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# Assessment of Reproductive Performance of Albino Rats Fed with Gm and Non-Gm Cotton Seeds for Three Generations

KEYWORDS: Bt cotton seeds, albino rat, reproductive performance, growth rate, developmental parameters

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**ABSTRACT** *Advances in technologies enabling transfer of foreign gene in plants have overcome several barriers to crop improvement. These technologies offer immense benefits in terms of increased yield, better quality and resistance to biotic and abiotic factors. There is a world-wide debate concerning the safety and regulatory approval process of genetically modified crops and foods. The present study was designed to compare the feeding value and reproductive performance of a line of genetically modified cotton seeds (GM cottonseeds) to that of standard diet and reference diet (Non-GM cottonseeds). GM and Non- GM treated cotton seeds were incorporated into the diet of the rats at a concentration of 20% for continuously three generations. Growth rate, litter size, weekly body weight, gross abnormality and developmental aspects were compared between control, Non- GM and GM seed fed animals for all generations. The results showed that maternal body weight gain and gestation period was not affected by the treatment as compared to control rats. It was observed that cotton seed supplementation in feed had significant effect on body weight gain of pups during 7th-42nd days of postnatal development as compared to control pups. No effect of treatment was observed in developmental parameters like eye opening, hair appearance. The production parameters, average litter size as well as number of weaned pups were in favour of the control and Non GM fed lines.*

**Introduction**

GM cotton is one of the predominant transgenic crops all over the world and the global area under GM crops has increased nearly 73.5 fold during last 13 year's period (1996 to 2008) (James, 2008). India is the world second largest producer of cotton is estimated at 11.7 million hectares and the world's second largest cotton grower, is projected to produce 26.0 million bales in 2013-14 (Johnson et al 2013). Insertion of *Bacillus thuringiensis* in to the DNA of local cotton varieties gives them resistance towards lepidopteran insect pests. The adoption of transgenic crops like Bt cotton helped in protecting the crop against the potentially most damaging pest and thus have reduced the risk of crop failure. Therefore, interest in genetically modified (GM) crops is continuously increasing due to the possibility of higher agronomic productivity and more nutritious food without the use of pesticides (Shintani & Della-Penna, 1998 & Ye et al 2000).

Proximate analysis showed that there was no difference between Bt-cotton and its non-Bt counterpart in terms of protein, carbohydrates, ash and moisture contents. Forage composition of Bt-cotton is substantially equivalent to non-Bt cotton in respect of gossypol and other acid contents (Padgette et al 1996). Potential impact of transgenic feed on the digestion, metabolism and health in farm animals have been addressed in with goats, buffalos, cows, rabbits, birds and fish (Manjunath, 2006). Most of these feeding experiments have been short term feeding trials using conventional designs such as digestibility experiments or growth trials until slaughter. Long term adverse effects of GM crops have not been reported in peer-reviewed publications related to animal feed. Therefore, growth rate, litter size, weekly body weight, gross abnormality and biosafety of non Bt and Bt whole cottonseed based rations in albino rats were compared in this study for three generations.

**2. Materials and methods**

*2.1. Animals and housing*

The study was conducted on albino rats weighing 100-110 gms obtained from Guru AngadDev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The rats were maintained in laboratory under standard conditions of temperature (25±2°C) providing them laboratory pelleted feed and water *ad libitum*. The rats were acclimatized to new quarters for one week before starting the treatment. The experimental protocol met the National guidelines on the proper care and use of animals in the laboratory research. This experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC).

**2.2. Experimental diets**

Non-Bt and Bt seeds were procured from Plant Breeding and Genetics Department of Punjab Agricultural university, Ludhiana, Punjab, India. Rats were divided into three groups. Rats in group I were considered as control and fed only with standard diet, i.e. wheat:grams (50:50 w/w); rats those were in group II were considered as Non-Bt group fed with diet containing wheat: gram :Non-Bt cotton seeds (50:30:20 w/w) and the rats in group III were considered as Bt group was fed with diet containing 20% transgenic Bt cotton seeds, i.e wheat: gram: Bt cotton seeds (50:30:20 w/w). The rats were fed according to their group by soaking the diet overnight.

**2.3. Experimental design and treatment**

**2.3.1 Performance and reproduction data**

The parental generation (F0) was fed with either standard diet or diet having 20% Non-Bt cotton seeds or diet with 20% Bt cotton seeds depending on their groups and three generations were bred (Table 1). Eighteen female albino rats (6 rats/each group) were mated with 9 male rats (one male for two female rats) overnight. Vaginal smears of the females were taken on the following morning. The presence of spermatozoa was considered day 0 of gestation. Dams and their offspring were fed with the same diets during the periods of mating, gestation, lactation, offspring care and pubescence. The offsprings of F1 and F2 generation in a group of each generation were mated among themselves to obtain subsequent generation. The collected data were divided into parental data and offspring data (Table 2) for each generation.

*2.3. Statistical Analysis*

All statistical comparisons were presented as the mean ±

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standard error of mean (S.E.M). Comparisons were made between control, Non-Bt and Bt groups on computer using "Analysis of Variance (ANOVA)" as a statgraphics statistical package. A "P" value of 0.05 was selected as a criterion for statistically significant differences.

## 3. Results

### 3.1. Parental Performance

Growth rate during pregnancy was observed to be more in Non-Bt and Bt group as compared to control group in F0 and F1 generation female parental rats. At time of delivery, the decrease in growth rate was observed in female parental rats of F1 generation in control, Non-Bt and Bt group as compared to F0 and F2 generational parental rats (Table 3). During three weeks of weaning the significant increased growth rate was observed in Bt cottonseeds fed parental rats as compared to Non-Bt and control group parental rats.

### 3.2. Reproduction data

No statistically significant differences were observed in reproductive performance of females between the feeding groups, but litter size with n> 8 were observed in the F1 female rats of all the three feeding groups (Table 4). Litter size decreased in subsequent generations and minimum size was observed in Bt cottonseeds fed female rats. The number of pups at weaning per pair was minimum in the Bt fed rats as compared to the Non- Bt and control group rats (Table 4). Sex ratio was observed to be 1:1 (male:female) in all the three generations in control, Non- Bt and Bt feeding groups of rats (Fig. 1).

### 3.3 Offspring performance

Growth rate during weaning of pups of three generation was observed to be maximum in Bt group as compared to control and Non-Bt group. After 42 days after birth non-significant change in percent growth rate was observed in the control group while the pups fed with Non-Bt and Bt cotton seeds showed significant change in % growth rate in all the three generations (Table 5).

### 3.4. Developmental aspects

The developmental landmarks like pinna detachment, testes descent and incisor eruption was delayed by one or two days in the different feeding groups. Hair appearance was not affected in any of the feeding group. Significantly higher delay was observed in vaginal opening and start of estrous cycle in pups born to Non-Bt and Bt fed mother where vaginal opening started on 38- 40$^{th}$ day. However in control group rats it appeared on 30$^{th}$ day (Fig. 2).

## 4. Discussion

Significant increase in growth rate was observed in cottonseeds fed rats. Rats fed with Bt corn also showed increased in the body weight as compared to the parental corn or reference diet (Kilic & Akay, 2008). Li et al. (2004) evaluated the effects of genetically modified rice with Xa21 on the development of rat embryos. Malley et al. (2007) did not find adverse diet-related differences in rats fed given 59122 maize grain with respect to body weight/gain. On a similar approach, following studies (He et al 2009) showed no adverse diet-related differences in body weights, feed consumption/utilization between rats consuming diets with Y642 maize grain compared with rats consuming diets containing Nongda 108 maize grain (near-isogenic non-GM quality protein maize). No adverse effect on general behavior or body weight of monkeys was observed which were orally administered a high or low dose of transgenic rice containing 7Crp or the non-transgenic control during the long term study (Domingo & Bordonaba, 2011). In 90 days dietary experiment using Swiss male mice eating meat meal of genetically modified (GM) Japanese quail, demonstrated normal growth curve and no significant differences in body weights between the control and the treated groups in most weeks (Nahas et al 2011). No difference for average daily weight gain, feed intake and feed conversion ratio was recorded in broiler performance fed with transgenic *Bacillus thuringiensis* (Bt) corn containing the Cry1A(b) protein compared with the corresponding near isogenic corn (Rossi et al 2005).

Hammond et al. (2004) demonstrated that animals fed with Roundup Ready corn grain in the diet had similar body weights; body weight gains when compared to animals fed diet containing control or reference control grain from six commercial hybrids. The comparable responses of rats fed with Roundup Ready corn grain to rats fed with control grain supports the absence of pleiotropic effects in Roundup Ready corn as confirmed in comprehensive agronomic and composition studies as well as feeding studies in swine and poultry (Taylor et al 2003 & Hyung et al 2004). No statistically significant effect was observed in body weights in 90-days study in rats fed with 11% or 33% MON 810 corn (Hammond et al 2006). Likewise, grower and finisher swine fed Roundup Ready corn grain at diet incorporation rates between 68% and 82% w/w, respectively, showed comparable performance (body weight gain, feed efficiency) to swine fed control and reference control corn diets (Hyung *et al* 2004). Recently, Schroder et al. (2007) reported the results of a 90- day safety study of GM rice (KMD1) expressing Cry1Ab protein (*Bacillus thuringiensis* toxin) in Wistar rats reported no adverse effects on animal behavior or weight gain were observed during the study.

Chainark et al. (2006) demonstrated no marked differences in growth in fish fed different levels of GM or Non-GM diet. Similar results were also obtained in feeding studies of modified and unmodified feed has been reported for other animals, like swine, poultry and dairy cattle (Donkin et al 2000, Cromwell et al 2002 & Rossi et al 2005). No significant difference in growth and feed performance between the GM and non-GM Soybean meal groups at either inclusion level at the end of 12$^{th}$week in juviline fish (Satosh & Chainark, 2010). Chainark et al. (2008) reported degradation and the possible carryover of foreign DNA fragments by means of measuring it from transgenic plants and host plants contained in GM or non-GM SBM (non-GM Soybean meal) formulated diets and evaluated the safety for fish.

Multi-Generational Studies performed by Velimirov et al. (2008) over 4 generations did not show significant differences in reproductive performance of the parental mice between the feeding groups isogenic line (ISO) and genetically modified (GM) corn. The number of pups weaned, the average litter size and weight at weaning tended to be lower in the GM group as compared to the isogenic line. Birthrate and survival of the offspring's did not change among groups demonstrating successful reproduction in rats fed with Bt corn for three generations (Kilic & Akay 2008).

Mice fed with GM maize and bred over four generations had less offspring in the third and fourth generations and these differences were statistically significant (Ho, 2008). High rate of pup mortality (~ 55,6%) was observed in the litter of mothers, whose diet was supplemented with the genetically modified, Roundup-Ready soya flour, in comparison with the pups of both the positive control (6,8%), and the traditional soya flour supplemented (9%) groups (Ermakova, 2005). Seralini et al. (2012) showed that the rate of mortality in rats fed with Roundup-tolerant genetically modified maize for 2 years. In the present study, non-significant effect was observed in the developmental parameters of pups, except slight delay in the vaginal opening due to intake of cottonseeds in the diets of Non-Bt and Bt group.

The biological phenomenon observed suggested that genetically modified crops have no adverse effects on the multigenerational reproductive-developmental ability because both diets were covering the energy and nutrient requirements and fulfilled the prerequisite of nutritional equivalence.

**Acknowledgement**
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**Table 1: Overview of succession of generations**

| GENERATION | PAIRS/GROUP | DATE |
|---|---|---|
| F0 parents | n= 6 | August/Sept 2011 |
| F1 offspring | | Sept 2011/Oct 2011 |
| F1 parents | n= 6 | Nov 2011/ Dec 2011 |
| F2 offspring | | Jan 2012/Feb 2012 |
| F2 parents | n= 6 | March/ April 2012 |
| F3 offspring | | May/June 2012 |

**Table 2:Data collection of parental and offspring rat per generation**

| **Parental Performance data** | **Reproduction data** | **Offspring Performance data** |
|---|---|---|
| Females body mass [g] | Deliveries/group at birth | Individual pup mass [g] |
| mating | Litter size distribution | Birth |
| Three weeks after mating | Number of pups | 7/14/21/28/35/42 d after birth |
| at delivery | at birth/pair | |
| 1 week after delivery | at birth/group | |
| 2 weeks after delivery | at weaning/ pair | |
| 3 weeks after delivery | at weaning/ group | |
| | Index of libido | |
| | (number of mated/number of paired) x 100 | |
| | Quantal pregnancy | |
| | (number of pregnant/number of mated) x 100 | |

**Table 3:Effect of 20% Bt cotton seeds in diet on growth rate (g/day/100g bw) of female albino rats of F0, F1, F2 generation as compared to control and Non-Bt group**

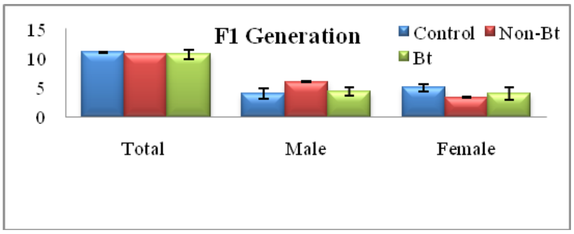
| Traits | F0 | | | F1 | | | F2 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Non-Bt | Bt | Control | Non-Bt | Bt | Control | Non-Bt | Bt |
| Mating | 0.92 ± 0.76 | 0.94 ± 0.02 | 1.11± 0.04 | 1.13± 0.04 | 1.09± 0.03 | 1.08 ± 0.04 | 0.66± 0.27 | 1.29 ± 0.25* | 1.07±0.15* |
| One week after mating | 0.43 ± 0.01 | 0.72± 0.07 | 2.01± 0.53* | 1.75± 0.22 | 2.01± 0.18 | 2.09 ± 0.38 | 1.88± 0.14 | 1.78± 0.35 | 1.27± 0.32 |
| Two week after mating | 0.42 ± 0.77 | 1.01± 0.18* | 1.68± 0.39* | 0.78± 0.09 | 0.53± 0.18 | 0.84 ± 0.14 | 2.51± 0.22 | 1.78± 0.36 | 1.73± 0.23 |
| Three week after mating | 3.46 ± 0.07 | 3.06± 0.96 | 0.87± 0.07* | 1.26± 0.73 | 2.50±0.57* | 2.78±0.49* | 1.81± 0.66 | 1.77± 0.82 | 2.36±0.65* |
| Delivery | 3.13 ± 0.10 | 3.27± 0.11 | 2.69± 0.53 | 1.44± 0.50 | 1.51± 0.23 | 1.13 ± 0.29 | 3.25± 0.63 | 0.83± 0.36* | 2.22± 0.35 |
| One week after Delivery | 0.44 ± 0.01 | 0.448± 0.17 | 2.11± 0.47* | 0.93± 0.42 | 0.62± 0.39 | 1.13 ± 0.40 | 1.07± 0.03 | 1.83± 0.30* | 1.10±0.19 |
| Two week after Delivery | 0 | 1.88± 0.31* | 1.89± 0.32 | 1.47± 0.30 | 0.49±0.24* | 2.87±0.95* | 0.65±0.09 | 1.34± 0.30* | 0.86± 0.18 |
| Three week after Delivery | 0.43 ± 0.56 | 1.70± 0.46* | 1.30± 0.15* | 0.96± 0.16 | 0.67±0.22 | 0.62 ± 0.17 | 2.04± 0.17 | 1.13± 0.17* | 1.60±0.41* |

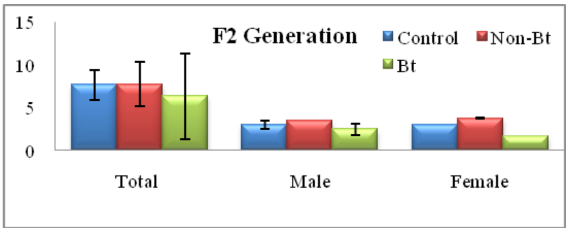
**Values are Mean ± SE**
***Significant difference at (p≤0.05) as compared to control**

**Table 4: Reproduction data of rat fed 20% Bt cotton seed or Non-Bt cotton seeds in their diet along with control group over several generations**

| Traits | F1 | | | F2 | | | F3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Non-Bt | Bt | Control | Non-Bt | Bt | Control | Non-Bt | Bt |
| Pairs/group | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Deliveries/group | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 5 |
| Non Deliveries/ group (%) | - | - | - | - | - | - | - | - | 16.66 |
| Number of pups at birth/pair | 11 ± 1.09 | 10.66±0.83 | 10.66±0.38 | 7 ± 1.20 | 7 ± 1.15 | 6.33±1.12 | 7 ± 6.75 | 9.33 ± 1.01 | 7.16 ± 0.89 |
| Sum of pups at birth/group | 66 | 64 | 64 | 42 | 41 | 38 | 42 | 56 | 43 |
| Number of pups at weaning/pair | 9 ± 0.89 | 9 ± 0.66 | 8.33 ± 0.38 | 6 ± 0.66 | 5.83 ± 1.06 | 4.83 ± 0.79 | 5.33 ± 1.00 | 5 ± 1.002 | 4.5 ± 1.17 |
| Sum of pups at weaning/group | 54 | 54 | 50 | 36 | 35 | 29 | 32 | 30 | 36 |
| Index of libido (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Quantal pregnancy (%) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 83.33 |

**Values are Mean ± SE**



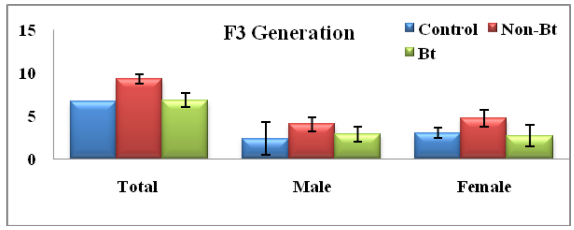




**Fig. 1: Effect of Bt cotton seeds on litter size and sex ratio of pups born**

**Table 5: Individual pup %growth rate (g/day/100g bw) at several timepoints of F1, F2 and F3 generations after birth of offsprings from parental rats fed 20% Bt cotton seeds or Non-Bt cotton seeds in their diet along with control group**

| Parameters | F1 | | | F2 | | | F3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Control | Non-Bt | Bt | Control | Non-Bt | Bt | Control | Non-Bt | Bt |
| 7th Day | 16.69±0.09 | 15.58±0.45 | 14.07 ± 2.66 | 14.29 ± 1.33 | 16.87±3.00 | 14.11±2.88 | 14.19 ± 2.45 | 14.34±0.01 | 15.59±0.45 |
| 14th Day | 9.89 ±0.67 | 13.50±1.22 | 16.33± 2.33* | 17.20 ± 2.54 | 12.26±2.12 | 26.05±1.90* | 13.60 ± 0.44 | 12.52±0.01 | 18.13±2.90* |
| 21st Day | 7.14 ± 0.67 | 6.96 ± 1.56 | 7.90 ± 2.90 | 9.55 ±1.90 | 7.07 ± 2.34 | 10.76±1.67 | 9.91 ± 0.06 | 4.81 ± 1.09* | 7.09±0.01 |
| 28th Day | 12.12 ±1.88 | 10.93±0.44 | 12.56 ± 2.90 | 7.19 ± 3.87 | 6.83 ± 1.90 | 5.89 ± 0.54 | 7.39 ± 0.56 | 6.55 ± 0.45 | 7.07 ± 0.78 |
| 35th Day | 7.12 ± 0.45 | 6.29 ± 0.56 | 3.62 ± 0.80* | 3.27 ± 0.01 | 6.40±2.67* | 7.14±0.01* | 3.71 ± 0.08 | 12.77±2.99* | 3.86±0.09 |
| 42nd Day | 3.08 ±0.56 | 3.62 ± 0.78 | 10.38± 0.56* | 5.49 ± 0.56 | 4.52±0.06 | 5.11±0.08 | 4.41 ± 0.98 | 9.58 ± 0.01 | 7.25±0.01* |

**Values are Mean ± SE**
***Significant difference at ($p \leq 0.05$) as compared to control**

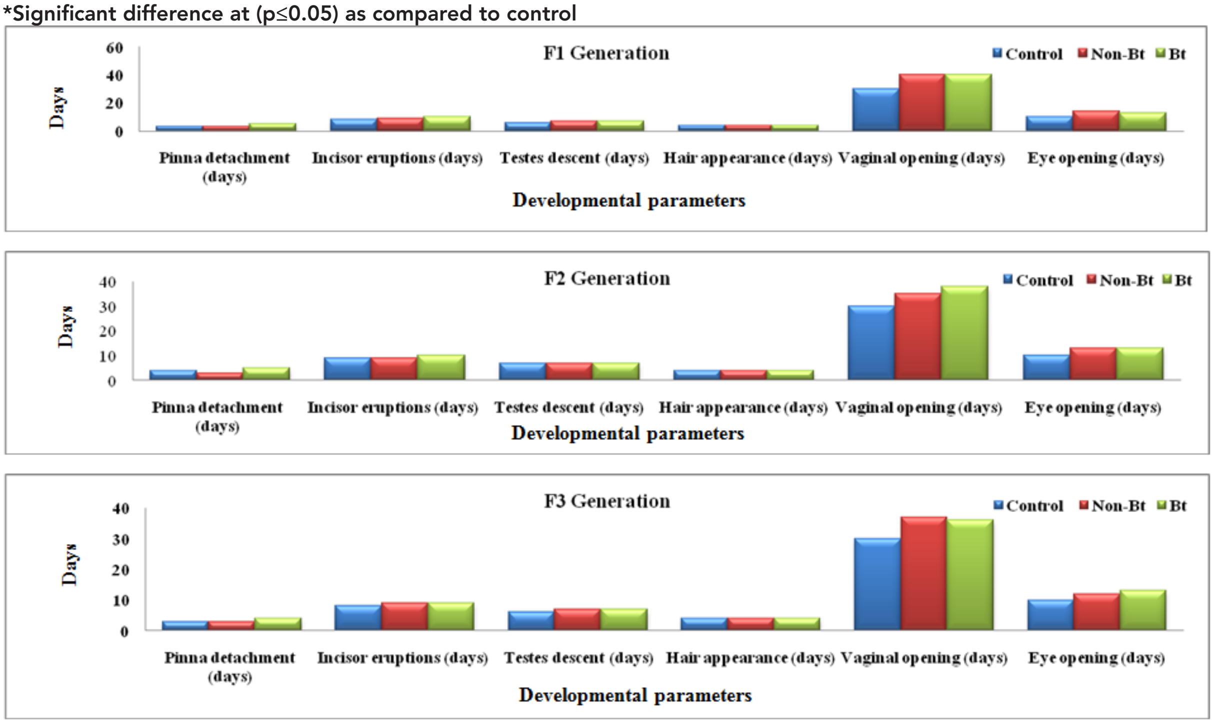



**Fig. 2: Effect of Bt cotton seeds on some developmental aspects of pups born in all the three generations**

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