

Effect of feeding transgenic cottonseed (Bt-cry1Ac gene) on nutrient utilization, production performance and blood biochemical status in lactating dairy cows

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ABSTRACT

The study was made to compare the effect of transgenic (Bt, Cry1Ac) linted whole cottonseed (WCS) on nutrient intake and utilization, milk production and biosafety with that of isogenic (on Bt) WCS. Lactating multiparous cows (16) adapted to non Bt cottonseed based concentrate mixture (40%) were divided randomly in 2 groups of 8 each. Non-Bt group (control) continued on same ration while in Bt group, non-Bt WCS in concentrate mixture was replaced with Bt WCS in a feeding trial of 4 weeks. BW gain, nutrient intake and digestibility, milk yield and composition, body condition score, and blood parameters did not vary significantly between the groups. Non detection of Bt protein (Cry1Ac) in milk or blood plasma indicated the degradation of the protein in the gastrointestinal tract of cows. It was concluded that Bt (Cry1Ac) cottonseed is as safe and nutritious as conventional cottonseed, when fed to lactating crossbred cows for 4 weeks.

Key words: Biosafety, Bt protein, Digestibility, Feed intake, Transgenic cottonseed

GM cotton is one of the predominant transgenic crops all over the world and the global area under GM crops was increased nearly 73.5 fold during last 13 year's period (1996 to 2008) (James 2008). India, one of the leading cotton producers, introduced the Bt cotton cultivation in the year 2002. Insertion of *Bacillus thuringiensis* into the DNA of local cotton varieties gives them resistance towards lepidopteran insect pests. Cottonseed is a traditional protein and energy supplement in the ration of lactating cows and buffaloes. Reports about the absence of transgenic proteins in milk and blood in ruminants (Phipps *et al.* 2003, Faust *et al.* 2007, Huls *et al.* 2008), and in tissues of pigs and poultry (Jennings *et al.* 2003, Elangovan *et al.* 2006) following the feeding of GM cottonseed based rations suggested that these are safe for animals and the consumers of products from such animals. However, the bio-safety aspects of feeding transgenic cottonseed having Cry1Ac protein, developed by Central Institute for Cotton Research (ICAR), Nagpur, to lactating cows has not been investigated for its effect on productivity and biosafety of crossbred cows. Therefore, feed intake, nutrient utilization, milk production and biosafety of non Bt and Bt (Cry1Ac) whole cottonseed based rations in crossbred cows were compared in this study.

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MATERIALS AND METHODS

Two WCS, i.e. non-transgenic (Bikaneri Nerma) and transgenic (Event BN-Bt) ones, designated as non-Bt and Bt cottonseed, were procured in whole linted form from Central Institute for Cotton Research (CICR), Nagpur, India. Other components of concentrate mixture, wheat straw and green berseem fodder were procured locally. The experiment was conducted from January to February (average minimum temperature 7.3°C, maximum temperature 28.5°C) inside the shed.

Sixteen multiparous crossbred cows (*Bos taurus* × *Bos indicus*), free from internal as well as external parasites and maintaining good health status, were maintained in a well-ventilated barn having an arrangement for individual feeding. Each cow was having an access to 2 mangers, one for wheat straw with concentrate mixture and other for green fodder. Initially, all the cows were continued on concentrate mixture having crushed linted non-Bt cottonseed (control) 40, maize 20, groundnut oil cake 15, wheat bran 25, mineral mixture 2 and common salt 1 parts. In addition to the green berseem (*Trifolium alexandrinum*) *ad lib.*, each cow was supplied 2 kg wheat straw to fulfill their DM requirement and to maintain fecal consistency. After an adaptation period of 19 days to cottonseed based diet, the cows were distributed into 2 groups of 8 each on the basis of BW, stage of lactation and milk yield. Control group (Non Bt) continued on the same

concentrate mixture while in experimental group (Bt) non Bt WCS was replaced with Bt WCS. The ration was offered to meet or exceed nutrient requirements (NRC 2001) of experimental cows and concentrate mixture was offered 3 times a day in equal parts at the time of each milking, i.e. 0500, 1200 and 1800 h. Known quantity of fresh berseem fodder was offered at 1000, 1300 and 1900 h, while wheat straw (kg) was offered to each cow at 0800 h. Orts were weighed next morning. DM content of fodder and leftover was determined daily to calculate the DM intake. Fresh and clean water was provided free choice to each cow 3 times a day after each milking during an experimental period of 4 weeks.

Cows were milked manually by full hand milking and milk yield of individual cow was recorded daily. Milk samples from each cow were collected on 0, 7, 14, 21 and 28th day of experimental feeding. Pooled milk samples, consisting milk of each milking in proportion of milk yield of individual cow, were analyzed for chemical composition and part of milk was frozen at -20°C till the analysis of Cry 1 Ac protein. Milk samples were analyzed for fat, protein, lactose, solids-not-fat (SNF) and total solids using precalibrated milk analyzer. Somatic cell counts (SCC) were recorded microscopically (IDF 1984).

Apparent nutrient digestibility of the diets was estimated on 6 cows in each group selected randomly during last 8 days of experimental feeding. Faecal grab samples from each cow were collected at 12 h intervals, continuously for 8 days, and dried in a hot air oven at 80°C . Apparent digestibility of nutrients was worked out taking acid insoluble ash (AIA) as internal marker (Van Keulen and Young 1977).

Blood sample (10 ml) from each cow was collected at 0900 h on 28th day of experimental feeding in a heparinized tube, by puncturing the jugular vein. The plasma was separated at 5 000 rpm for 15 min and analyzed for the quantitative analysis of Bt protein. Haemoglobin (Hb) was estimated by cyanohaemoglobin method and total leukocyte counts (TLC) in whole blood were estimated microscopically using haemocytometer. Alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN) and creatinine values were determined in blood plasma using standard procedures of commercial kits. Cry1Ac in transgenic cottonseeds, milk and blood plasma were estimated using kit. The minimum limit of detection was 100 ng/g seed.

The seed index was measured as the average weight of 100 seeds in gram in 10 replicates (Bertrand *et al.* 2005). Representative samples of both types of crushed cottonseeds were ground (1 mm) after drying for 24 h at 80°C and analyzed for proximate composition, fibre fractions and minerals. The dried samples of seeds, concentrate mixtures, green fodder, left over material and faecal samples from each cow were also ground (1 mm), pooled and analyzed for proximate (AOAC 2005) and fibre fractions including acid

detergent lignin (AOAC 2005). Sodium sulphate and heat stable amylase were not added to the neutral detergent solution and the values were expressed exclusive of ash. Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were also estimated in the NDF and ADF fractions. Calcium, phosphorus (Talpatra *et al.* 1940) and trace elements including heavy metals (atomic absorption spectrophotometer) were estimated in both types of cottonseeds.

The body condition score (BCS) was determined on a 6-point scale (Prasad 1994). Net energy of lactation (NE_L) secretion in Bt and non-Bt groups was estimated on the basis of chemical composition of milk as per NRC (2001). 4% FCM was determined as per Tyrrell and Reid (1965). The Bt protein in concentrate was calculated from that of cottonseed included in concentrate.

Data on feed intake, digestibility, milk yield, milk composition and body weight change were subjected to 't' test using SYSTAT 7.0 software. Significant differences were accepted if $P \leq 0.05$.

RESULTS AND DISCUSSION

The seed index for the linted whole Bt and non-Bt cottonseeds was 8.34 ± 0.12 and 8.40 ± 0.23 , respectively, and the values were close to those reported by Bertrand *et al.* (2005). Bt and non-Bt cottonseeds were having similar proximate composition, fibre fractions and minerals (Table 1) with minor variations. Chemical composition of cottonseeds, feeds and fodders were in their normal range reported by Mujahid *et al.* (2000) and NRC (2001) and supported by Kumar and Singhal (2004), Hamilton *et al.* (2004) and Bertrand *et al.* (2005). The EE content of Bt based concentrate was higher (attributed to the higher EE content of Bt cottonseeds (Table 1)) than non-Bt cottonseeds. NDF and ADF contents of wheat straw were lower than that reported by NRC (2001), possibly due to the expression on ash free basis. The ADICP and NDICP values, estimated for determination of available CP to the ruminal microbes, were within the normal range (NRC 2001). The ratio of calcium and phosphorus was higher in Bt cottonseed (1:2) than in non Bt cottonseed. Concentration of other minerals was higher in Bt cottonseed than in non Bt cottonseed, however, levels in both types of cottonseed were within the range for their optimal utilization in animal diets.

Average DMI during the adaptation period was 15.37 ± 0.22 kg/d and the DMI/100 kg body weight was 3.70 kg. Feeding of Bt and non-Bt cottonseed based concentrate mixture did not affect the voluntary intake of DM and DMI per 100 kg BW in Bt and non Bt groups (Table 2). DMI in both groups was sufficient to fulfill the nutritional requirement, recommended by NRC (2001). Similar level of DMI was recorded earlier when Bt and non-Bt cottonseed based diets, having similar level of cottonseeds, were fed to crossbred lactating cows (Singhal *et al.* 2006 a,b). In present

Table 1. Chemical composition of feeds and fodders (g/kg on DM basis)

Parameter	Non-Bt cottonseed	Bt cottonseed	Non-Bt concentrate	Bt concentrate	Berseem	Wheat straw
Crude protein	244.1	251.0	225.9	227.5	164.5	23.5
Ether extract	170.5	177.8	68.7	75.5	26.4	4.6
Ash	39.1	40.4	67.5	92.0	231	92.5
Acid insoluble ash	3.3	4.6	5.9	6.6	53.5	45.1
NDF	542.6	474.1	512.9	467.7	312.5	644.6
ADF	480.5	395.5	227.7	172.9	257.4	347.8
NDICP	54.2	50.2	25.5	13.9	54.7	17.2
ADICP	22.3	25.3	12.2	7.9	26.5	4.4
ADL	134.6	132.7	50.9	42.4	152.4	75.8
Calcium	2.6	2.1	12.9	14.1	-	-
Phosphorus	4.5	5.1	6.6	7.0	-	-
Copper (ppm)	22.36	21.55	-	-	-	-
Manganese (ppm)	4.20	5.26	-	-	-	-
Zinc (ppm)	44.62	67.55	-	-	-	-
Iron (ppm)	61.87	146.56	-	-	-	-
Lead (ppm)	0.59	0.88	-	-	-	-
Cadmium (ppm)	0.02	0.05	-	-	-	-

ADF, acid detergent fibre; ADICP, acid detergent insoluble crude protein; ADL, acid detergent lignin; NDF, neutral detergent fibre.

Table 2. Influence of transgenic cottonseed based ration on feed intake during the whole trial (mean± standard error)

Feed intake (kg/cow/d)	Non-Bt	Bt (Cry1Ac)	P
Dry matter intake	16.51±0.65	16.64±0.65	0.892
Berseem	11.11±0.32	11.22±0.33	0.942
Wheat straw	0.90±0.02	0.98±0.03	0.163
Concentrate	4.49±0.03	4.45±0.02	0.152
DMI/100 kg BW	3.88	3.64	-

study both types of cottonseed constituted about 11% of total DMI. NDF intake in Bt and non-Bt groups was 43.82 and 45.86% of total DMI, which was similar in both groups and exceeded the minimum value recommended by NRC (2001). Body weight gain during 4 weeks of experimental feeding was similar in both groups (Table 3). All cows maintained normal health and improved their BCS during the trial, showing that the inclusion of Bt cottonseed in the ration did not affect the general health and BCS of cows as recorded by Castillo *et al.* (2004).

During the adaptation period when all cows were fed on non-Bt cottonseed based ration, the average milk yield was 12.94 kg/d. Milk yield was improved during the experimental period in both groups, reflecting the effect of adaptation on experimental diets. The average milk yield and milk composition including the somatic cell counts (SCC) of cows during the experimental period (Table 3) and daily milk yield (Fig. 1) were not affected significantly by the dietary treatments. Net energy of lactation (NE_L) in Bt and non-Bt groups was 10.26 and 10.51 Mcal/d, respectively, and the variation between the groups was not significant. The daily milk yield and 4% FCM yield/d were similar in both groups.

Table 3. Impact of cottonseed based rations on productivity and body parameters

Parameter	Non-Bt	Bt	P
Milk production (kg/day)			
Milk	13.53±0.75	13.12±0.92	0.734
4% Fat corrected milk	14.73±0.82	14.18±0.99	0.909
Protein	0.42±0.02	0.41±0.03	0.708
Fat	0.64±0.04	0.62±0.04	0.761
Lactose	0.63±0.03	0.62±0.04	0.752
Solids-not-fat	1.17±0.06	1.13±0.08	0.680
Milk (kg)/DMI (kg)	0.82	0.79	-
Milk composition (g/kg)			
Protein	31.0±0.3	30.9±0.3	0.628
Fat	45.9±0.3	45.4±0.5	0.161
Lactose	47.0±0.5	46.9±0.6	0.883
Solids-not-fat	87.2±0.9	87.0±1.0	0.822
Somatic cell counts (× 10 ⁵ /ml)	1.09±0.03	1.03±0.03	0.14
Body weight (kg)			
Initial mean	401.63±15.6	428.25±18.3	0.260
Final mean	428.25±14.6	456.66±19.2	0.287
Change in 4 weeks	26.62±2.19	28.41±3.36	0.664
Body condition score			
Initial mean (units)	3.60±0.12	3.58±0.19	0.938
Final mean (units)	3.70±0.12	3.64±0.10	0.794
Change (units/4 weeks)	0.10±0.03	0.06±0.04	0.472

Values represent mean± standard error.

Milk yield/kg DM intake in non-Bt and Bt groups was 0.82 and 0.79, respectively, and the variation between the groups was not significant. Though the trend of milk yield in Bt and non Bt groups was similar to that recorded by Singhal *et al.* (2006a), however, values were higher than that recorded in

Table 4. Intake and nutrient digestibility in lactating cows during digestibility trial period

	Non-Bt	Bt (Cry 1 Ac)	P
Feed intake (kg/cow/d)			
Dry matter intake	14.46±0.33	14.69±0.32	0.508
Berseem	9.23±0.06	9.17±0.06	0.052
Wheat straw	0.55±0.09	0.93±0.09	0.876
Concentrate	4.64±0.19	4.53±0.25	0.779
Apparent digestibility			
Dry matter	0.693±0.009	0.690±0.009	0.852
Organic matter	0.765±0.006	0.757±0.009	0.491
Crude protein	0.776±0.014	0.769±0.011	0.237
Ether extract	0.885 ^a ±0.010	0.846 ^b ±0.004	P<0.01
Neutral detergent fibre	0.683±0.007	0.671±0.013	0.444
Acid detergent fibre	0.634±0.013	0.664±0.019	0.237

Values represent mean±standard error, means with different superscripts in a row differ significantly (P< 0.01).

earlier study, which was possibly due to the difference in the stage of lactation as well as quality of fodder made available to the cows. Singhal *et al.* (2006 a,b) and Castillo *et al.* (2004) did not record any adverse effect of feeding cottonseed having Cry1Ac alone or in combination with Cry2Ab to the lactating cows. Milk SCC ($\times 10^5$ of cell/ml) in both groups were within the normal range, reported by Singh and Ludri (2001), which indicated the normal udders status of experimental cows.

The apparent nutrient digestibility was similar in both groups, except that for ether extract, which was higher (P<0.05) in non-Bt group than that in Bt group (Table 4). Similar DM digestibility was recorded by Moreira *et al.* (2004) however, lower values were reported by Sullivan *et al.* (2004) on feeding similar or higher levels of cottonseed. Higher CP digestibility in both the groups than that reported by Singhal *et al.* (2006a,b) for Bt and non Bt cottonseed based diets may be attributed to the higher availability of nitrogen from early cut berseem fodder. It was also recorded that mean intake of Cry1Ac protein through Bt cottonseed was approximately 400 µg/cow/d, which appeared to be degraded during the course of its rumen fermentation as per the fate of dietary proteins (Kumar and Singhal 2004).

Nonsignificant variation between the groups for TLC and haemoglobin as well as plasma ALP, AST, ALT, BUN and creatinine values (Table 5) showed normal blood biochemical profile in all cows of both groups (Kaneko 1997). Similar values were recorded by Saijpal *et al.* (2006) following the feeding of similar level of cottonseed based rations. However, higher Hb, serum ALP, AST and ALT values were reported by Singh *et al.* (2003) on feeding transgenic cottonseed (Cry1Ac) based rations to lactating buffaloes but they also did not find any significant variation between Bt and non-Bt groups. These observations suggested that from Bt as well as non-Bt cottonseed did not affect the liver functioning in crossbred cows adversely. Higher BUN level in Bt group may be attributed to the higher intake of DM from berseem

Table 5. Blood biochemical profile of lactating crossbred cows

	Non-Bt	Bt (Cry 1 Ac)	P
ALP (units/L)	53.55±4.69	49.68±10.23	0.739
ALT (units/L)	32.38±1.86	28.13±3.72	0.330
AST (units/L)	47.38±3.71	44.00±5.56	0.623
BUN (mg/100 ml)	22.01±1.49	25.99±2.88	0.169
Creatinine (mg/100 ml)	1.28±0.29	1.15±0.23	0.890
Hb (g/100 ml)	9.92±0.29	10.05±0.20	0.344
TLC ($\times 10^5$ /ml)	10.45±1.87	10.69±1.98	0.682

Values represent mean± standard error.

fodder, rich in nitrogen. The BUN and creatinine values of Bt and non-Bt groups were within the normal range (Kaneko 1997) and similar to those reported by Saijpal *et al.* (2006). Coppock *et al.* (1985) reported nonsignificant variation (P>0.05) in the serum profiles such as blood urea N, creatinine and ratio of blood urea N to creatinine following the feeding of whole cottonseed based ration up to 430 days. Biochemical and enzymatic profile of blood of lactating crossbred cows indicated that presence of Cry proteins in the ration did not affect their liver and kidney functions adversely.

Feeding of Bt cottonseeds (having Cry 1C protein 0.195 mg/g) to dairy cows resulted in intake of Cry 1Ac protein to the level of about 400 µg/cow/d but it was neither detected in milk nor in blood samples, collected at different intervals during 4 weeks period, which suggested that it was either digested in the gastrointestinal tract or not absorbed across the intestinal mucosa. It was possibly due to the degradation of these proteins along with other dietary proteins during the course of rumen fermentation as hypothesized earlier by Phipps *et al.* (2003), Singh *et al.* (2003) and Singhal *et al.* (2006 a, b), without any adverse effect. Castillo *et al.* (2004) on feeding BG II cottonseed and Faust *et al.* (2007) on feeding Bt corn (Cry 1F) silage to lactating cows also recorded non detection of Bt protein in milk and blood. Thus, the DNA and proteins may be undergoing degradation in

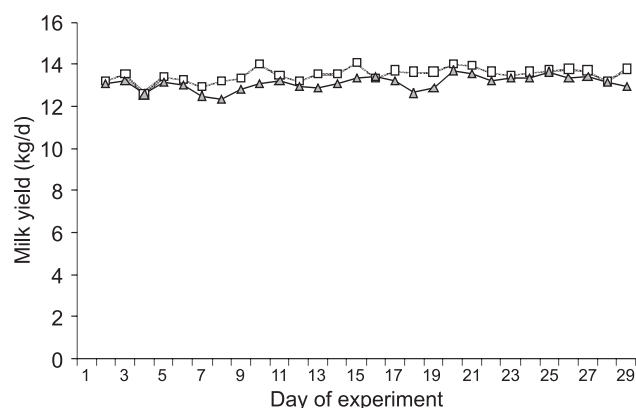


Fig 1. Milk yield (kg/d) of cows fed Bt and Non-Bt cottonseed based rations

gastrointestinal tract (McAllan and Smith 1973). This is supported by the finding of whole or some degradation products of Cry proteins and recombinant genes in the rumen or stomach, but not in the intestine, body tissues or secretions like milk (Deaville and Maddison 2005, Sharma *et al.* 2006) and also not found in gastric and intestinal simulating fluid (Ream 1994).

Incorporation of transgenic crushed whole delinted cottonseed @ 10.7% of total DMI in the lactating dairy cows had shown similar DMI, BW gain, nutrient digestibility, milk yield, milk composition and blood biochemical parameters to non-transgenic cottonseed based rations under similar feeding conditions for 4 weeks. Thus, cottonseed expressing Cry1Ac protein is as safe and nutritious as conventional cottonseed when fed to lactating dairy cows.

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