Report

on

Effect of feeding cottonseed produced from cotton hybrids carrying Cry1C gene on feed intake, milk production and composition in lactating crossbred dairy cows

By

Dr. K.K. Singhal
Dr. A.K. Tyagi
Dr. Y.S. Rajput &
Dr. Shiv Prashad

National Dairy Research Institute
Karnal, 132001, India

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Laboratory	Dairy Cattle Nutrition Division National Dairy Research Institute, Karnal
Principal Investigator	Dr. K.K. Singhal
Designation	Principal Scientist & Head
Signature	Dr. K.K. Singhal
Date	23.2.08

Statement of particular of Study

Title	**Effect of feeding cottonseed produced from cotton hybrids carrying Cry1C gene on feed intake, milk production and composition in lactating crossbred dairy cows**
Facility	Dairy Cattle Nutrition Division National Dairy research Institute Karnal 132 001, India Fax Email: k_ksinghal@yahoo.co.in
Study Director	Dr. K.K. Singhal
Co-study Director	Dr. A.K. Tyagi Dr. Y.S. Rajput Dr. Shiv Prashad
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	Date
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Abbreviations

ADF	Acid detergent fibre
ad lib.	*Ad libitum*
BCS	Body Condition Score
Bt	*Bacillus thuringiensis*
?C	degree Celsius
FCM	Fat corrected milk
DM	Dry matter
g	gram
GM	Genetically modified
l	liter
kg	Kilogram
KF	Karn Friasian
KS	Karn Swiss
mg	milligram
NDF	Neutral detergent fibre
NDRI	National Dairy Research Institute
SNF	Soil not fat
?g	microgram

ABSTRACT

Twenty crossbred (KS and KF) multiparous cows, housed in well ventilated sheds, were fed individually on concentrate mixture consisting of crushed cottonseed (Non Bt) 40 parts, groundnut cake 10 parts, maize 25 parts, wheat bran 22 parts, common salt 1 part and mineral mixture 2 parts along with green maize and berseem (*Trifolium alexandrian*um) fodders *ad libitum* according to their nutritional requirements for 15 days to adapt them to the cottonseed based diet. Thereafter, cows were divided in two groups of 10 each on the basis of their lactation number, stage of lactation and milk yield. Group 1 was continued on the same ration and designated as Non Bt (control) group while in the concentrate mixture of group 2, Non Bt cottonseed was replaced with Bt cottonseed and designated as Bt group. Average milk yield during adaptation period of 15 days in the cows assigned to Non Bt and Bt group was 11.40 and 11.2 kg, respectively. Milk yield and voluntary feed intake were recorded daily while milk samples were collected at the start of experimental feeding and thereafter at weekly intervals during the four week experimental period for the analysis of milk composition and to test for the presence of Bt proteins. At the end, a blood sample from each cow was collected and plasma was separated to test for the presence of Cry 1C protein. Cry1C protein in cottonseed, milk and blood samples was measured by ELISA method. The amount of Cry1C protein in Bt cottonseed was 2.44 ?g/g on fresh basis respectively. Corresponding values in Bt concentrate mixture was 0.976 ?g/g on fresh basis. Concentrate mixture provided to the Non Bt and Bt groups were isonitrogenous. Mean voluntary DM intake /100 kg body weight was 3.48 in Non Bt group and 3.45 in Bt group and there was no significant difference between the groups. Cows in both the groups improved their body weight during the study period and body weight gain in Bt group was higher than that in Non Bt group. Average milk yield in Non Bt (11.4 kg/day) and Bt (12.0 kg/day) groups was not different. Mean fat, protein lactose, SNF and total solids contents in the milk of control group was 4.70%, 3.13, 4.68, 8.64 and 13.35%, respectively and the corresponding value in Bt group was 4.74, 3.12, 4.69, 8.59 and 13.34%. Milk composition was similar in both the groups The 4% fat-corrected milk (4%FCM) and 4%FCM/kg DM intake in Non Bt (12.56 kg/day; 0.929, respectively) and Bt (12.0 kg/day; 0.970, respectively) groups were also not varied significantly between the groups. Cry1C protein was not detected in milk

samples, drawn at 0, 7, 14, 21 and 28 day of feeding the experimental diet, as well as in plasma samples drawn on day 28 from the cows fed the Bt cottonseed based ration. Lactating dairy cows of both the groups did not show symptoms of any disease, maintained their health and performed in a similar fashion when fed Non Bt and Bt cottonseed as a source of energy and protein supplement during the four-week long experimental period. Milk production of all the 20 cows remained uninfluenced after their shifting on normal cottonseed based ration under similar housing and feeding management system.

INTRODUCTION

Cottonseed is a traditional protein and energy supplement in the ration of lactating cows and buffaloes. Cotton is prone to the lepidopteran insect pests, which causes extensive damage to the crop and results not only in financial losses to the farmers but reduces the much needed cottonseed for the feeding of dairy animals and oil extraction for human consumption. In order to provide resistance to insect damage, genetically modified (GM) cotton has been developed. GM cotton, having an insect tolerant trait with gene coding for Cry 1 Ac protein derived from *Bacillus thuringiensis* var. Kurstaki, has been introduced in India for large scale production after evaluation of its safety aspects including that of feeding to the dairy cows (Singhal *et al.*, 2001), who reported the absence of Bt protein in the milk as well as in blood plasma of cows fed on Bt cottonseed and the milk production as well as feed intake were similar to those fed on non Bt cottonseed based diet. Similar observations were recorded by Singh *et al.* (2002) in lactating buffaloes following the feeding of Bt cottonseed based ration in buffaloes. Feeding of Bt corn grain and corn silage (Folmer *et al.*, 2001) based diets also did not have any adverse effect on milk production performance of dairy cows. Singh *et al.*, (2003) concluded that feeding of transgenic (Cry I Ac) and non-transgenic cottonseed based rations to the lactating Murrah buffaloes did not have any adverse effects on their feed intake and health status as assessed from haemato-biochemical constituents. Another protein (Cry2Ab), derived from the naturally occurring soil bacterium *Bacillus thuringiensis* (Bt), has been safely used to control lepidopteran insect pests for more than 40 years (Betz *et al.*, 2000). Therefore, this insect controlling gene (Cry 2Ab) in addition to Cry 1Ac gene in cotton genome was also incorporated for effective and season-long control of key lepidopteran insect pests. Incorporation of cottonseed having Cry1Ac and Cry 2Ab genes in the ration of lactating cows was found safe for lactating cows and their milk production, which was comparable to those fed on isogenic cottonseed without these genes (Castillo *et al.*, 2004; Singhal *et al.* 2006a). Incorporation of foreign protein (Cry 1 Ac) in cottonseed neither affected the nutrient digestibility (Singh *et al.*, 2002; Singhal *et al.*, 2006b) nor the degradability of protein of cottonseed (Senthil Kumar and Singhal, 2002). Now another foreign protein (Cry1C) has been incorporated into genome of cotton to make it insect resistant and it will be designated in this communication as Bt

cottonseed. It is essential to investigate the biosafety of the new GM feed ingredients, therefore, efforts have been made in present study to compare the effect of feeding of Bt cottonseed and near isogenic Non Bt cottonseed on biosafety of lactating cows and also their influence on milk production performance before commercial application of cotton having Cry1C protein.

MATERIALS AND METHODS:

Twenty multiparous crossbred cows (KS and KF), maintained under loose housing system and fed on a groundnut cake based concentrate mixture as per their milk yield and green maize fodder *ad lib.*, were selected from the general herd of National Dairy Research Institute, Karnal. Details of lactation number (Parity), stage of lactation and body weight of these cows are given in Table 1.

Table1. Details for the crossbred cows selected for the study

Animal No.	Parity	Days in Milk	B. wt. (kg)	Animal No.	Parity	Days in Milk	B. wt. (kg)
	Non Bt Group			Bt group			
KS 4316	4	197	302	KS 4353	2	96	426
KS 4345	2	187	382	KF 5818	4	229	485
KS 4359	3	114	438	KF 6205	4	178	335
KF 6367	4	122	379	KF 6215	3	227	497
KF 6429	2	236	416	KF 6223	4	114	409
KF 6441	2	122	413	KF 6245	2	200	334
KF 6476	2	191	418	KF 6574	2	235	311
KF 6481	2	101	429	KF 6624	2	123	363
KF 6537	2	168	352	KF 6698	1	118	339
KF 6568	1	236	352	KF 6755	1	65	407
Average ± SE	**2.4 ± 0.31**	**167.4 ± 15.85**	**388.1 ± 12.89**	**Average ± SE**	**2.5 ± 0.37**	**158.5 ± 19.80**	**390.6 ± 19.54**

Housing of Animals:

All cows, having a unique identification number tattooed on their hip region as well as in their ears, were shifted to a well ventilated shed having *pacca* floor and arrangement for protecting the animals from cold individual feeding (Fig. 1). Cows were individually tied with ropes during the two-week adaptation period and the four-week experimental period enabling individual feed recording. To monitor the DM intake for individual cow, arrangements were made for individual feeding of each cow (Fig. 2). Maximum and minimum temperature in the shed during the study period was recorded daily (**Appendix I**).

Fig. 1. Housing of the experimental cows

Feeding of Animals:

Initially, all the cows were maintained on the concentrate mixture having groundnut cake as protein supplement for four days for their adaptation to the changed housing system. Concentrate mixture was replaced gradually with that containing crushed cottonseed (Delinted Non Bt, supplied by Metahelix Life Sciences Pvt. Ltd., Bangalore in crushed form). Thereafter, cows were switched over to the cottonseed based concentrate

mixture completely. In addition to the concentrate mixture, given as per nutritional requirements recommended by NRC (2001), green berseem (*Trifolium alexandrinum*) and maize fodders were fed *ad libitum* to each cow.

Fig. 2. Arrangements for individual feeding of crossbred cows

Feeding of crushed cottonseed (Non Bt) based concentrate mixture continued for 15 days for the adaptation of rumen microbes for the changed nutrient supplement. Thereafter, the cows were distributed in two groups of 10 each in a manner so that each group was having similar lactation no., days in milk and daily milk yield. Both the groups were housed in separate shed having the arrangements for individual feeding and protection from the cold weather. Control group, continued to receive the crushed Non Bt cottonseed based concentrate mixture, while in treatment group (Bt) it was replaced with similarly processed Bt cottonseed, supplied by the same firm Composition of both types of concentrate mixtures containing Non Bt and Bt cottonseeds is given in Table 2.

Preparation of Concentrate Mixture:

Consignments of delinted and crushed cottonseeds (Non Bt and Bt) received from Metahelix Life Sciences Pvt. Ltd., Bangalore were stored in a go down (well ventilated

store room) safely. The other ingredients, received from Central Store of NDRI were crushed using hammer mill in a feed unit. Non Bt and Bt cottonseeds, both were checked for the presence of Cry1C proteins using ELISA test prior to the preparation of concentrate mixtures. The ingredients of concentrate mixture were mixed homogeneously using a mixer and both types of concentrate mixtures were stored safely. Freshly chopped green maize fodder, green berseem fodder and wheat straw were supplied by Farm section of the institute, daily.

Table 2. Ingredient composition of concentrate mixture

Name of ingredient	**parts**
Maize grain	25
Cottonseed (Non Bt or Bt)	40
Groundnut cake (Expeller pressed)	10
Wheat bran	22
Common salt	1
Mineral mixture	2

Fig.3. Appearance of concentrate mixture based on Bt cottonseed

Feeding schedule:

Each cow in both groups was offered weighed amount of chopped maize fodder and berseem fodders three times a day i.e. 5 am, 12 noon and 5.30 pm in addition to 2 kg wheat straw, which was offered at 8.00 am. Known quantity of respective concentrate mixture was provided to each cow to fulfill their nutritional requirements according to NRC (2001). Total quantity of concentrate mixture was divided in three equal parts, which were given prior to each milking. Left over concentrate mixture, if any, and residual fodder as well as wheat straw of individual cow was weighed next morning. DM content of fodder as well as left over was determined to calculate the DM intake. Fresh and clean water was provided free choice to each cow three times a day.

Milking Schedule:

Cows were milked three times a day i.e. 5 am, 12 noon and 6 pm daily by employing full hand milking and milk yield for individual cow was recorded at each milking.

Sampling of Milk

Milk sample from each cow were collected on 0, 7, 14, 21 and 28th day of initiating the experimental feeding. Milk samples consisted milk of each milking in proportion of milk yield of individual cow.

Health Status:

Health of all the experimental cows was monitored daily by a veterinarian during the course of study. Body weight of each cow was recorded prior to their feeding and watering in the morning before starting the experimental feeding and was again recorded after completion of four weeks of experimental feeding.

Disposal of Milk:

Total milk collected at each milking from the cows fed the Bt cottonseed was discarded in a pit which was inaccessible to the animals including birds and dogs.

Blood Sampling:

A blood sample from each cow was collected on the last day of experimental feeding period (day 28) by puncturing the jugular vein in a heparinized tube. Plasma was separated and stored till its analysis for the presence of Bt protein.

Analysis of Samples:

Feed Samples:

Each type of cottonseed was sampled from the lot provided by Metahelix Life Sciences Pvt. Ltd., Bangalore and preserved in polyethylene bags. Representative samples of both types of crushed cottonseed and their respective concentrate mixtures were analyzed for dry matter, crude protein, ether extract, total ash (AOAC, 1995), neutral detergent fibre (NDF) and acid detergent fibre (ADF) as per Goering and van Soest (1967). Pooled dry samples (28 days) of green maize, berseem and feed refusals were also analyzed for the aforementioned proximate and fibre fractions. In addition, control and Bt cottonseeds were also analyzed for quantitatively estimation of the Cry1C protein using ELISA test and for free gossypol content as per Smith (1968).

Milk and blood plasma samples:

Milk samples were analyzed for fat, protein, total solids, lactose, and solids-not-fat (SNF) using precalibrated Milk Analyzer (LactoStar, FUNKE GERBER, Article No 3510, Berlin). All the milk samples, collected on particular day, were frozen in liquid nitrogen and stored at -20^0C prior to Cry1C protein determination. Blood plasma samples, collected on day 28 of experimental feeding were also analyzed for the presence of Bt proteins using the ELISA kit provided by Metahelix, Bangalore. Values of Bt protein were obtained as per the following procedure.

Estimation of Cry 1C

The level of Cry 1C protein in blood or milk was estimated using QuantiPlate kit from M/s Envirologix (supplied by M/s Metahelix Life Sciences Pvt. Ltd. Bangalore). Standards at 1, 5 and 10 ppb Cry 1C were used in every assay. The 96 well plates were precoated with anti-Cry 1C antibodies. Milk samples, blood samples and crushed Bt- and non Bt- cottonseeds were treated with extraction buffer for extraction of Cry1C protein. Milk samples were diluted 1X and 5X with extraction buffer. Blood samples were diluted 5X, 10X and 20X with extraction buffer. Cottonseeds (10 mg) were put into Eppendorf tube and then 500 µl of extraction buffer was added. The contents were vortexed for 2 minutes and centrifuged (7000 rpm, 5 min.). Undiluted supernatant at 5 and 10 fold dilutions (with extraction buffer) were used for estimating Cry1C protein.

The supplied ELISA kit uses 96 well plates precoated with anti-Cry1C.

- 100 µl of sample (cotton seed extract or diluted blood or diluted milk or Cry 1C standard) were added to wells of multi-well plate.
- After 1 hour, 100 µl of anti-Cry1C antibodies-enzyme conjugate were added. The contents were mixed. The plate was incubated at room temperature (25^{o} C) for 1 hour.
- The contents from well were removed; each well of plate was washed with wash buffer. The washing was repeated three times.
- 100 µl of substrate was added to each well. After 30 min., 100µl of stop solution was added.
- Absorbance was recorded at 450 nm.
- Standard curve was plotted and concentrations of Cry1C in samples were calculated.

Statistical Analysis:

Statistical analysis of daily dry matter intake, daily milk yield as well as milk composition determined at various intervals was done as per student's 't' test (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Change in body weight of cows during experimental period

Body weight of cows were recorded before starting the experimental feeding as well as at the end of trial period and it was found that there was an increase in body weight of each, irrespective of the group (Table 3). Average body weight gain in cows fed on non Bt cottonseed based ration was 0.8 kg, whereas the increase in cows fed on Bt cottonseed based ration was to the tune of 16.4 kg during the experimental period of 4 weeks. Higher body weight gain/ day in Bt group (0.580 g) than in Non Bt group (0.028 g) may be due to the high energy density of Bt cottonseed based ration due to the higher EE content in it as a result of incorporation of Bt cottonseed which was having higher EE content than that of Non Bt cottonseed (Table 4). These

results revealed that body weight and appearance of the cows were not affected by the incorporation of Bt cottonseed in the ration of lactating cows.

Table 3. Change in body weight of crossbred cows assigned to Non Bt and Bt groups.

Animal No.	Non Bt Group		Animal No.	Bt Group	
	Initial B. Wt (kg)	final B. wt (kg)		Initial B. Wt (kg)	Final B. Wt (kg)
KS 4316	302	302	KS 4353	426	416
KS 4345	382	391	KF 5818	485	471
KS 4359	438	451	KF 6205	335	343
KF 6367	379	390	KF 6215	497	501
KF 6429	416	420	KF 6223	409	435
KF 6441	413	407	KF 6245	334	383
KF 6476	418	430	KF 6574	311	325
KF 6481	429	404	KF 6624	363	385
KF 6537	352	350	KF 6698	339	367
KF 6568	352	344	KF 6755	407	444
AVG±SE	**388. 1±12.89**	**388.9±13.48**	**AVG±SE**	**390.6±19.54**	**407.0±16.93**

Chemical composition of feeds

The chemical composition of Bt and Non Bt cottonseeds, respective concentrate mixtures, berseem and maize fodder has been presented in Table 4. Oil content in Bt cottonseed was higher than that of Non Bt cottonseed. CP content as well as the cell wall constituents were similar in both types of cottonseeds. The Cry1C protein in Bt cottonseed was 2.44 µg/ g on fresh basis and it was not detected in Non Bt cottonseed. The gossypol content in Bt and Non Bt cottonseed was 0.182 and 0.160 %, respectively. The chemical composition of concentrate mixtures containing Non Bt and Bt cottonseeds was identical and quite close to the specifications given by Bureau of Indian Standards for Type I cattle feed (BIS, 1975); however, their ether extract content was higher than the recommended value as a result of high level of oil rich cottonseed (40%) in the concentrate mixtures. This level of cottonseed was maintained in the concentrate mixture to supply a minimum 2 kg of cottonseed intake per cow per day. Singhal *et al.* (2006a) also recorded higher ether extract content in

concentrate mixture following the replacement of Non Bt cottonseed with Bt cottonseed and similar to present observations they found that the EE content of Bt cottonseed was higher than that of Non Bt cottonseed.

Table 4. Chemical composition of cottonseeds, concentrate mixtures based on them and green fodder (% DM basis)

Attributes	Conc. Mix.		Cottonseed		Green maize	Berseem fodder	Wheat Straw
	Bt	Non Bt	Bt	Non Bt			
DM	90.19	90.67	92.19	92.71	18.24	11.04	93.84
Organic matter	93.64	94.31	95.99	95.81	91.27	80.81	91.38
Ash	6.36	5.69	4.00	4.19	8.73	19.19	8.62
Crude protein	22.90	23.36	26.74	27.03	7.33	23.36	3.25
Ether extract	9.60	5.76	22.84	17.23	0.46	3.16	0.50
Crude fiber	11.38	10.74	17.44	20.02	26.68	17.04	43.23
NDF	30.38	27.45	35.04	36.86	52.45	38.97	72.28
Calcium (%)	0.99	1.10	1.34	1.39	ND	ND	ND
Phosphorus (%)	0.51	0.54	0.42	0.39	ND	ND	ND
Cry1C (? g/g)*	0.976	-	2.44	-	-	-	-
Free Gossypol (%)*	0.073	0.064	0.182	0.160	-	-	-

* On fresh basis, ND Not determined

Cry1C protein in the test concentrate mixture was calculated to be 0.976 µg/ g on DM basis. Gossypol content of Bt and non Bt cottonseed based concentrate mixture was 0.073 and 0.064 %, respectively. Gossypol content of both types of cottonseeds was within the range reported by Senthil and Singhal (2004) in Bt and Non Bt cottonseed. Similar range of gossypol in various varieties of cottonseed was also reported by Mujahid *et al* (2000). Proximate composition of maize and berseem fodders as well as wheat straw was similar to that reported by Sen and Ray (1977).

FEED INTAKE AND MILK PRODUCTION PERFORMANCE OF COWS

Adaptation period

Data on feed intake and milk yield in Non Bt and Bt groups during adaptation period of 2 weeks are presented in Table 5.

During the adaptation period, total DM intake in groups, later on assigned to Non Bt and Bt was 14.0±0.2 and 14.1 ± 0.1 kg/day, respectively, and the average concentrate to roughage ratio in corresponding groups was 1.86:1 and 1.87:1. Total DM intake/ 100 kg body weight in Non Bt and Bt groups was 3.59 and 3.60 kg, respectively. Milk yield in corresponding groups was 11.40 ± 0.90 and 11.20 ± 0.80 kg/ day. Statistical analysis of data revealed non-significant difference between the groups for the milk yield as well as DM intake. Lower DM intake and milk yield were recorded when crossbred lactating cows were fed a ration containing similar levels of Non Bt cottonseeds (Singhal *et al.*, 2001) either with berseem (*Trifolium alexandrinum*) or chopped maize fodder (Singhal *et al.*, 2006b) *ad libitum,* which may be attributed to the stage of lactation of the experimental cows, season of the feeding trial or the quality of fodder. Milk yield/ kg DM intake in groups, later on assigned to the Non Bt and Bt cottonseed based rations was 0.815 and 0.795, respectively, and the values were comparable to those reported by Singhal *et al.* (2001) in lactating crossbred cows.

Table 5. Performance of cows to be allotted to Non Bt and Bt cottonseed based diets during adaptation period

Parameter	**Non Bt (Control)**	**Bt**
Av. B. Wt. (kg).	388.1 ± 13.48	390.6 ± 19.54
DM intake through Conc. mix (kg/day)	4.89	4.89
DM intake through green fodder (kg/day)	8.00 ± 0.15	8.02 ± 0.14
DM intake through straw (kg/day)	1.09 ± 0.05	1.17 ± 0.06
Roughage: concentrate	1.86:1	1.87:1
Total DM intake (kg/day)	13.98 ± 0.15	14.08 ± 0.146
Dry Matter intake/ 100 kg B. Wt (kg)	3.59	3.60
Milk yield (kg/day)	11.4 ± 0.9	11.2 ± 0.80
4% FCM yield (kg/day)	12.59 ± 1.06	12.49 ±0.90
Milk yield/ kg DM intake	0.815	0.795

Each value is an average of 10 observations

Experimental period

DM intake and milk production performance of cows on shifting them to Non Bt and Bt cottonseed based ration are presented in Table 6.

It was recorded that the average DM intake was 13.51 ± 0.19 kg/ day in Non Bt and 13.75 ± 0.16 kg/ day in Bt group and the variation between the groups was not significant. In general, lower DM intake was recorded during experimental period than in adaptation period, which may be attributed to the maturity of maize fodder and lower DM content of berseem fodder. DM intake/ 100 kg b. wt in Non Bt and Bt cottonseed fed groups was 3.48 and 3.45 kg, respectively. These values were lower than those recorded during adaptation period in both the groups. However, variation between the groups was not significant and DM intake in both groups was enough to fulfill the nutritional requirement recommended by NRC (2001). It was recorded that DM intake/ 100 kg body weight was 36% higher than the recommended value (NRC 2001) but lower than those recorded by Castillo *et al.* (2004) in lactating cows and this may be attributed to the type of animal and the difference in the feedstuffs. Folmer *et al.* (2001) reported that there was no difference of feeding Bt and Non Bt corn or its silage on DM intake when both these ingredients constituted 38% of total ration. Anderson *et al.* (1979) studied the effect of whole cottonseed feeding (20%) of ration during 4 week period on DM intake and milk production in cows under the conditions similar to the present experiment and reported that the DM intake was 2.74 to 2.80 kg/ 100 kg body weight, however, in present study DM intake was higher than that recorded by Anderson *et al.* (1979), possibly due to the better quality of fodder and cold climate, which encourages the animals to consume more to compensate the energy loss in maintaining the body temperature. In present study cottonseed constituted 16% of the total DM consumed in both the groups.

Roughage to concentrate ratio in Non Bt and Bt groups was 1.70:1 and 1.75:1, respectively. Though this ratio was narrowed down during the experimental period in comparison to the adaptation period, obviously due to the lower consumption of DM from roughages, however, variation between the groups was not significant. Similar DM intake under the similar roughage to concentrate ratio were recorded in crossbred

lactating cows by Singhal *et al* (2001 & 2006) and Thakur *et al.* (2005). Singh *et al.* (2003) reported that DM intake/ 100 kg body weight in lactating buffaloes was 3.64 and 3.52 kg on feeding Non Bt and Bt cottonseed based ration, which were similar to those recorded in present study. Average milk yield/ day in Non Bt and Bt group was 11.4 ± 1.0 and 12.0 ± 0.9 kg, respectively and the variation between the groups was not significant. On comparing the milk production performance of cows during adaptation and experimental period it was observed that Milk yield/day was improved on replacing the Non Bt cottonseed with Bt cottonseed during the experimental period, but the improvement was not significant.

It was also recorded that the average intake of Cry1C protein through Bt cottonseed was 5.4 mg/ day, which appeared to be degraded during the course of its rumen fermentation as most of the protein gets degraded during their digestion in rumen. Senthil and Singhal (2004) reported that Bt protein (Cry1Ac), present in GM cottonseed, did not affect the rumen fermentation *in vitro*, indicating that it got degraded in the rumen. The average intake of free gossypol in Non Bt and Bt cottonseed was 3.9 and 3.4 g/day, respectively, which appeared to be not affecting the milk production performance of lactating crossbred cows.

Table 6. Performance of crossbred cows fed on Non Bt and Bt cottonseed based diets during experimental period

Parameter	Non Bt (Control)	Bt
Av. B. Wt. (kg).	388.5	398.8
DM intake through Conc. mix (kg/day)	5.00	5.00
DM intake through green fodder (kg/day)	7.35 ± 0.19	7.36 ± 0.17
DM intake through straw (kg/day)	1.16 ± 0.05	1.39 ± 0.03
Roughage: concentrate	1.70:1	1.75:1
Total DM intake (kg/day)	13.51 ± 0.19	13.75 ± 0.16
Dry Matter intake/ 100 kg B. Wt (kg)	3.48	3.45
Milk yield (kg/day)	11.4 ± 1.0	12.0 ± 0.90
4% FCM yield (kg/day)	12.56 ± 1.05	13.35 ± 0.90
Milk yield/ kg DM intake	0.84	0.87

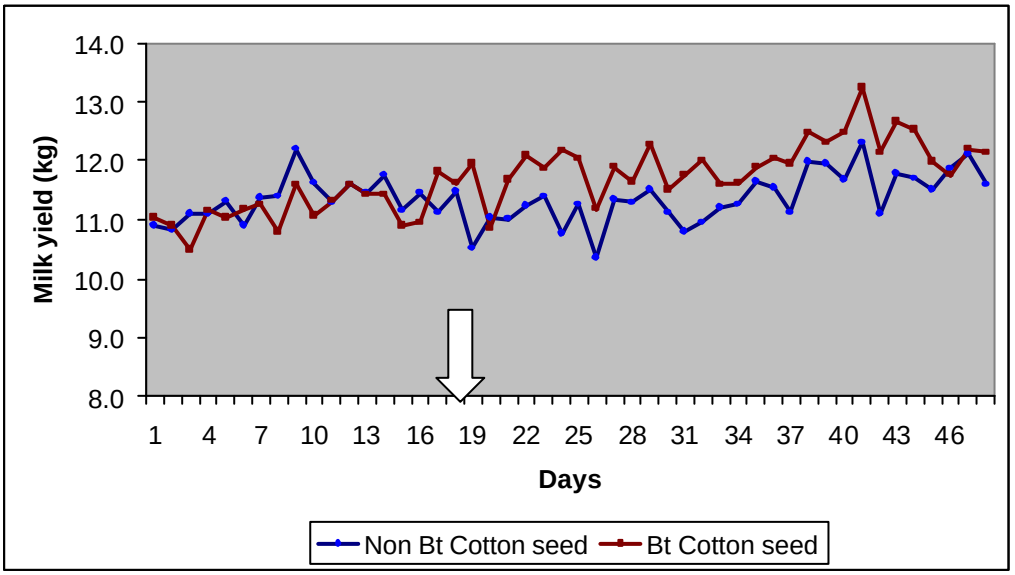
Each value is an average of 10 observations

Milk production efficiency in terms of milk yield/ kg DM intake in Non Bt and Bt groups was 0.84 and 0.87, respectively. Milk production efficiency was improved during experimental period than that recorded during the adaptation period, but the variation between periods was not significant. In an earlier experiment, Singhal *et al.* (2001) reported that the milk yield / kg DM intake was similar when cows were fed Bt as well as non Bt cottonseed based ration, however, milk production efficiency in terms of milk produced per kg DM consumed was 0.72 kg, which was far lower than the values recorded in present experiment. Better DM utilization in the present experiment may be attributed to the early stage of lactation of the experimental cows.

Milk Production performance

Record of milk yield of each cow was maintained during adaptation period and experimental period and presented graphically in Fig.4. Milk yield of cows during the adaptation period was very close as all the cows were fed on similar ration having Non Bt cottonseed for another 20 days during post experimental period to study any carry-over effect on the productivity of the cows. The daily milk yield during adaptation period, experimental period and post experimental period in Bt and Non Bt groups have been presented in Fig 4.

Fig. 4 Average milk yield (kg) in Non Bt and Bt cotton seed based diet in cross bred cows during adaptation and experimental period



Arrow indicated the day of starting the experimental feeding

Milk composition

The composition of milk samples drawn on 0, 7, 14, 21 and 28th day of experimental feeding in Bt and Non Bt groups are presented in Table 7.

Table 7. Composition of milk samples drawn at various intervals

Date	Non Bt group	Bt group
FAT (%)		
19th Dec.	4.78 ± 0.25	4.93 ± 0.15
26th Dec	4.81 ± 0.15	4.79 ± 0.05
1st Jan	4.60 ± 0.23	4.72 ± 0.22
8th Jan.	4.85± 0.27	4.78 ± 0.11
17th Jan.	4.48 ± 0.22	4.56± 0.16
Avg	**4.7**	**4.75**
PROTEIN (%)		
19th Dec.	3.11 ± 0.06	3.19 ± 0.07
26th Dec	3.12 ± 0.21	3.13 ± 0.28
1st Jan.	3.12 ± 0.32	3.10 ± 0.11
8th Jan.	3.27 ± 0.03	3.21 ± 0.02
17th Jan.	3.0 ± 0.03	2.99 ± 0.03
Avg	**3.13**	**3.12**
LACTOSE (%)		
19th Dec.	4.64 ± 0.08	4.76 ± 0.10
26th Dec	4.64 ± 0.20	4.67 ± 0.21
1st Jan.	4.69 ± 0.04	4.67 ± 0.34
8th Jan.	4.90 ± 0.05	4.80 ± 0.03
17th Jan.	4.52 ± 0.05	4.52 ± 0.05
Avg	**4.68**	**4.69**
SNF (%)		
19th Dec.	8.52 ± 0.16	8.61 ± 0.09
26th Dec	8.66 ± 0.15	8.64 ± 0.25
1st Jan	8.62 ± 0.25	8.57 ± 0.12
8th Jan.	8.97 ± 0.10	8.81 ± 0.07
17th Jan.	8.44 ± 0.11	8.34 ± 0.07
Avg	**8.64**	**8.59**
TOTAL SOLIDS (%)		
19th Dec.	13.30 ± 0.41	13.54 ± 0.21
26th Dec	13.53 ± 0.05	13.26 ± 0.25
1st Jan	13.19 ± 0.23	13.42 ± 0.20
8th Jan.	13.82 ± 0.35	13.59 ± 0.14
17th Jan.	12.92 ± 0.32	12.89 ± 0.20
Avg	**13.35**	**13.34**

Each value is the average of 10 observations ± SE

All the cows were milked three times-a-day by full hand milking as practiced during the course of the experiment.

Plan of Nutrition

NDF intake as percentage of total DM intake during experimental period in Non Bt and Bt groups was 38.63 and 38.11 %, which was similar in both groups and the level of NDF intake was as per recommendations of Mertens (1985) for high yielding cows. CP intake in Non Bt and Bt cottonseed based ration was 2.18 and 1.95 kg, respectively, which was similar to the recommendations of NRC (2001) indicating that the CP requirements of cows yielding about 12 kg milk fulfilled their nutritional requirements.

During the experimental period the milk composition in terms of fat, protein, lactose, SNF and total solids content in Non Bt groups was 4.70, 3.13, 4.68., 8.64, and13.35 % respectively. Corresponding values in Bt group was 4.75, 3.12, 4.69, 8.59 and 13.34%. Variation between the groups for each parameter was not significant. Presence of Bt protein (Cry IC) was not detected in the milk samples, collected at various intervals after incorporation of Bt cottonseed in the ration of cows. Statistical analysis of data revealed that milk composition was not altered by the sampling day or by the type of cottonseed.

Average milk composition in Non Bt (control) and Bt groups are presented in Table 7.

No milk sample, irrespective of day of sampling or cow, showed the presence of Cry1 C proteins. These observations were similar to that reported by Singhal *et al.*, (2001) while feeding Bt cottonseed, Castillo *et al.* (2001) while feeding BG II cottonseed and Folmer *et al.* (2002) on feeding Bt corn silage to lactating cows. The SCC values were within the normal range reported by Nazem *et al.* (1998), irrespective of the type of cottonseed or sampling interval. Singhal *et al.* (2001) also reported similar observations in crossbred cows fed on Bt and Non Bt cottonseed for four weeks. Cry1C proteins were not detected in the blood plasma samples, drawn at the end of experimental feeding period. These results suggest that the Cry1C proteins in transgenic cottonseed did not have any adverse effect on crossbred cows. None of the milk samples, drawn at various intervals, or blood samples, drawn at the end of the four week feeding of Bt cottonseed were positive to these proteins. The lack of detection of these proteins suggested that the

proteins were digested in the GI tract and/ or were not absorbed across the intestinal mucosa.

Health status

In general, the cows maintained good health and their body weight was improved during the experimental period, which may be attributed to the feeding of cottonseed having high calorific value and berseem fodder *ad lib***.** Dutta and Dogra (1998) reported that feeding of transgenic cottonseed (Cry 1Ac) did not affect the body weight gain and health of goat kids Three cows in Non Bt group and 1 in Bt group exhibited the symptoms of mastitis. One cow in Bt group had fever. All these cows were given proper treatment immediately and recovered. Two cows in each group had some leg injury and proper dressing was done by veterinarian. Sixty percent of cows, assigned to Bt group and 50 % of cows in Non Bt group came into heat during experimental period and all these cows were inseminated artificially. Data on the animals exhibited the heat symptoms and the body condition score (BCS) of each cow have been presented in Table 8

Table 8. Heat detection and the body condition score of cows in Non Bt and Bt groups

Non Bt Group			Bt Group		
Animal No.	**BCS***	**Heat Detection**	**Animal No.**	**BCS***	**Heat Detection**
KS 4316	2.85	✍ ✍ ✍	KS 4353	3.71	✍ ✍ ✍
KS 4345	3.71	?	KF 5818	3.57	✍ ✍ ✍
KS 4359	4.78	✍ ✍ ✍	KF 6205	2.57	✍ ✍ ✍
KF 6367	3.64	?	KF 6215	3.78	✍ ✍ ✍
KF 6429	3.86	✍ ✍ ✍	KF 6223	3.07	✍ ✍ ✍
KF 6441	3.29	✍ ✍ ✍	KF 6245	3.07	?
KF 6476	3.43	?	KF 6574	3.14	?
KF 6481	2.86	?	KF 6624	4.71	?
KF 6537	3.93	✍ ✍ ✍	KF 6698	4.07	✍ ✍ ✍
KF 6568	4	?	KF 6755	3.64	?
Average ±SE	**3.64±0.18**		**Average ±SE**	**3.53 ± 0.19**	

*On a scale of 6 as per Prashad (1994)

Post experimental period

After the experimental period of four weeks, cows were maintained in the same sheds under the similar feeding and milk ing management except that all the cows were fed on Non Bt cottonseed based concentrate mixture. Cows were continued to be fed on concentrate mixture and Berseem fodder as per their nutritional requirements and their milking schedule was continued to be the same. Average daily milk yield of cows during 20 days of withdrawing the Bt cottonseed was about 12.3 ± 1.2 kg. These observations indicated that there was no carry over effect of Bt cottonseed on the performance of crossbred cows in terms of milk yield its composition and reproduction.

Conclusion

It can be concluded from this study that feed intake and milk production performance of crossbred cows fed Bt and Non Bt cottonseeds @ 2 kg /day or about 17% of their ration was similar during the feeding trial of 4 weeks. Cry1C proteins were neither detected in the milk nor in blood of cows fed Bt cottonseed during the four week trial. Hence, feeding of Bt cottonseed as a source of protein and energy in the ration of crossbred cows is safe and as nutritious as Non Bt cottonseed.

Acknowledgement

Authors acknowledge the encouragement provided by Dr. Sushil Kumar, Director, National Dairy Research Institute, Karnal. Help provided by Dr. Rao and Mr. Prasanna from Metahelix Life Sciences Pvt. Ltd, Bangalore in analyzing the Cry1C protein in cottonseed, milk and blood samples is also acknowledged. Help rendered by Mr Kewal Krishnan, (technician) and Dr Ranjan Mohanty (M.V.Sc. student) in Dairy Cattle utrition Division for conducting the study is also acknowledged.

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APPENDIX 1

Maximum and minimum temperature recorded during the study

Days	Maximum Temperature(^{0}C)	Minimum Temperature(^{0}C)
19th DEC	28.0	7.0
20th DEC	29.0	8.0
21st DEC	26.0	5.0
22nd DEC	27.0	8.0
23rd DEC	27.0	6.0
24th DEC	27.0	7.0
25th DEC	26.0	6.5
26th DEC	20.0	11.0
27th DEC	26.0	7.5
28th DEC	28.0	6.0
29th DEC	28.0	7.0
30th DEC	27.0	7.0
31st DEC	27.0	5.5
1ST JAN	25.0	5.0
2nd JAN	25.0	4.5
3rd JAN	26.0	5.0
4th JAN	27.0	5.0
5th JAN	26.0	5.5
6th JAN	26.0	6.5
7th JAN	31.0	6.0
8th JAN	31.0	6.5
9th JAN	27.0	8.0
10th JAN	27.0	6.0
11th JAN	27.0	5.5
12th JAN	26.0	4.0
13th JAN	26.5	-1.0
14th JAN	26.0	-1.0
15th JAN	24.0	2.0
16th JAN	26.0	2.0
17th JAN	28.0	8.0

CATTLE YARD
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
Karnal – 132 001 (Haryana)

Dr. Shiv Prasad, Sr. Scientist
Incharge Cattle yard &
Chairman, IAEC

F. No. IAEC/2006/Vol-III
Dt, 29.01.2007

The Expert Consultant,
Sub Committee on Large Animals
Committee for the Purpose of Control and
Supervision of Experiments on Animals (CPCSEA)
No. 13/1 3rd Seaward Road, Balmiki Nagar
Thiruvanmiyur (Chennai)- 600041.

Subject: Submission of the research Project Proposals for the approval of SCLA of CPCSEA.

Sir,

I am enclosing herewith the proceedings of the Institute Animals Ethics Committee (IEAC) meeting held on 13.01.2007 along with the recommendations against each project along with 5 copies of the requisite proposal for the kind consideration and speedy approval of the Sub Committee for Large Animals (SCLA) of CPCSEA. It is requested to kindly convey the approval of the SCLA at the earliest. In case no communication is received within 45 days, it will be assumed that the SCLA has no objection to these proceedings and the project work will be initiated in the best interest of the institute research programme.

As most of the research projects are routine in nature involving routine management operations and the feeding trials for the benefit of the animals, it is requested that the powers of allotment of animals to the research projects not involving any invasive procedures may also kindly be delegated to the institute Animal Ethics Committee (IAEC), wherein a nominee of CPCSEA is a regular member. This gesture on the part of SPCSEA will not only help to lessen the burden of the committee, it will also help in expediting the process of approval.

With kind regards

Yours Faithfully

(Shiv Prasad)

Encl-As stated above

EPABX Tel. No. : 0184-2250366 / 2250716 / 2270066 / 2270166 / 2270266/ 2259306 (cy)
Fax No (Office): 0184-2250042
Gram. DAIRYSEARCH

Proceedings of the Meeting of Institutional Animal Ethics Committee of NDRI held on 13.1.2007 at 1.30 PM in the Office of Chairman IAEC & I/C Cattle Yard.

As per earlier intimation the Meeting of Institutional Animal Ethics Committee was held on 13.01.2007 at 1.30 PM. The following members attended the meeting:-

1) Dr. Shiv Prasad, I/C Cattle Yard	-	Chairman
2) Dr. Pradeep Kumar Kapoor	-	Member CPCSEA
3) Dr. Avtar Singh, Sr. Scientist, DCB Divn.	-	Member
4) Dr. Dheer Singh, Sr. Scientist, Ani.Biochem.	-	Member
5) Dr. A.K. Tyagi, Sr. Scientist	-	Member
6) Dr. Praveen Kumar, TO (Veterinary)	-	Member
7) Dr. T. K. Mohanty, Sr. Scientist, Cattle Yard	-	Member Secretary

The following issues were discussed: -

1) The proceedings of the last meeting held on 20.03.2006 were confirmed.

2) The issue of the outside members who are not attending the meetings of the IAEC regularly was discussed and it was observed that the outside Member from NBAGR Dr. Gautam Sahana, Scientist could not attend the past few meetings as he is on long leave. The committee empowered the chairman to request Director, NBAGR, Karnal to nominate an alternative scientist member to ensure regular participation of the outside member to IAEC meetings.

3) The issue of allotment of large animals to various experiments was discussed at length. It was felt that the approval of the large animals is often not received or is received very late as a result the Institute Research agenda suffers. It was recommended to request the sub committee for large animals (SCLA) of CPCSEA to convey the approval at the earliest (within 30-45days) so that the time bound research work of the scientists, masters and Ph.D. scholars does not suffer. It was unanimously decided to request the SCLA of CPCSEA for delegating powers of allotment of animals to the research projects not involving any invasive procedures to IAEC of the Institute.

4) The concerned Scholars/project leaders should process the proposal for the IAEC approval before submission at higher bodies i.e. Inter Disciplinary Seminars/SRC. The summary of results of experiments on animals should also be submitted to the IAEC before submission of thesis/project reports to the University office/SRC.

5) The protocol forms for Research proposal duly typed should be submitted to the IAEC on proper proforma provided by CPCSEA (Part B) with relevant literature. The electronic version of the same should also be sent to the secretary IAEC which is to be sent to the nominee CPCSEA for verification and early disposal.

Contd. 2...

In the meeting a total of 31 Research projects receive
Divisions were taken up for discussion. The decisions of IAEC
individual projects have been indicated in the enclosed table. Howe
involving small animals were cleared by the IAEC and remaining 20 pr
animals have been recommended for approval of Sub committee for
(SCLA) of CPCSEA.

The meeting ended with Vote of thanks to the Chairman.

29/01/2007

(Dr. T .K. Mohanty)
Member-Secretary

(Dr.Shiv Prasa
Chairman, IAE

LIST OF THE PROJECTS DISCUSSED IN THE INSTITUTE ANIMAL ETHICS COMMITTEE MEETING HELD ON 16.12.2006 IN CATTLE YARD.

IAEC No.	Name of Project	Division	Name of PI	Nos. of Animals required	Kind of Animals	Remarks
1/06	Nutrient utilization for growth in calves fed rations supplemented with bypass fat.	Dairy Cattle Nutrition Division	Dr S. S. Thakur, Principal Scientist	15	Cross-bred calves/buffalo calves for 90 days	Recommended for approval from CPCSEA as it involve only feeding of bypass fat for better utilization of nutrients.
2/06.	Effect of sun drying of aflatoxin contaminated feed on growth and certain blood parameters in calves	Dairy Cattle Nutrition Division	Dr R. C. Chopra Principal Scientist	15	Cross-bred, Approximately 4 months for 120 days	As it involves aflatoxin feeding PI may furnish following information i.e. Minimum Toxicity dose of aflatoxin in bovine is given, the large animal committee may please see and accord necessary approval. Recommended to CPCSEA for approval.
3/06	Status of certain contaminants in animal feeds and measures to minimize their carry over to milk in goats	Dairy Cattle Nutrition Division	Dr. (Ms.) Aruna Chabra, PS	20	Female Goats, 1.5-2.0 Years/40KG/ Medium size, for 70 days	Recommended for approval from CPCSEA The project proposal in detail with organo phosphorus compound feeding, relevant articles are attached for such studies.
4/06	Effect of feeding cottonseed produced from Bt cotton cultivar on feed intake, milk production and composition in dairy cows	Dairy Cattle Nutrition Division	Dr. K. K. Singhal, PS & Head DCN	20	KS Cows, 20 numbers for 50 days	Proposal is recommended for approval of CPCSEA. However, the milk should not be used for human consumption till the time it is proved safe. Recommended for approval of LASC.
5/06	Biotechnology of Ruminant Nutrition Practical (BT-612) , Rumen and its ecosystem, (AN-521), Practical and In vitro studies	Dairy Cattle Nutrition Division	Dr. S.K. Sirohi, Sr. Scientist	4+4	Buffalo calves and. Cattle calves for Rumen Fistulation	Recommended to CPCSEA for approval. Fistulated animals are essentially needed for PG students' demonstration and practical exercise and in vitro studies of masters and Ph.D. scholars. Course content of BT-612 and AN-521 is attached with justifications for four cattle and four buffaloes Rumen Fistulation for approval of LAC.
6/06	Impact of Fibrocystic enzyme on nutrient digestibility and growth in buffalo and cattle	Dairy Cattle Nutrition Division	Dr. S.K. Sirohi, Sr. Scientist	15	Buffalo calves (6 month to two year of age) for five months	Recommended for CPCSEA approval as it involves experimentation on fibrocystic enzymes on nutrient digestibility only.

DAIRY MICROBIOLOGY DIVISION
NATIONAL DAIRY RESEARCH INSTITUTE

1. 5. 07

Proceedings of the meeting of IBSC held on 1.5.07

The second meeting of the newly constituted IBSC was held on 1.5.07 at 3.00 PM in the office of JD(R). All the members of IBSC were present except the nominee from DBT and Dr. V. K. Batish. At the outset, the Chairman welcomed the new members Dr. Misra and Dr.Rekha Sharma. Discussions were held for the three consultancy projects viz. **"Effect of feeding cottonseed produced from Bt cotton cultivar on feed intake, production and consumption of milk in dairy cows"; "Effects of feeding grain produced from YieldGard corn containing cry1Ab gene for four weeks on feed intake, milk production and composition in lactating crossbred cows in India" and "Effects of feeding cottonseed produced from BF II cotton on feed intake, milk production and composition in lactating crossbred dairy cows"** submitted by Head, DCN. As the clarifications given by the PI were in order, the IBSC members accorded their approval for carrying out the three consultancy projects at NDRI.

As DBT has nominated the name of Dr. Jitender Sharma, Assoc. Prof., KUK. vide letter no. BT/17/02/95-PID dated 23.4.07, hence, committee decided to hold a meeting in the first week of June again.

Member Secretary informed the house that in response to her circular for furnishing the information regarding the project proposals including inhouse, externally funded, M.Sc and Ph.D projects related to GMOs, pathogens or use of hazardous chemicals on small animals ruminants etc. or involving any Biosafety aspects, response has been received from Drs. V. K. Batish, Jai K.Kaushik, Ananadlaxmi and Sunita Grover only. The committee also decided to take appropriate initiatives for monitoring health of the workers involved in genetic engineering expts / biosafety studies.

Dr. S. L. Goswami, Joint Director (R)
Chariman

Dr. B. P. Misra, Sr. Sci., NBAGR
Member

Dr. Y. S. Rajput, Head, DC Div.
Member

Dr. A. A. Patel, Head, DT Div.
Member

(SUNITA GROVER)
Member Secretary (IBSC)
Sr. Sci., MBU/DM Div.

Submitted for approval please

APPROVED

(DIRECTOR)
NDRI, Karnal

F.No.25/18/2007-AWD
Government of India
Ministry of Environment & Forests
(Animal Welfare Division)
***

8th Floor, Jeevan Prakash Building,
25, Kasturba Gandhi Marg, New Delhi-110 001
Dated : 22nd October, 2007.

To

The Director
National Dairy Research Institute,
Karnal, Haryana-132 001.

Sir,

I am directed to inform you that the CPCSEA has reconsidered the following project proposals of your institute in its meeting held on 18.9.2007 and to convey decision of the CPCSEA as under: -

S. No.	Name of the institute/Name of the Investigator	Project Title	Decision of XXI CPCSEA meeting
1.	National Dairy Research Institute, Karnal, Haryana-132 001. / Dr. S.N. Rai Received on 23.7.2007	Studies on Acacia nilotica pods replacing energy sources in ration of dairy cows on production performance and neutraceutical value of milk and milk products	**Approved.**
2.	National Dairy Research Institute, Karnal, Haryana-132 001. / Dr. K.K. Singhal Received on 4.5.2007	Effect of feeding cottonseed produced from Bt cotton cultivar on feed intake, production and composition on milk in dairy cows	**Approved.**

Yours faithfully,

(Anjani Kumar)
Dy. Secretary (AW) & Member-Secretary, CPCSEA
Tele: 23318553

Copy to : Expert Consultant, CPCSEA, 13/1, 3rd Seaward Road, Valmiki Nagar, Thiruvanmiyur, Chennai-600 041.

JD(A)
HOP/DCN
PA
Director

डेरी प्रौद्योगिकी विभाग
DIVISION OF DAIRY TECHNOLOGY
राष्ट्रीय डेरी अनुसंधान संस्थान
NATIONAL DAIRY RESEARCH INSTITUTE
(मान्य विश्वविद्यालय)
(Deemed University)
(भारतीय कृषि अनुसंधान परिषद)
(Indian Council of Agricultural Research)
करनाल (हरियाणा) भारत KARNAL-132001 (Haryana) India

SPEED Post

Dr. A.A. Patel, Chairman
Consultancy Processing Cell

Ref. No. Cons./2007-08 954
Dated Feb., 2008

Dr. M.J Vasudeva Rao,
Metahelix Life Science Private Limited,
Plot No.3, KIADB 4th Phase,
Bommasandra,
Bangalore 560 099

Fax No.80-783 6084

Subject: Report on Sponsored Project entitled " Effect of feeding cottonseed produced from cotton hybrids carrying Cry 1C gene on feed intake, milk production and composition in lactating crossbred dairy cows."

Dear Dr. Rao,

The above-said project has been successfully concluded. Please find enclosed the relevant report from the concerned scientists at this institute.

With warm regards.

Yours faithfully,

(A.A.PATEL) 27/2/08

Encl: As above.

Copy : Dr. K. K. Singhal, Head, DCN.

Tel. 0184-2259268, 2259270(O)
EPABX Nos. 1268, 1270, 1210, 1257
E.mail : ashok.as@sify.com
Gram : DAIRYSEARCH Fax : 0184-2250042

METAHELIX
Germinating Hope

PCR and ELISA CONFIRMATION OF *B.T.* & NON *B.T.* COTTON SEED

Objective: Quality control of the seed material from Cry1C-9124 intra hirsutum cotton hybrids to be used for the biosafety studies at NDRI, Karnal and baseline susceptibility studies conducted at Metahelix Life Sciences Pvt. Ltd., Bangalore.

1. Confirmation of the transgenic nature by PCR based testing
2. Confirmation of presence of protein by ELISA and Quantification of Cry1C protein in the seed material.

1. PCR confirmation:

PCR confirmation was done using the following primers and conditions:

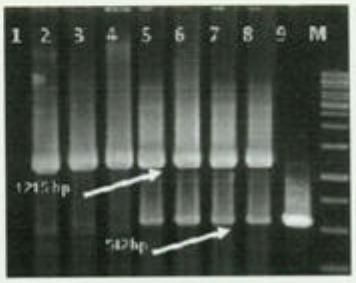
Primers Used
Internal Control: (1) Primer 229 Gh 2S alb U and (2) Primer 230 Gh 2S alb L
Cry1C Specific: (1) Primer 117 MH1CGh2-U and (2) Primer 118 MH1CGh2-L

PCR Conditions: (Eppendorf master Cycler)

Step	Temp°C	Duration	No. of cycles
1	94	2 min	1
2	94	15 sec	
3	60	20 sec	Minus 0.5 deg for 10 cycles
4	72	1 min	
5	94	15 sec	
6	55	20 sec	30 cycles
7	72	1 min	
8	72	5 min	1
9	END		

Expected Band Sizes: 1215 (internal band) & 542 bp (Cry1C specific band)

Legend
1. Water control
2. Non transgenic leaf DNA (-ve)
3. Non *B.t.* seed DNA 1
4. Non *B.t.* seed DNA 2
5. *B.t.* seed DNA 1
6. *B.t.* seed DNA 2
7. Transgenic cotton leaf DNA 1
8. Transgenic cotton leaf DNA 2
9. Plasmid cry1c



Conclusion: The expected 542 bp amplicon has been observed in the transgenic seed powder DNA only, 1215 bp cotton internal control amplicon was observed in all the cotton DNA samples, as expected water and negative controls were clear.

Metahelix Life Sciences Private Limited
Plot No. 3, KIADB 4th Phase, Bommasandra, Bangalore 560 099, India.
Tel: +91-80-787 0236, 783 6086 Fax: +91-80-783 6084 www.meta-helix.com

METAHELIX
Germinating Hope

2. ELISA confirmation and Quantification

Confirmation and quantification of Cry1C protein was done using the Quantiplate kit for Cry1C (Envirologix, USA, Catalog number AP 007)

No.	Sample	Concentration
1	Blank	NA
2	Std 1 ppb	1.20 ng /ml
3	Std 5 ppb	5.17 ng/ml
4	Std 10 ppb	9.71 ng /ml
5	B.t. -5 X diluted	2.32 µg/g
6	B.t. 10X diluted	2.55 µg / g
7	Non B.t. 5X diluted	NA

*All blank reduction values

Result: The absorbance value observed for the Non B.t. cotton seed sample was similar to the blank values and the colour development was not seen. Blue colour development was seen in transgenic samples, which was clearly absent in negative controls and non transgenic cotton seed sample. The average amount of Cry1C protein in the seed samples was 2.44 µg / g of seed powder.

Declaration:
I hereby declare that the certificate of quality presented in the above results are true to my knowledge and is made on the basis of experiments conducted at our facility

17th Oct 2007
(Vai. Ramanathan)
Head- Genomics

METAHELIX LIFE SCIENCES PRIVATE LTD. BANGALORE

Metahelix Life Sciences Private Limited
Plot No. 3, KIADB 4th Phase, Bommasandra, Bangalore 560 099, India.
Tel: +91-80-787 0236, 783 6086 Fax: +91-80-783 6084 www.meta-helix.com