Comparative nutritional evaluation of transgenic cottonseeds containing Cry1C protein for ruminant feeding

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Abstract

An experiment was designed to compare the compositional and *in vitro* fermentation characteristics of chemically delinted transgenic cottonseed (Bt, *cry1C*) and its non-transgenic counterpart (non-Bt). The chemical compositions of both types of cottonseeds including minerals were in their normal range. Total gas production (ml/ 500 mg substrate) from Bt and non-Bt cottonseeds was similar (P>0.05) and both types of cottonseeds, irrespective of presence or absence of Cry protein, were having similar degradability for dry matter and organic matter (P>0.05). The ammonia nitrogen concentration in the incubation medium was similar (P>0.05) for both substrates. Similar NH_3-N concentrations in the inoculums and methane concentration in the total gas produced during the fermentation of 24 h indicated that degradation of cottonseed proteins in the rumen, irrespective of presence or absence of foreign protein (Cry1C), was similar. Thus, fermentability of cottonseeds in the rumen, having Cry1C protein, was similar to its traditional counterpart under *in vitro* conditions, which shows its potential for ruminant feeding.

Keywords: Degradability, genetically modified cottonseed, in vitro, ruminal fermentation

Introduction

Cotton, being one of the predominant crops in India and its vulnerability to bollworm insect infestation, India has adopted the transgenic cotton cultivation, having Crystal genes (such as *cry1Ac, cry2Ab, cry1C etc)* isolated from soil bacterium, *Bacillus thuringiensis* (Bt). Transgenic cotton is being cultivated on about 90% of total area under cotton crop in India and area under transgenic cotton is increasing in many countries (James 2009). However, several doubts have been raised about the safety aspects of cottonseed, a byproduct of this crop being extensively used for feeding of lactating cows and buffaloes. As farmers from developing countries are adopting them the nutritional and biosafety aspects needs to be well regulated. The transgenic cottonseeds were found similar to their near isogenic or commercial

counterparts (Bertrand et al 2005) or showed minor variations (Kumar and Singhal 2004; Tang et al 2006) in their chemical composition and feeding values (Mohanta et al 2010). Though the health and the quality of milk was found similar in Bt and non Bt cottonseed fed cows besides non-detection of the Bt proteins in milk or blood, as the Cry protein gets degraded in to smaller fragments in the rumen and still smaller fragments during the subsequent degradation in the digestive tract of ruminants. However, only a few reports are there about the effect of Bt cottonseed on the rumen fermentation pattern (Kumar and Singhal 2004; Bertrand et al 2005). Therefore, in this experiment, efforts were made to compare the effect of a newly developed variety of whole cottonseed (WCS), having *cry1C* gene (event MLS9124) on compositional attributes and rumen fermentation.

Materials and methods

Procurement of samples

Chemically delinted transgenic whole cottonseeds (Bt, with *cry1C* gene), using sulphuric acid, were procured along with their commercial counterparts (Non-Bt) from M/s. Metahelix Life Sciences Pvt. Ltd., Bangalore, India.

Chemical Analysis

The seeds were sampled from the lot and dried at 80^0C for 24 h before grinding to pass 1 mm sieve. The Proximate principles were analyzed as per (AOAC 2005) and fibre fractions (Goering and Van Soest 1970). The fibre values were expressed exclusive of ash. Acid detergent insoluble crude protein (ADICP) values were estimated for determination of available CP for the ruminal microbes by estimating the crude protein content in the ADF fraction. Calcium and phosphorus in both types of cottonseeds and concentrate mixtures were analyzed as per Talapatra et al. (1940), other minerals using atomic absorption spectrophotometer (Hitachi Z-5000 Polarized Zeeman) and free gossypol content (Smith 1968). Cottonseeds were analyzed for the quantitative detection of Cry1C protein using ELISA kit from Envirologix, USA. The limit of detection was 1 ppb.

Rumen fermentation study

The experiment was done by using randomized block design in triplicates for degradability parameters and in six numbers for other parameters. Incubations were carried out in 100 ml calibrated glass syringes (Haberle Labortechnik, Lonsee-Ettlenschie, Germany) as described by Menke et al (1979) and Menke and Steingass (1988). Syringes were incubated in triplicate for 24 h in a water bath at 39 ± 0.5^0C (Blummel and Ørskov 1993). Rumen inoculum was obtained before morning feeding from two rumen fistulated male bullocks fed on a forage based diet (1.0 kg concentrate mixture in equal proportions at 10:00 and 16:00 h and wheat straw *ad libitum*). The proportion of buffer medium to rumen fluid was 2:1. Buffered rumen fluid (30 ml) was used per syringe having powdered Bt or non-Bt cottonseed (500 mg) as substrate or blank syringe (n=3) without substrate. Mulberry (*Morus alba*) leaves powder having similar particle size was used as internal standard. Syringes were shaken every 30 min for first 2 h from the start of incubation and thereafter every 2 h up to 10 h.

In vitro gas production test

After 24 h incubation, total gas production was estimated by recording the displaced position of piston during incubation and gas produced from fermentation of substrates were expressed after correcting for the blank. An aliquot of the total gas (5 ml) was taken from the headspace of syringe in an airtight syringe and injected into gas chromatograph (Nucon-5700) equipped with flame ionization detector and stainless steel column packed with Porapak-Q for methane estimation. The gas flow rates for nitrogen, hydrogen and air were 40, 30 and 300 ml/min. The temperature of injector, oven and detector was maintained at 100, 60 and 200^{0}C, respectively. A 50:50 mixture of methane and carbon dioxide (Spancan, Spantech Products Ltd., England) was used as a standard.

Degradability analysis

True dry matter degradability (TDMD) was estimated as per Goering and Van Soest (1970). Truly organic matter degradability (TOMD) was calculated as the amount of substrate organic matter (OM) incubated minus the amount of substrate OM recovered as residue after neutral detergent solution treatment. Ammonia nitrogen (NH_3-N) concentration in the incubation medium was determined after centrifugation taking 5 ml supernatant from centrifuge tube using Kjeldahl method and expressed as mg/100 ml of incubation medium. The statistical analysis of data on *in vitro* parameters was done with SYSTAT 7.0 software using paired 't' test.

Results and discussion

The chemical composition of both types of cottonseeds was similar irrespective of the presence or absence of *cry1C* gene (Table 1).

Table 1. Chemical composition of cottonseeds (% Dry matter basis unless mentioned)

Ingredients	Bt cottonseed	Non-Bt cottonseed
Proximate constituents		
Crude protein	26.7	27.0
Ether extract	22.8	21.2
Ash	4.01	4.19
Acid insoluble ash	0.22	0.51
Fiber composition		
Neutral detergent fibre	35.0	36.9
Acid detergent fibre	23.3	26.2
Acid detergent insoluble CP	1.43	1.69
Acid detergent lignin	8.21	9.12
Mineral composition		
Calcium	0.16	0.15
Phosphorus	0.45	0.42
Copper, ppm	22.5	22.4
Manganese, ppm	4.24	4.20
Zinc, ppm	40.8	44.3
Iron, ppm	47.2	61.9
Lead, ppm	0.63	0.59
Cadmium, ppm	0.04	0.02

The Bt cottonseed had Cry1C protein concentration of 2.44 mg/g of seed on fresh basis. The free gossypol content in Bt and Non-Bt cottonseed (delinted) was 0.182 and 0.160%, respectively. Free gossypol content of both types of cottonseeds was lower than the values reported by Bertrand et al (2005) in Bt and Non-Bt cottonseed. The other compositional values were within the range described by Mujahid et al (2000) and Ranjhan (1998) for cottonseeds of South Asian region. Similar findings were obtained by Kumar and Singhal (2004) and Bertrand et al (2005) while comparing Bt and non-Bt cottonseeds. The mineral composition and heavy mineral content were also similar in both types of whole cottonseeds (Table 1). The chemical compositions indicate that there are no alterations in chemical composition induced by insertion of the genetic alteration in the cotton plant.

Total gas production (ml/500 mg substrate) from Bt and non-Bt cottonseeds also followed the same trend (Table 2) indicating that rumen microbes were not influenced by the 'foreign' protein as the quantity of gas produced during the *in vitro* fermentation of a substrate is closely related to its digestibility and consequently to its energetic value (Getachew et al 2002).

Methane production from Bt cottonseed and non-Bt cottonseed inoculums' was also similar ($P>0.05$) both in terms of quantity and as a percentage of total gas produced (Table 2) indicating similar usage of nutrient fractions in the inoculum by rumen microbes. True dry matter degradability (TDMD) of Bt and non-Bt cottonseeds was not significant ($P>0.5$). True organic matter degradability (TOMD) followed the same trend as recorded for TDMD (Table 2). However, the values were higher than those reported by Bertrand et al (2005) for transgenic and isogenic cottonseeds *in vitro*. These observations further confirmed that transgenic cottonseed did not exert any adverse effect on ruminal microbes and their activity.

Table 2. Influence on rumen fermentation parameters and *in vitro* degradability

Attribute	**Bt (Cry1C)**	**Non-Bt**	***P* value**
Total gas, ml/ 24 h	35.4±0.88	35.7±1.43	0.89
Methane, ml/ 24 h	7.61±0.38	7.44±0.27	0.72
Methane, %	21.3±0.24	21.1±0.58	0.69
Ammonia nitrogen, mg/100ml	16.8±2.34	16.9±1.45	0.99
True dry matter degradability, %	69.2±3.27	68.9±2.93	0.94
True organic matter degradability, %	69.4±2.67	67.5±1.90	0.53

The ammonia concentration in the incubation medium was similar ($P>0.05$) for both substrates indicating that ruminal degradation of cottonseed proteins, irrespective of presence or absence of foreign protein (Table 2). Earlier, Kumar and Singhal (2004) did not find any difference in the rumen degradable and rumen undegradable protein fractions of Bt cottonseed having *cry1Ac* gene.

Sung et al (2006) also did not observe any significant difference on *in vitro* fermentation characteristics of transgenic corn (Mon 810 and Event 176) with respect to isogenic counterpart (DK729). Wiedemann et al (2007) found no adverse effect of transgenic maize feeding on the dynamics of six ruminal bacterial strains (investigated by real-time PCR) compared to the conventional maize silage. Ream (1994) reported that Bt protein and its associative functional activity were readily degraded upon exposure to simulated gastric and intestinal fluid *in vitro* suggesting degradation of Bt protein in the mammalian digestive tract.

Phipps et al (2003) reported that *cry* DNA was degraded progressively in the gastrointestinal tract starting from rumen with other endogenous plant DNA in lactating dairy cows without appearing in the blood or milk which has been further demonstrated for both cry gene and Cry protein by Guertler et al (2010)

Conclusion

Similar *in vitro* fermentation pattern of Bt cottonseeds and non-Bt cottonseeds in the present experiment reflected similar degradation pattern and ruminal fermentation irrespective of presence or absence of Cry protein. This finding also supports that fate of Cry protein follows that of other proteins in rumen. However, more elaborative study on this aspect is required both *in vitro* and *in vivo* to ascertain this claim.

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