05/03

Regression Statistics	
Multiple R	0.982
R Square	0.964
Adjusted R Square	0.963
Standard Error	0.026
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.042	1.042	1540.035	0.000
Residual	58	0.039	0.001		
Total	59	1.081			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.755	0.051	-34.548	0.000	-1.856	-1.653	-1.856	-1.653
X Variable 1	2.913	0.074	39.243	0.000	2.764	3.061	2.764	3.061

Tank 7/II Date 28/05/03

Regression Statistics	
Multiple R	0.964
R Square	0.930
Adjusted R Square	0.928
Standard Error	0.030
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.682	0.682	766.277	0.000
Residual	58	0.052	0.001		
Total	59	0.733			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.797	0.073	-24.717	0.000	-1.943	-1.652	-1.943	-1.652
X Variable 1	2.960	0.107	27.682	0.000	2.746	3.174	2.746	3.174

Tank 7/I Date 12/06/03

Regression Statistics	
Multiple R	0.952
R Square	0.906
Adjusted R Square	0.898
Standard Error	0.022
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.063	0.063	124.872	0.000
Residual	13	0.007	0.001		
Total	14	0.070			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.372	0.152	-9.049	0.000	-1.699	-1.044	-1.699	-1.044
X Variable 1	2.403	0.215	11.175	0.000	1.938	2.867	1.938	2.867

Tank 7/II Date 12/06/03

Regression Statistics	
Multiple R	0.972
R Square	0.945
Adjusted R Square	0.941
Standard Error	0.026
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.151	0.151	222.679	0.000
Residual	13	0.009	0.001		
Total	14	0.159			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.829	0.144	-12.724	0.000	-2.139	-1.518	-2.139	-1.518
X Variable 1	3.060	0.205	14.922	0.000	2.617	3.503	2.617	3.503

Tank 7/I Date 26/06/03

Regression Statistics	
Multiple R	0.97191432
R Square	0.944617446
Adjusted R Square	0.94035725
Standard Error	0.03337368
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.247	0.247	221.731	0.000
Residual	13	0.014	0.001		
Total	14	0.261			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.727524586	0.141529472	-12.20611	1.7032E-08	-2.033280363	-1.4217688	-2.033280363	-1.421768809
X Variable 1	2.963025402	0.198985855	14.890633	1.5109E-09	2.53314268	3.39290812	2.53314268	3.392908124

Tank 7/II Date 26/06/03

Regression Statistics	
Multiple R	0.982816981
R Square	0.965929218
Adjusted R Square	0.963308389
Standard Error	0.026759757
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.264	0.264	368.559	0.000
Residual	13	0.009	0.001		
Total	14	0.273			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.899	0.113	-16.811	0.000	-2.143	-1.655	-2.143	-1.655
X Variable 1	3.167	0.165	19.198	0.000	2.811	3.523	2.811	3.523

Tank 7/I	Date 10/07/03

Regression Statistics	
Multiple R	0.958
R Square	0.917
Adjusted R Square	0.911
Standard Error	0.043
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.270	0.270	143.509	0.000
Residual	13	0.024	0.002		
Total	14	0.294			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-2.058	0.202	-10.215	0.000	-2.494	-1.623	-2.494	-1.623
X Variable 1	3.324	0.277	11.980	0.000	2.725	3.924	2.725	3.924

Tank 7/II	Date 10/07/03

Regression Statistics	
Multiple R	0.935961664
R Square	0.876024237
Adjusted R Square	0.86648764
Standard Error	0.039161097
Observations	15

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.141	0.141	91.859	0.000
Residual	13	0.020	0.002		
Total	14	0.161			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.793	0.224	-8.020	0.000	-2.276	-1.310	-2.276	-1.310
X Variable 1	2.969	0.310	9.584	0.000	2.300	3.639	2.300	3.639

Tank 7/I	Date 24/07/03

Regression Statistics	
Multiple R	0.966
R Square	0.934
Adjusted R Square	0.929
Standard Error	0.032
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.185	0.185	183.003	0.000
Residual	13	0.013	0.001		
Total	14	0.198			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.502	0.160	-9.367	0.000	-1.848	-1.155	-1.848	-1.155
X Variable 1	2.659	0.197	13.528	0.000	2.234	3.084	2.234	3.084

Tank 7/II	Date 24/07/03

Regression Statistics	
Multiple R	0.951
R Square	0.905
Adjusted R Square	0.898
Standard Error	0.039
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.187	0.187	123.784	0.000
Residual	13	0.020	0.002		
Total	14	0.206			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-2.168	0.236	-9.192	0.000	-2.677	-1.658	-2.677	-1.658
X Variable 1	3.502	0.315	11.126	0.000	2.822	4.182	2.822	4.182

Tank 8/I Date 28/05/03

Regression Statistics	
Multiple R	0.968
R Square	0.937
Adjusted R Square	0.936
Standard Error	0.031
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.824	0.824	869.678	0.000
Residual	58	0.055	0.001		
Total	59	0.879			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.609	0.062	-26.068	0.000	-1.733	-1.485	-1.733	-1.485
X Variable 1	2.708	0.092	29.490	0.000	2.524	2.892	2.524	2.892

Tank 8/II Date 28/05/03

Regression Statistics	
Multiple R	0.975
R Square	0.951
Adjusted R Square	0.950
Standard Error	0.026
Observations	60.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.772	0.772	1121.265	0.000
Residual	58	0.040	0.001		
Total	59	0.812			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.875	0.063	-29.763	0.000	-2.001	-1.749	-2.001	-1.749
X Variable 1	3.099	0.093	33.485	0.000	2.913	3.284	2.913	3.284

Tank 8/I	Date 12/06/03

Regression Statistics	
Multiple R	0.976
R Square	0.952
Adjusted R Square	0.949
Standard Error	0.029
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.224	0.224	259.647	0.000
Residual	13	0.011	0.001		
Total	14	0.235			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.658	0.119	-13.905	0.000	-1.916	-1.401	-1.916	-1.401
X Variable 1	2.810	0.174	16.114	0.000	2.433	3.186	2.433	3.186

Tank 8/II	Date 12/06/03

Regression Statistics	
Multiple R	0.969
R Square	0.939
Adjusted R Square	0.934
Standard Error	0.033
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.214	0.214	200.481	0.000
Residual	13	0.014	0.001		
Total	14	0.228			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.897	0.156	-12.184	0.000	-2.233	-1.560	-2.233	-1.560
X Variable 1	3.161	0.223	14.159	0.000	2.679	3.643	2.679	3.643

Tank 8/I Date 26/06/03

Regression Statistics	
Multiple R	0.963
R Square	0.927
Adjusted R Square	0.921
Standard Error	0.028
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.133	0.133	165.071	0.000
Residual	13	0.010	0.001		
Total	14	0.144			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.688	0.157	-10.759	0.000	-2.026	-1.349	-2.026	-1.349
X Variable 1	2.876	0.224	12.848	0.000	2.392	3.359	2.392	3.359

Tank 8/II Date 26/06/03

Regression Statistics	
Multiple R	0.936
R Square	0.876
Adjusted R Square	0.867
Standard Error	0.029
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.07997884	0.079979	92.121108	2.89022E-07
Residual	13	0.0112865	0.000868		
Total	14	0.09126534			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.395	0.184	-7.595	0.000	-1.792	-0.998	-1.792	-0.998
X Variable 1	2.461	0.256	9.598	0.000	1.907	3.015	1.907	3.015

Tank 8/I	Date 10/07/03

Regression Statistics	
Multiple R	0.975
R Square	0.950
Adjusted R Square	0.946
Standard Error	0.025
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.151	0.151	246.884	0.000
Residual	13	0.008	0.001		
Total	14	0.159			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.782	0.137	-13.021	0.000	-2.077	-1.486	-2.077	-1.486
X Variable 1	2.975	0.189	15.713	0.000	2.566	3.384	2.566	3.384

Tank 8/II	Date 10/07/03

Regression Statistics	
Multiple R	0.963564929
R Square	0.928457372
Adjusted R Square	0.922954093
Standard Error	0.030230024
Observations	15

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.154	0.154	168.710	0.000
Residual	13	0.012	0.001		
Total	14	0.166			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.724	0.164	-10.520	0.000	-2.078	-1.370	-2.078	-1.370
X Variable 1	2.878	0.222	12.989	0.000	2.399	3.357	2.399	3.357

Tank: 8/I	Date 24/07/03

Regression Statistics	
Multiple R	0.977
R Square	0.955
Adjusted R Square	0.952
Standard Error	0.029
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.224	0.224	275.771	0.000
Residual	13	0.011	0.001		
Total	14	0.235			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.806	0.143	-12.650	0.000	-2.114	-1.498	-2.114	-1.498
X Variable 1	3.048	0.184	16.606	0.000	2.652	3.445	2.652	3.445

Tank 8/II	Date 24/07/03

Regression Statistics	
Multiple R	0.401
R Square	0.161
Adjusted R Square	0.096
Standard Error	0.265
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.175	0.175	2.493	0.138
Residual	13	0.911	0.070		
Total	14	1.085			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.656	1.396	-1.186	0.257	-4.673	1.360	-4.673	1.360
X Variable 1	2.927	1.854	1.579	0.138	-1.078	6.932	-1.078	6.932

Tank 9/I	Date 28/05/03

Regression Statistics	
Multiple R	0.970
R Square	0.941
Adjusted R Square	0.940
Standard Error	0.029
Observations	60.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.801	0.801	924.627	0.000
Residual	58	0.050	0.001		
Total	59	0.851			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.717	0.064	-26.811	0.000	-1.845	-1.589	-1.845	-1.589
X Variable 1	2.858	0.094	30.408	0.000	2.669	3.046	2.669	3.046

Tank 9/II	Date 28/05/03

Regression Statistics	
Multiple R	0.968
R Square	0.937
Adjusted R Square	0.936
Standard Error	0.029
Observations	60.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.733150138	0.73315014	867.761479	1.3895E-36
Residual	58	0.049002761	0.00084488		
Total	59	0.782152898			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.735	0.066	-26.283	0.000	-1.867	-1.603	-1.867	-1.603
X Variable 1	2.886	0.098	29.458	0.000	2.690	3.083	2.690	3.083

Tank 9/I	Date 12/06/03

Regression Statistics	
Multiple R	0.970402257
R Square	0.94168054
Adjusted R Square	0.937194428
Standard Error	0.030476746
Observations	15

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.195	0.195	209.910	0.000
Residual	13	0.012	0.001		
Total	14	0.207			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.785	0.143	-12.504	0.000	-2.093	-1.476	-2.093	-1.476
X Variable 1	2.966	0.205	14.488	0.000	2.524	3.408	2.524	3.408

Tank 9/II	Date 12/06/03

Regression Statistics	
Multiple R	0.925484734
R Square	0.856521992
Adjusted R Square	0.845485222
Standard Error	0.046914251
Observations	15

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.171	0.171	77.606	0.000
Residual	13	0.029	0.002		
Total	14	0.199			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-2.114	0.270	-7.819	0.000	-2.698	-1.530	-2.698	-1.530
X Variable 1	3.455	0.392	8.809	0.000	2.608	4.303	2.608	4.303

Tank 9/I Date 26/06/03

Regression Statistics	
Multiple R	0.903
R Square	0.816
Adjusted R Square	0.801
Standard Error	0.067
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.256	0.256	57.503	0.000
Residual	13	0.058	0.004		
Total	14	0.314			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.459	0.237	-6.146	0.000	-1.971	-0.946	-1.971	-0.946
X Variable 1	2.550	0.336	7.583	0.000	1.823	3.276	1.823	3.276

Tank 9/II Date 26/06/03

Regression Statistics	
Multiple R	0.931
R Square	0.866
Adjusted R Square	0.856
Standard Error	0.034
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.099	0.099	84.088	0.000
Residual	13	0.015	0.001		
Total	14	0.114			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.511	0.189	-8.012	0.000	-1.918	-1.104	-1.918	-1.104
X Variable 1	2.556	0.279	9.170	0.000	1.954	3.158	1.954	3.158

Tank 9/I Date 10/07/03

Regression Statistics	
Multiple R	0.950
R Square	0.903
Adjusted R Square	0.895
Standard Error	0.033
Observations	15.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.12844304	0.12844304	120.797752	5.94929E-08
Residual	13	0.01382277	0.00106329		
Total	14	0.14226581			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-1.851	0.200	-9.243	0.000	-2.283	-1.418	-2.283	-1.418
X Variable 1	3.043	0.277	10.991	0.000	2.444	3.641	2.444	3.641

Tank 9/II Date 10/07/03

Regression Statistics	
Multiple R	0.966
R Square	0.933
Adjusted R Square	0.928
Standard Error	0.038
Observations	16.000

ANOVA

	df	SS	MS	F	Significance F
Regression	1	0.285	0.285	194.899	0.000
Residual	14	0.020	0.001		
Total	15	0.305			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	-2.179	0.170	-12.806	0.000	-2.544	-1.814	-2.544	-1.814
X Variable 1	3.493	0.250	13.961	0.000	2.957	4.030	2.957	4.030

Tank 9/I	Date 24/07/03

Regression Statistics	
Multiple R	0.809
R Square	0.654
Adjusted R Square	0.629
Standard Error	0.108
Observations	16.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.307	0.307	26.431	0.000
Residual	14	0.163	0.012		
Total	15	0.469			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.344	0.324	-4.149	0.001	-2.039	-0.650	-2.039	-0.650
X Variable 1	2.379	0.463	5.141	0.000	1.387	3.371	1.387	3.371

Tank 9/II	Date 24/07/03

Regression Statistics	
Multiple R	0.843
R Square	0.711
Adjusted R Square	0.688
Standard Error	0.085
Observations	15.000

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.232	0.232	31.935	0.000
Residual	13	0.094	0.007		
Total	14	0.327			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-1.423	0.313	-4.549	0.001	-2.099	-0.747	-2.099	-0.747
X Variable 1	2.473	0.438	5.651	0.000	1.528	3.418	1.528	3.418

EXPLANATION OF PHOTOGRAPHS

Inside cover page

1. Set up of the experiment.
2. Stocking of the fish in the pool.
3. Conditioning of the fish before transport from the farm.
4. Oxygen packing of the fish before transportation from the farm.
5. Fish samples of *Cyprinus carpio*.
6. Showering of the fish samples before transportation.
7. Transportation of the fish in the tub.
8. Conditioning of fish before release in the plastic pool.
9. Demonstrating oxygen packing of the fish.

Back inside cover page

10. Feed manufacturing for experimental fish.
11. Counting of the fish before oxygen packing.
12. Conditioning of the fish at the farm in the oxygen pack.
13. Demonstrating oxygen pack fishes stock.
14. Demonstration of oxygen packing preparatory.
15. Counted fish before packing.
16. Fish packing demonstration before oxygen.
17. Fish samples just before segregation.

Cover page

BG = Bollgard II seed

NBG = Non-Bollgard-II

Photo of *Cyprinus carpio*

Back page

Photo of Cyprinus carpio lot