

## **Report On**

# **Effect of feeding cottonseed produced from Bt cotton cultivar on feed intake, production and composition of milk in dairy cows**

By

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## Statement of particular of Study

**Title:- Effect of feeding cottonseed produced from Bt cotton cultivar on feed intake, production and composition of milk in dairy cows**

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**Study Initiation Date** **January 18th, 2007**

**Experiment termination Date.** **March 5th, 2008**

**Record Retention** Raw data of the study will be retained by the Study Director and this will be made available to the authorized personal from the sponsors

### Signature of Approval

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**Study Director**

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**Date**

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**Co study Director**

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**Date**

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**In charge, Consultancy Cell**

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**Date**

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**Study Sponsor**

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**Date**

## Abbreviations

ADF	Acid detergent fibre
<i>ad lib.</i>	<i>Ad libitum</i>
BCS	Body Condition Score
°C	degree Celsius
DM	Dry matter
g	gram
l	liters
kg	Kilogram
KF	Karan Fries
KS	Karan Swiss
mg	milligram
NDF	Neutral detergent fibre
NDRI	National Dairy Research Institute
µg	microgram

## ABSTRACT

Sixteen 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 fresh berseem (*Trifolium alexandrinum*) fodders *ad libitum* according to their nutritional requirements for 19 days to adapt them to the cottonseed based diet. Thereafter, cows were divided in two groups of 8 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 19 days in the cows assigned to Non Bt and Bt group was  $12.80 \pm 0.86$  and  $13.08 \pm 0.98$  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 protein. At the end, a blood sample from each cow was collected and plasma was separated to test for the presence of Cry 1Ac protein. Cry 1Ac protein in cottonseed, milk and blood samples was measured by ELISA method. The amount of Cry 1Ac protein in Bt cottonseed was  $195.04 \pm 15.90$  ng/g on fresh basis. Corresponding values in Bt concentrate mixture was 78 ng/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 3.85 kg in Non Bt group and 3.64 kg 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 both groups was similar. Average milk yield during 28 days of experimental period in Non Bt ( $13.53 \pm 0.75$  kg/day) and Bt ( $13.12 \pm 0.92$  kg/day) groups did not vary significantly. During the experimental period the milk composition in terms of fat, protein, lactose, SNF and total solids content in Non Bt groups was 4.59, 3.11, 4.70., 8.72, and 13.31 % respectively. Corresponding values in Bt group was 4.54, 3.10, 4.71, 8.71 and 13.25% which revealed that milk composition was similar in both the groups. Cry 1Ac 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. The present study results revealed that the feed intake and milk production performance of crossbred cows fed Bt and Non Bt cottonseeds @ 2 kg /day on fresh basis or about 11% of their ration on DM basis was similar during the feeding trial of 4 weeks. Cry1Ac proteins were neither detected in the milk nor in blood of cows fed Bt cottonseed during the four week trial and there is no effect of Bt cottonseed containing Cry protein on milking cows. 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.

## 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 the 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 the insect's 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 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. An 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 (Cry2Ab) 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). A new version of Cry 1Ac gene has been incorporated in to the genome of cotton and new event has been developed by Central Cotton Research Institute (ICAR), Nagpur to make indigenous cotton variety having insect resistant characteristics 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 Cry 1Ac protein.

## MATERIALS AND METHODS:

Sixteen 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)
<b>Non Bt Group</b>				<b>Bt group</b>			
KS 4316	4	245	311	KS 4353	2	144	425
KS 4345	2	235	404	KF 6205	4	226	374
KF 6367	4	170	408	KF 6215	3	275	520
KF 6429	2	284	422	KF 6223	4	162	456
KF 6441	2	170	421	KF 6245	2	248	410
KF 6476	2	239	460	KF 6624	2	171	400
KF 6481	2	149	416	KF 6698	1	166	369
KF 6568	1	284	371	KF 6755	1	113	472
Average± SE	2.38 ± 0.38	222 ± 18.62	401.63 ± 15.58	Average ± SE	2.38 ± 0.42	188.13 ± 19.65	428.25 ± 18.27

### Housing of Animals:

All cows, having a unique identification number tattooed on their hip region as well as in their ears, cows 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 cottonseed supplied by Central Cotton Research Institute, Nagpur (MS)). 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 straw were fed *ad libitum* to each cow. Feeding of crushed cottonseed (Non-Bt) based concentrate mixture continued for 19 days for the adaptation of rumen microbes for the changed nutrient supplement. Thereafter, the cows were distributed in two groups of 8 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.



**Fig. 2. Arrangements for individual feeding of crossbred cows**

### **Preparation of Concentrate Mixture:**

Consignments of delinted and crushed cottonseeds (Non Bt and Bt) received from Central Cotton Research Institute, Nagpur (MS) 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 Cry 1Ac 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. Green berseem fodder and wheat straw were supplied by Farm section of the institute, daily.



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

**Table 2. Ingredient composition of concentrate mixture**

	<b>parts</b>
Maize grain	25
Cottonseed ( Non Bt or Bt)	40
Groundnut cake (Expeller pressed)	10
Wheat bran	22
Common salt	1
Mineral mixture	2

### **Feeding schedule:**

Each cow in both groups was offered weighed amount of berseem fodders three times a day i.e. 10 am, 1 pm and 6.30 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. 5.45 am, 11.45 am and 5.45 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 28<sup>th</sup> 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 prior 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 Central Cotton Research Institute, Nagpur (MS) 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 as well as minerals (Calcium, Copper, Manganese) using Atomic Absorption Spectrophotometer and Phosphorus using calorimetric technique. In addition, Non Bt and Bt cottonseeds were also analyzed for quantitative estimation of the Cry 1Ac protein using ELISA test.

### **Milk and blood plasma samples:**

Representative milk samples were analyzed for fat, protein, total solids, lactose, and solids-not-fat (SNF) on the day of sampling using pre-calibrated Milk Analyzer (LactoStar, FUNKE GERBER, Article No 3510, Berlin). A portion of all the milk samples, collected on a particular day, were frozen in liquid nitrogen and stored at  $-20^{\circ}\text{C}$  for the determination of Cry1Ac protein. Blood plasma samples, collected on day 28 of experimental feeding were also analyzed for the presence of Bt proteins using the ELISA kit (EnviroLogix, QualiPlate, USA) on same day. Values of Bt proteins were obtained as per the following procedure.

### **Estimation of Cry 1Ac**

The level of Cry 1Ac protein in blood or milk was estimated using QualiPlate kit from M/s Envirologix. Standards at 1, 5 and 10 ppb Cry 1Ac were used in every assay. The 96 well plates were precoated with anti-Cry 1Ac antibodies. Milk samples, blood samples and crushed Bt- and non Bt- cottonseeds were treated with extraction buffer for extraction of Cry 1Ac protein. Milk samples were diluted 1X and 5X with extraction buffer. Blood samples were diluted 10X and 20X with extraction buffer. Cottonseeds (10 mg) were put to Eppendorf tube and then 500  $\mu\text{l}$  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 Cry 1Ac protein.

The supplied ELISA kit uses 96 well plates precoated with anti-Cry1Ac.

- 100 µl of sample (cotton seed extract or diluted blood or diluted milk or Cry 1Ac standard) were added to wells of multi-well plate.
- After incubation of plate for 45 min. at room temperature, the plate was washed with wash buffer. The washing was repeated three times.
- 100 µl anti Cry 1 Ac-peroxidase conjugate (1:1 diluted with wash buffer) was added.
- The ELISA plate was then incubated for 30 min. at room temperature.
- The plate was washed three times with wash buffer.
- 100 µl pf substrate was then added
- The plate was incubated for 15 min. at room temperature.
- 100µl of 1N HCl was added to stop the reaction
- Absorbance was recorded at 450 nm.
- Standard curve was plotted and concentrations of Cry 1Ac in samples were calculated.
- For positive control, Bt cottonseed kernel with known concentration of Cry 1 Ac protein was used.

### **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 students‘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 cow, irrespective of the group (Table 3 ). Average body weight gain in cows fed on non Bt cottonseed based ration was  $26.63 \pm 2.19$  kg, whereas the increase in cows fed on Bt cottonseed based ration was to the tune of  $28.41 \pm 3.36$  kg (Table 4). Higher body weight gain in both groups may be due to the high energy density of Bt as well as Non Bt cottonseed due to their higher EE content as well as feeding of nutritious berseem fodder *ad lib*. 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	311	339.2	KS 4353	425	441.2
KS 4345	404	439.0	KF 6205	374	408.6
KF 6367	408	441.6	KF 6215	520	545.6
KF 6429	422	444.4	KF 6223	456	491.0
KF 6441	421	444.2	KF 6245	410	450.5
KF 6476	460	476.8	KF 6624	400	423.0
KF 6481	416	439.6	KF 6698	369	385.0
KF 6568	371	401.2	KF 6755	472	508.4
AVG±SE	401.63 ± 15.58	428.25 ± 14.61	AVG±SE	428.25 ± 18.27	456.66 ± 19.19

### 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. Both types of cottonseed were having fuzz on their surface. Oil content in Bt cottonseed was higher than that of Non Bt cottonseed, however, CP content as well as the cell wall constituents were similar in both types of cottonseeds. The Cry1Ac protein in Bt cottonseed was  $0.195\mu\text{g/g}$  on fresh basis and it was not detected in Non Bt cottonseed. 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. High 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		Berseem fodder	Wheat Straw
	Bt	Non Bt	Bt	Non Bt		
DM	91.15	90.48	90.53	90.73	15.72	90.45
Organic matter	90.80	93.25	95.96	96.09	76.90	90.75
Ash	9.20	6.75	4.04	3.91	23.10	9.25
Crude protein	22.75	22.59	25.10	24.41	16.45	2.26
Ether extract	9.46	5.83	17.78	14.17	2.64	0.46
NDF	46.77	51.29	47.41	54.26	31.25	64.46
ADF	17.29	22.77	39.55	48.05	25.74	34.78
Calcium (%)	ND	ND	1.29	1.69	ND	ND
Phosphorus (%)	ND	ND	0.60	0.63	ND	ND
Copper (ppm)	ND	ND	21.55	24.12	ND	ND
Manganese (ppm)	ND	ND	0.37	0.37	ND	ND
Cry1Ac (ng/g)*	0.078	-	0.195	-	ND	ND

\* On fresh basis, ND - Not determined

Cry 1Ac protein in the test concentrate mixture was calculated to be 0.078 ng/ g on fresh basis. Proximate composition of berseem fodder 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  $15.37 \pm 0.22$  and  $15.31 \pm 0.21$  kg/day, respectively, and the average roughage to concentrate ratio in corresponding groups was 1.90:1 and 2.00:1. Total DM intake/ 100 kg body weight in Non Bt and Bt groups was 3.83 and 3.58 kg, respectively. Milk yield in corresponding groups was  $12.80 \pm 0.86$  and  $13.08 \pm 0.98$  kg/ day. Statistical analysis of data revealed non-significant difference between the groups for DM intake as well as for milk yield. Lower DM intake and milk yield were recorded when crossbred lactating cows were fed a ration containing similar levels of Non Bt cottonseeds with berseem (*Trifolium alexandrinum*) (Singhal *et al.*, 2001) 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 0.832 and 0.854, respectively, and the values were comparable to those reported by Singhal *et al.* (2001).

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

Parameter	Non Bt (Control)	Bt
Av. B. Wt. (kg).	$401.63 \pm 15.58$	$428.25 \pm 18.27$
DM intake through Conc. mix (kg/day)	5.3	5.1
DM intake through green fodder (kg/day)	$8.97 \pm 0.22$	$8.99 \pm 0.23$
DM intake through straw (kg/day)	$1.11 \pm .047$	$1.22 \pm 0.55$
Roughage: concentrate	1.90:1	2.00:1
Total DM intake (kg/day)	$15.37 \pm 0.22$	$15.31 \pm 0.21$
Dry Matter intake/ 100 kg B. Wt (kg)	3.83	3.58
Milk yield (kg/day)	$12.80 \pm 0.86$	$13.08 \pm 0.98$
4% FCM yield (kg/day)	$14.15 \pm 0.95$	$14.55 \pm 1.10$
Milk yield/ kg DM intake	0.832	0.854

## Experimental period

DM intake and milk production performance of cows on Non Bt and Bt cottonseed based rations are presented in Table 6.

It was recorded that the average DM intake was  $16.51 \pm 0.65$  kg/ day in Non Bt and  $16.64 \pm 0.65$  kg/ day in Bt group and the variation between the groups was not significant. In general, higher DM intake was recorded during experimental period than in adaptation period, which may be attributed to the maturity of berseem fodder. DM intake/ 100 kg b. wt in Non Bt and Bt cottonseed fed groups was 3.85 and 3.64 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 (See Appendix 1), which encourages the animals to consume more to compensate the energy loss in maintaining the body temperature. In present study cottonseed constituted 11% of the total DM consumed in both the groups.

Roughage to concentrate ratio in Non Bt and Bt groups was 2.68:1 and 2.74:1, respectively. Though this ratio was broaden during the experimental period in comparison to the adaptation period, obviously due to the higher consumption of DM from roughages, however, variation between the groups was not significant. Lower DM intake under the similar roughage to concentrate ratio was recorded earlier in crossbred lactating cows by Singhal *et al* (2001 & 2006) and Thakur *et al.* (2005). However, similar to present observations, 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 rations. Average

milk yield/ day in Non Bt and Bt group was  $13.53 \pm 0.75$  and  $13.12 \pm 0.92$  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 similar on replacing the Non Bt cottonseed with Bt cottonseed during the experimental period.

It was also recorded that the average intake of Cry 1Ac protein through Bt cottonseed was 0.386 mg/ day, which appeared to be degraded during the course of its rumen fermentation as per the fate of most of the dietary proteins. Senthil and Singhal (2004) reported that Bt protein (Cry 1Ac), present in GM cottonseed, did not affect the rumen fermentation *in vitro*, indicating that it got degraded in the rumen. It was reported that the intake of higher amount of Cry 1 Ac alone or in combination of Cry 2 Ab through cottonseed (Singhal *et al.*, 2001 and 2006) as well as Cry 1 C through cottonseed (Singhal *et al.* 2008) did not adversely affect the milk production and health aspects of crossbred cows. These workers reported that the cottonseed having these proteins even at higher levels were safe for feeding 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).	$428.25 \pm 14.61$	$456.66 \pm 19.19$
DM intake through Conc. mix (kg/day)	$4.49 \pm 0.02$	$4.45 \pm 0.01$
DM intake through green fodder (kg/day)	$11.11 \pm 0.32$	$11.22 \pm 0.33$
DM intake through straw (kg/day)	$0.90 \pm 0.02$	$0.98 \pm 0.03$
Roughage: concentrate	2.68:1	2.74:1
Total DM intake (kg/day)	$16.51 \pm 0.65$	$16.64 \pm 0.065$
Dry Matter intake/ 100 kg B. Wt (kg)	3.88	3.64
Milk yield (kg/day)	$13.53 \pm 0.75$	$13.12 \pm 0.92$
4% FCM yield (kg/day)	$14.73 \pm 0.82$	$14.18 \pm 0.99$
Milk yield/ kg DM intake	0.82	0.79

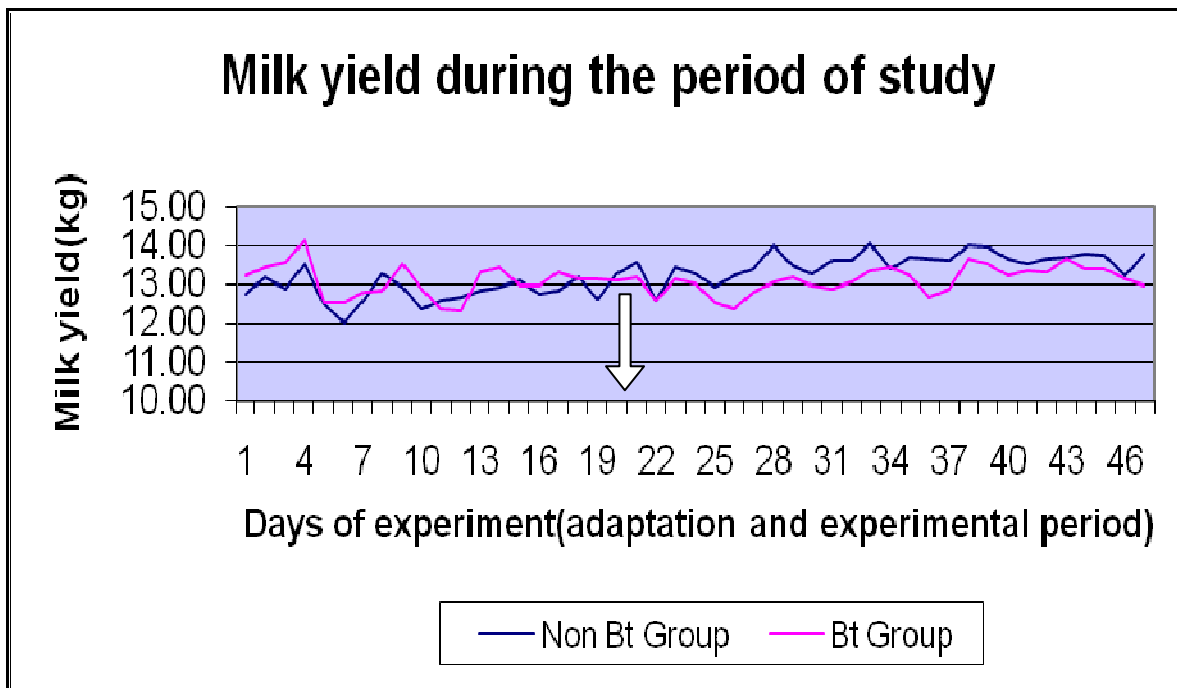
Milk production efficiency in terms of milk yield/ kg DM intake in Non Bt and Bt groups was 0.82 and 0.79, respectively. Milk production efficiency was lower during experimental period than that recorded during the adaptation period, but it varied in the range reported

earlier by various workers in crossbred cows (Singhal et al., 2001, 2006, 2008) and the variation between groups was not significant. Similar to these observations, Singhal *et al.* (2001) reported that the milk yield / kg DM intake was not affected by the feeding of Bt and non Bt cottonseed based ration, however, they reported that milk production efficiency in terms of milk yield per kg DM intake 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

Daily milk yield of cows during adaptation period as well as during experimental period in Bt and non Bt groups have been presented graphically 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 28<sup>th</sup> 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
<b>FAT (%)</b>		
6 <sup>th</sup> Feb	4.59 ± 0.12	4.62 ± 0.11
13 <sup>TH</sup> Feb	4.67 ± 0.08	4.65 ± 0.06
20 <sup>th</sup> Feb	4.50 ± 0.10	4.44 ± 0.07
27 <sup>th</sup> Feb	4.53 ± 0.09	4.56 ± 0.07
5 <sup>th</sup> Feb	4.64 ± 0.16	4.43 ± 0.16
<b>Avg</b>	<b>4.59</b>	<b>4.54</b>
<b>PROTEIN (%)</b>		
6 <sup>th</sup> Feb	3.11 ± 0.03	3.12 ± 0.04
13 <sup>TH</sup> Feb	3.12 ± 0.03	3.14 ± 0.01
20 <sup>th</sup> Feb	3.16 ± 0.03	3.13 ± 0.03
27 <sup>th</sup> Feb	3.18 ± 0.02	3.13 ± 0.02
5 <sup>th</sup> March	3.00 ± 0.04	2.99 ± 0.04
<b>Avg</b>	<b>3.11</b>	<b>3.10</b>
<b>LACTOSE (%)</b>		
6 <sup>th</sup> Feb	4.70 ± 0.06	4.77 ± 0.13
13 <sup>TH</sup> Feb	4.70 ± 0.04	4.76 ± 0.07
20 <sup>th</sup> Feb	4.69 ± 0.04	4.74 ± 0.06
27 <sup>th</sup> Feb	4.91 ± 0.05	4.80 ± 0.05
5 <sup>th</sup> March	4.51 ± 0.06	4.50 ± 0.05
<b>Avg</b>	<b>4.70</b>	<b>4.71</b>
<b>SNF (%)</b>		
6 <sup>th</sup> Feb	8.71 ± 0.09	8.80 ± 0.14
13 <sup>TH</sup> Feb	8.73 ± 0.04	8.83 ± 0.07
20 <sup>th</sup> Feb	8.76 ± 0.06	8.77 ± 0.07
27 <sup>th</sup> Feb	8.99 ± 0.06	8.84 ± 0.05
5 <sup>th</sup> March	8.41 ± 0.09	8.31 ± 0.09
<b>Avg</b>	<b>8.72</b>	<b>8.71</b>
<b>TOTAL SOLIDS (%)</b>		
6 <sup>th</sup> Feb	13.31 ± 0.17	13.42 ± 0.19
13 <sup>TH</sup> Feb	13.40 ± 0.06	13.48 ± 0.13
20 <sup>th</sup> Feb	13.26 ± 0.13	13.21 ± 0.10
27 <sup>th</sup> Feb	13.52 ± 0.12	13.40 ± 0.09
5 <sup>th</sup> March	13.05 ± 0.23	12.75 ± 0.22
<b>Avg</b>	<b>13.31</b>	<b>13.25</b>

*Each value is the average of 8 observations ± SE*

There were variations in milk composition at different sampling days, but the variations for each parameter were within the normal range and statistical analysis did not reveal significant variations during sampling days. During the experimental period the milk composition in terms

of fat, protein, lactose, SNF and total solids content in Non Bt groups was 4.59, 3.11, 4.70., 8.72, and 13.31 % respectively. Corresponding values in Bt group was 4.54, 3.10, 4.71, 8.71 and 13.25%. Milk composition of crossbred cows was found similar to that reported by Singhal *et al.* (2006).

Presence of Bt protein (Cry1Ac) was not detected in the milk samples, collected at various intervals after incorporation of Bt cottonseed in the ration of cows. These observations were similar to those 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.

Cry 1Ac protein was also not detected in the blood plasma samples, drawn at the end of experimental feeding period. These results suggest that the Cry1Ac protein consumed by cows through Bt cottonseed either got degraded in the rumen or not absorbed across the intestinal mucosa in to the blood circulation and the absence of Cry 1 Ac in the milk was obvious.

#### Plane of Nutrition

CP intake in Non Bt and Bt cottonseed based ration was 2.865 and 2.87 kg, respectively, which was similar to the recommendations of NRC (2001) indicating that the CP requirements of cows yielding more than 12 kg milk fulfilled their nutritional requirements.

#### 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. Similarly, other group of workers (Castillo *et al.*, 2001; Singh *et al.* 2003; Singhal *et al.* 2006, 2008) did not report any adverse affect of feeding Bt cottonseed on the health and productivity of cows or buffaloes. Similar health status of all the cows under the feeding of Bt and Non Bt cottonseed based ration was also event from the data on their body condition score (Table 8).

**Table 8. Body condition score of cows in Non Bt and Bt groups**

Non Bt Group			Bt Group		
Animal No.	BCS*	BCS*	Animal No.	BCS*	BCS*
KS 4316	3.00	3.14	KS 4353	3.71	3.79
KS 4345	3.79	3.93	KF 6205	2.86	3.14
KF 6367	3.79	3.86	KF 6215	3.79	3.86

<b>KF 6429</b>	<b>3.86</b>	<b>4.07</b>	<b>KF 6223</b>	<b>3.14</b>	<b>3.21</b>
<b>KF 6441</b>	<b>3.50</b>	<b>3.64</b>	<b>KF 6245</b>	<b>3.29</b>	<b>3.36</b>
<b>KF 6476</b>	<b>3.57</b>	<b>3.57</b>	<b>KF 6624</b>	<b>4.64</b>	<b>4.57</b>
<b>KF 6481</b>	<b>3.29</b>	<b>3.36</b>	<b>KF 6698</b>	<b>3.79</b>	<b>3.79</b>
<b>KF 6568</b>	<b>4.00</b>	<b>4.00</b>	<b>KF 6755</b>	<b>3.43</b>	<b>3.43</b>
<b>Average ± SE</b>	<b>3.60 ± 0.12</b>	<b>3.70 ± 0.12</b>	<b>Average ± SE</b>	<b>3.58 ± 0.19</b>	<b>3.64± 0.16</b>

\*On a scale of 6

## 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 on fresh basis or about 11% of their ration on DM basis was similar during the feeding trial of 4 weeks. Cry1Ac proteins were neither detected in the milk nor in blood of cows fed Bt cottonseed during the four week trial and there is no effect of Bt cottonseed containing Cry protein on milking cows. 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. Ramdev from Dharwad Agricultural University and Dr. Balasubramanian from Central Cotton Research institute, Nagpur in analyzing the Cry1Ac protein in cottonseed, milk and blood samples is also acknowledged. Help provided by Dr. Ranjan Mohanta (M.V. Sc. Scholar) and Mr. Kewal Krishan (Technical Officer) from Dairy Cattle Nutrition Division for looking after the experimental animals and in analysis of samples is deeply acknowledged.

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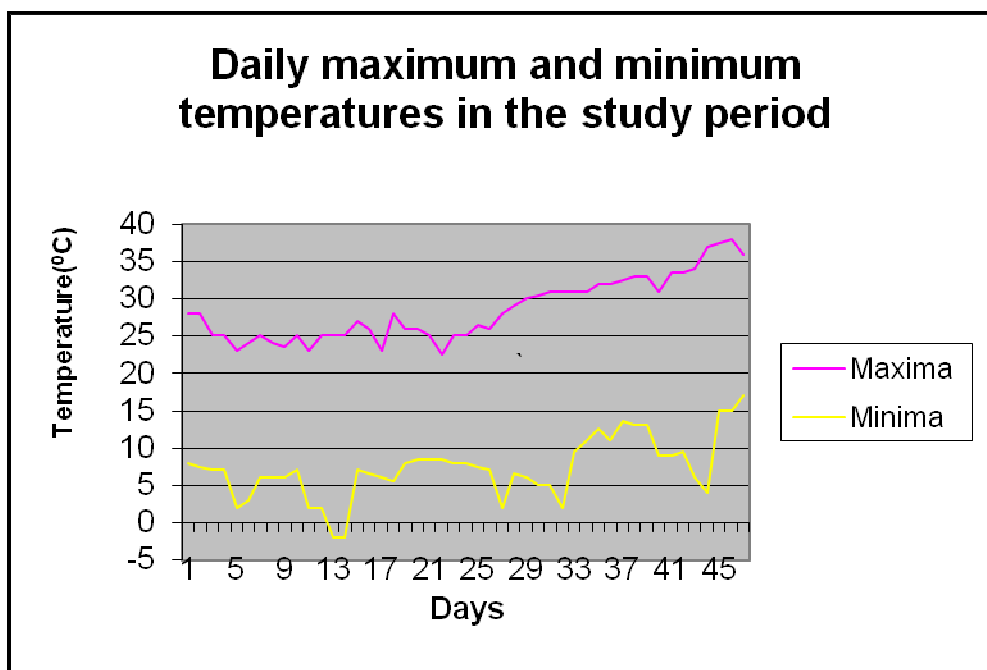
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## APPENDIX 1

### Maximum and minimum temperature recorded during the study

Date	Maximum Temperature (°C)	Minimum Temperature (°C)
<b>Adaptation Period</b>		
18th JAN	28.0	8.0
19th JAN	28.0	7.5
20th JAN	25.0	7.0
21st JAN	25.0	7.0
22nd JAN	23.0	2.0
23rd JAN	24.0	3.0
24th JAN	25.0	6.0
25th JAN	24.0	6.0
26th JAN	23.5	6.0
27th JAN	25.0	7.0
28th JAN	23.0	2.0
29th JAN	25.0	2.0
30th JAN	25.0	-2.0
31st JAN	25.0	-2.0
1st Feb	27.0	7.0
2nd Feb	26.0	6.5
3rd Feb	23.0	6.0
4th Feb	28.0	5.5
5th Feb	26.0	8.0
<b>Experimental Period</b>		
6th Feb	26.0	8.5
7th Feb	25.0	8.5
8th Feb	22.5	8.5
9th Feb	25.0	8.0
10th Feb	25.0	8.0
11th Feb	26.5	7.5
12th Feb	26.0	7.0
13th Feb	28.0	2.0
14th Feb	29.0	6.5
15th Feb	30.0	6.0
16th Feb	30.5	5.0
17th Feb	31.0	5.0
18th Feb	31.0	2.0
19th Feb	31.0	9.5
20th Feb	31.0	11.0
21st Feb	32.0	12.5
22nd Feb	32.0	11.0
23rd Feb	32.5	13.5
24th Feb	33.0	13.0
25th Feb	33.0	13.0
26th Feb	31.0	9.0
27th Feb	33.5	9.0
28th Feb	33.5	9.5
29th Feb	34.0	6.0
1st March	37.0	4.0
2nd March	37.0	15.0
3rd March	38.0	15.0
4th March	36.0	17.0