Effect of Feeding Transgenic Cottonseed vis-à-vis Non-transgenic Cottonseed on Haematobiochemical Constituents in Lactating Murrah Buffaloes

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ABSTRACT : An experiment was conducted to investigate the effect of feeding transgenic cottonseed (Bt.) vis-à-vis non-transgenic (non-Bt.) cottonseed on blood biochemical constituents in lactating Murrah buffaloes. Twenty Murrah buffaloes in mid-lactation were divided into 2 groups of 10 each. Animals of group I were fed with 39.5% non-transgenic cottonseed in concentrate mixture while the same percentage of transgenic (Bt.) cottonseed was included in the concentrate mixture fed to the animals of group II. Animals of both groups were fed with concentrate mixture to support their milk production requirements. Each buffalo was also offered 20 kg mixed green fodder (oats and berseem) and wheat straw *ad libitum*. The experimental feeding trial lasted for 35 days. There was no significant difference in the dry matter intake between the two groups of buffaloes. All the buffaloes gained body weight, however, the differences were non significant. Total erythrocyte count, hemoglobin content and packed cell volume were $9.27\pm0.70\times10^6/\mu l$, 13.01±0.60 g/dl and 34.87±1.47%, respectively in group I with the corresponding figures of 8.88±0.33, 12.99±0.52 and 31.08±1.52 in group II. The values of total erythrocyte count, haemoglobin content and packed cell volume did not differ significantly between the two groups of buffaloes. The concentration of plasma glucose, serum total proteins, albumin, globulin, triglycerides and high density lipoprotein were non significantly higher in buffaloes fed non-transgenic cottonseed than in buffaloes fed transgenic cottonseed. The cholesterol concentration was significantly ($p<0.01$) higher in buffaloes of group I (136.84±8.40 mg/dl) than in buffaloes of group II (105.20±1.85 mg/dl). The serum alkaline phosphotase, glutamic-oxaloacetate transaminase and glutamic-pyruate transaminase activities did not differ significantly between two groups of buffaloes. However, serum glutamic-pyruate transaminase activity was considerably high in buffaloes fed non-transgenic cottonseed as compared to buffaloes fed transgenic cottonseed. Bt. proteins in serum samples of animals of group II were not detected after 35 days of feeding trial. It was concluded that transgenic cottonseed and non-transgenic cottonseed have similar nutritional value without any adverse effects on health status of buffaloes as assessed from haematobiochemical constituents. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 12 : 1732-1737)***

Key Words : Transgenic Cottonseed, Bt. Protein, Lactating Murrah Buffaloes, Haematobiochemical Constituents, *Bacillus thuringiensis*

INTRODUCTION

High-producing dairy cows and buffaloes need good quality green fodders and concentrates containing high proportion of grains, protein supplements and other by-products. Traditionally, cotton (*Gossypium hirsutum*) seed is also incorporated in the ration of lactating cows and buffaloes as source of energy and protein by the farmers in cotton growing areas in order to increase milk yield and fat content in the milk. Recently, Monsanto Company, USA has developed a genetically modified (GM) cotton variety by inserting an insect tolerance trait with gene coding for Cry 1 Ac protein derived from *Bacillus thuringiensis* (Bt. protein) var. Kurstaki which encodes for the production of a protein that is insecticidal to lipidopteron insects/pests of cotton but is safe to mammals, birds, fish and beneficial insects. This genetically modified variety of cotton is resistant to certain insects and pests especially the 'ballworm complex'. In India, Maharastra Hybrid Seeds Company (MAHYCO) Ltd. has crossed genetically engineered cotton developed by Monsanto in USA with Indian cotton to insert Cry 1 Ac gene into an Indian cotton hybrid to minimize use of chemical sprays on cotton crop to provide a low cost alternative for insect and pest control. Prior to commercialization of newly developed variety of transgenic cotton (Bt. Cotton) in India, it is mandatory to investigate effect of Bt. protein expressed in it by inserted gene on domestic animals. The studies conducted in growing kids at Industrial Toxicology Research Centre, Lucknow, India has shown that the growth performance of kids fed ration containing Bt. and non-Bt. cottonseed was similar during 90 days of feeding trial (Dutta and Dogra, 1998). It was also observed that nutritional value of transgenic cottonseed was at par with that of non-transgenic cottonseed in terms of feed intake, nutrients digestibility, milk yield and milk composition in lactating Murrah buffaloes (Singh et al., 2002). In the present communication, effects of feeding transgenic cottonseed on certain haematobiochemical constituents in lactating Murrah buffaloes have been reported.

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MATERIALS AND METHODS

Experimental animals and their housing

Twenty lactating Murrah buffaloes in mid lactation were selected from the herd of Livestock Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar

Table 1. Ingredient composition of experimental concentrate mixtures (parts per quintal)

Ingredients	Concentrate mixtures	
	1 [Non-transgenic (Non-Bt.) group]	2 [Transgenic (Bt.) group]
Milk feed[1]	59.5	59.5
Non-transgenic (non-Bt.) cottonseed	39.5	-
Transgenic (Bt.) cottonseed	-	39.5
Mineral mixture[2]	1.0	1.0

[1] Milk feed contained barley/maize, 15%; mustard cake, 10%; de-oiled mustard cake, 10%; de-oiled groundnut cake, 5%; de-oiled rice polish, 32%; wheat bran, 13%; molasses, 10%; common salt, 2%; mineral mixture, 0.5%; and calcine powder, 2.5% and nutrisac 50 g/quintal. Milk feed was obtained from U.P. State Agro Industrial Corporation Limited, Harthala, Moradabad (U.P.).

[2] Mineral mixture supplied by Dabur Ayur Vet Ltd. contained 0.3 kg calcium, 0.325 kg phosphorus, 0.0312 g copper, 0.045 g cobalt, 2.114 g magnesium, 0.979 g iron, 2.13 g zinc, 0.166 g iodine and 1.82 g DL-methionine and 4.4 g L-lysine mono hydrochloride.

maintained in stalls and fed milk feed, a dairy concentrate mixture, according to milk yield and mixed green fodder (oats and berseem) to *ad libitum* intake. All the buffaloes were housed in a well ventilated shed having concrete *pucca* floor with individual feeding arrangement and tied with iron chains in a tail to tail arrangement during adaptation (2 weeks) and experimental (5 weeks) feeding periods. The buffaloes were tied so that they could move freely without getting access to the feed of the other buffaloes. All the buffaloes had unique identification numbers tattooed on the hip region. The animals were cleaned daily by splashing them with fresh, clean water. Proper hygienic conditions and healthy surroundings were maintained in the shed throughout the experimental feeding period.

Feeding schedule

Initially all the buffaloes were fed concentrate mixture consisting of 59.5 parts milk feed, 39.5 parts crushed cottonseed (non-transgenic) and 1 part mineral mixture as per the nutritional requirement (Kearl, 1982) alongwith 20 kg mixed green fodder (oats and berseem) following chaffing and wheat straw *ad libitum* for a period of two weeks which was observed as adaptation period in order to get the rumen microbes accustomed with changed feed supplement. Thereafter, the buffaloes were divided into 2 groups of 10 each on the basis of their average milk yield during adaptation period so that both groups had similar milk yield. The buffaloes in group I were continued on the same concentrate mixture containing non-transgenic (non-Bt.) cottonseed whereas, the buffaloes of group II were fed concentrate mixture containing 39.5 parts crushed transgenic (Bt.) cottonseed in place of non-transgenic cottonseed as per their nutritional requirements (Kearl, 1982) alongwith 20 kg mixed green fodder (oats and berseem) following chaffing and wheat straw *ad libitum* to fulfil their dry matter requirement and to maintain good faecal consistency. The ingredient composition of experimental concentrate mixtures is given in Table 1. The buffaloes in both the groups were offered weighed quantity of daily concentrate allowance in two equal installments at the time of milking at 4:30 and 16:30 h whereas, weighed quantities of mixed green fodder (oats and berseem) following chaffing and wheat straw were offered 2 times daily at 10:00 and 17:00 h. All the buffaloes were also offered clean drinking water *ad libitum* thrice daily at 8:00, 14:00 and 19:00 h. During the experimental feeding period, the leftover feed (wheat straw along with some green fodder) was weighed and recorded daily. The dry matter content of the feeds offered and leftover was recorded daily (AOAC, 1990). All the buffaloes were weighed at the start and end of the both adaptation and experimental feeding period before feeding and watering in the morning for 3 consecutive days.

Collection and sampling of blood

Blood samples of approximately 10 ml from each buffalo were collected from jugular vein at the onset and end of the experimental feeding period prior to feeding and watering of the animals in the morning. Sterilized sharp needles were used and blood samples were collected in clean, dry glass tubes containing ethylene diamine-tetra acetic acid (EDTA) as an anticoagulant at the rate of 1 mg/ml of blood for estimation of total erythrocyte count, packed cell volume and haemoglobin content. Plasma was separated by centrifugation of blood samples at 1,381 g and stored at -20°C in deep freezer until analysed for glucose. Simultaneously, 10 ml of blood from each buffalo was also collected for obtaining the serum for analysis of total protein, albumin, globulin, cholesterol, high-density lipoprotein (HDL), triglycerides and to determine the alkaline phosphatase, glutamic-oxaloacetate transaminase and glutamic-pyruvate transaminase activities.

Chemical analysis

The samples of feed offered and residue left were analysed for proximate principles according to AOAC (1990). Both transgenic (Bt.) and non-transgenic (non-Bt.) cottonseeds were tested for the presence of Bt. protein (Sims and Berberich, 1996) and the quantitative estimation of Bt. protein in Bt. cottonseed were done using ELISA (Sims and Berberich, 1996) method. The blood samples were analysed for total erythrocyte count, packed cell volume and haemoglobin content by the methods as described by Jain (1986). Plasma glucose, serum protein, albumin, cholesterol, high density lipoprotein (HDL), triglycerides, and serum enzymes (alkaline phosphatase,

Table 2. Chemical composition of feeding stuffs (% dry matter basis)

Feeding stuffs	Dry matter	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen-free extract	ash
Concentrate mixture 1 (Non-Bt.)	93.30	89.18	18.46	7.13	12.21	51.38	10.82
Concentrate mixture 2 (Bt.)	93.02	89.49	19.48	7.95	11.87	50.19	10.51
Milk feed[a]	93.10	86.61	16.04	1.85	8.76	59.96	13.39
Non-transgenic cottonseed (Non-Bt.)	91.57	94.98	22.40	14.87	17.48	40.23	5.02
Transgenic cottonseed (Bt.)	91.94	95.70	24.89	16.86	16.63	37.32	4.30
Mixed green fodder (oats and berseem)	29.14	91.43	8.50	3.12	32.62	47.19	8.57
Wheat straw	93.84	91.38	3.37	0.50	43.13	44.38	8.62

[a] Milk feed contained barley/maize, 15 parts; mustard cake, 10 parts; de-oiled mustard cake, 10 parts; de-oiled groundnut cake, 5 parts; wheat bran, 13 parts; de-oiled rice polish, 32 parts; molasses, 10 parts; common salt, 2 parts; mineral mixture, 0.5 parts; calcine powder, 2.5 parts and nutrisac 50 mg/100 kg.

Table 3. Average daily dry matter consumption, concentrate:roughage ratio and body weight change in lactating murrah buffaloes fed ration containing non-transgenic and transgenic cottonseed during experimental feeding period of 35 days

Particulars	Groups	
	Group I (Non-transgenic)	Group II (Transgenic)
Average body weight (kg)	450.75±13.73	476.15±15.48
Roughage dry matter intake (kg)	11.84±0.14	12.06±0.16
Concentrate dry matter intake (kg)	4.46±0.25	4.55±0.25
Concentrate: roughage ratio	1:2.79±0.15	1:2.72±0.14
Total dry matter intake (kg)	16.30±0.35	16.61±0.38
Dry matter intake/100 kg body weight (kg)	3.64±0.11	3.52±0.11
Dry matter intake/$W^{0.75}$ kg (g)	167.15±4.16	163.76±4.55
Body weight change (kg/d)	0.494±0.05	0.426±0.09

glutamic-oxaloacetate transaminase and glutamic-pyruvate transaminase) activity were determined following the method of Oser (1979) using Autopak reagent kit of Bayer Diagnostics, Baroda, India. The blood samples from each buffalo collected on 35th day of experimental feeding were analysed for the presence of Bt. protein by the method of Sims and Berberich (1996).

Statistical analysis

The data recorded were analysed statistically using students's paired 't' test by the methods given by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Chemical composition of feeding stuffs

The chemical composition of different feeding stuffs is presented in Table 2. The transgenic (Bt.) cottonseed had higher fat and protein contents by 13.38 and 11.12%, respectively than non-transgenic (non-Bt.) cottonseed. The chemical composition of both the varieties of cottonseeds corroborated with the analysis report of Subramaniam (2000). The total and free gossypol contents of the Bt. cottonseed were 1.23% and 1.14%, respectively, while the unmodified cottonseed contained 1.52% and 1.39% of the total and free gossypol on a dry matter basis. The genetic insertion of Bt. protein reduced the total and free gossypol content compared to the non-Bt. cottonseed. Each gram of transgenic cottonseed contained 20 μg Bt. protein.

Dry matter intake and body weight change

The dry matter intake and body weight change data are presented in Table 3. The average daily dry matter intake in buffaloes fed ration containing non-transgenic and transgenic cottonseed was 16.30±0.35 and 16.61±0.38 kg, respectively. There was no significant difference in daily dry matter intake between the two groups of buffaloes. The intake of non-transgenic and transgenic cottonseeds was 1.86 and 1.82 kg/d, respectively on dry matter basis in groups I and II, which was 11.16 and 11.20% of the total dry matter intake. The daily dry matter intake/kg $W^{0.75}$ in buffaloes was 167.15±4.16 g in group I and 163.76±4.55 g in group II and the values did not differ between the groups. In the present study, the dry matter intake in lactating buffaloes was considerably higher than those reported by Mudgal and Mulla (1985) and Tiwari and Patle (1983, 1985). The dry matter intake was 24.4% higher than that recommended (133 g/kg $W^{0.75}$) in feeding standards (Kearl, 1982) which might be due to fact that experimental rations contained higher level of energy due to supplementation of

Table 4. Average haematological values in blood of lactating Murrah buffaloes fed ration containing non-transgenic and transgenic cottonseed

Particulars	Groups	
	Group I (Non-transgenic)	Group II (Transgenic)
Total erythrocyte counts ($\times 10^6/\mu l$)	9.27±0.70	8.88±0.30
Haemoglobin (g/dl)	13.01±0.60	12.99±0.52
Packed cell volume (%)	34.87±1.47	31.08±1.52

cottonseeds. Kurar and Mudgal (1980) also opined that dry matter intake in lactating buffaloes varied in relation to the energy level in the diet as also the interaction of protein and energy and these increase the dry matter intake in lactating buffaloes. All the buffaloes in groups I and II gained body weight during experimental period. The average weight gain was 0.494±0.05 kg/d in group I and 0.426±0.09 kg/d in group II and there was no significant difference in body weight gain in buffaloes fed non-transgenic and transgenic cottonseed.

Haematological parameters

The mean values for total erythrocyte count, haemoglobin content and packed cell volume in the blood of lactating Murrah buffaloes fed non-transgenic and transgenic cottonseed are presented in Table 4. The mean total erythrocyte counts ($\times 10^6/\mu l$), haemoglobin content (g/dl) and packed cell volume (%), respectively were 9.27±0.70, 13.01±0.60 and 34.87±1.47 in buffaloes of group I fed non-transgenic cottonseed whereas, the corresponding values in buffaloes of group II fed transgenic cottonseed were 8.88±0.30, 12.99±0.52 and 31.08±1.52. There was no significant difference in the mean values of total erythrocyte counts, haemoglobin content and packed cell volume between the two groups of buffaloes. The values of haematological parameters were in the normal range as reported for bovines (Chauhan, 1995). Similar to the present observation, Lindsay et al. (1980) also reported that feeding of cottonseed meal containing 0.225% gossypol did not adversely affect the packed cell volume in cows, however, the haemoglobin content was depressed by 9th week in cows fed solvent extracted cottonseed. They also observed erythrocyte fragility. On the contrary, Hawkin et al. (1985) did not find any effect on erythrocyte fragility in lactating cows fed whole cottonseed. Coppock et al. (1987) opined that gossypol toxicity can occur in ruminants from feeding of whole cottonseed and cottonseed meal but it is unlikely to occur at 3 to 4 kg/d of either feed stuffs but may occur if large amounts of whole cottonseed or cottonseed meal are included in the diet.

Blood biochemical constituents

Table 5. Average blood biochemical constituents in lactating Murrah buffaloes fed ration containing non-transgenic and transgenic cottonseed

Particulars	Groups	
	Group I (Non-transgenic)	Group II (Transgenic)
Plasma glucose (mg/dl)	77.50±6.28	69.55±7.04
Serum protein (g/dl)	6.15±0.42	5.80±0.41
Serum albumin (g/dl)	2.82±0.11	2.67±0.12
Serum globulin (g/dl)	3.33±0.37	3.13±0.43
Albumin:Globulin ratio	0.98±0.15	0.96±0.18
Serum tryglycerides (mg/dl)	54.52±6.71	40.43±6.79
Serum cholesterol (mg/dl)**	136.84±8.40	105.20±1.85
Serum high density lipoprotein (mg/dl)	119.55±14.72	110.78±8.59

** Values in a row differ significantly ($p< 0.01$) from each other.

The mean values for plasma glucose, serum protein, albumin, globulin, albumin: globulin ratio, serum triglycerides, cholesterol and high density lipoprotein (HDL) are presented in Table 5. The concentration of plasma glucose, serum protein, albumin, globulin, triglycerides and HDL did not differ significantly between the two groups of buffaloes. However, serum cholesterol concentration was significantly higher in buffaloes fed non-transgenic cottonseed (136.84±8.40 mg/dl) than in buffaloes fed transgenic cottonseed (105.20±1.85 mg/dl) and this may be attributed to the variation within the groups of buffaloes fed non-transgenic cottonseed (94.25 to 170.85 mg/dl). Although the values for all the biochemical constituents were in the normal range reported for bovines (Chauhan, 1995), but these were lower in buffaloes fed ration containing transgenic cottonseed than in buffaloes fed ration containing non-transgenic cottonseed. The results of this study corroborate the findings of Colin-Negrete et al. (1996) who found no differences in the levels of serum albumin, globulin, total protein, blood glucose and total cholesterol due to feeding whole cottonseed to growing Holstein heifers. Similarly, Barraza et al. (1991) also did not find any effects on albumin and protein in blood of lactating cows due to cottonseed feeding, however, cholesterol concentration was considerably increased but no signs of gossypol toxicity were observed.

Serum alkaline phosphatase, glutamic-oxaloacetate transminase and glutamic-pyruvate transminase activities and detection of Bt. protein in blood.

The mean values of serum alkaline phosphatase, glutamic-oxaloacetate transaminase and glutamic-pyruvate transaminase activities are presented in Table 6. There were no differences in the activities of serum alkaline phosphatase, glutamic-oxaloacetate transaminase and glutamic-pyruvate transaminase between the two groups of buffaloes fed ration containing non-transgenic and

Table 6. Average serum alkaline phosphatase, glutamic-oxaloacetate transaminase and glutamic-pyruvate transaminase activities and Bt. protein in lactating murrah buffaloes fed ration containing non-transgenic and transgenic cottonseed

Particulars	Groups	
	Group I (Non-transgenic)	Group II (Transgenic)
Serum alkaline phosphatase (units/l)	114.35±9.38	124.10±12.47
Serum glutamic-oxaloacetate transaminase (Aspartate aminotransferase) (units/l)	319.95±46.93	348.75±49.79
Serum glutamic-pyruvate transaminase (Alanine aminotransferase) (units/l)	102.25±31.85	66.75±9.13
Bt. protein	Not detected	Not detected

transgenic cottonseed.

However, the values for serum alkaline phosphatase and glutamic- oxaloacetate transaminase were non-significantly higher in buffaloes fed transgenic cottonseed than in buffaloes fed non-transgenic cottonseed. On the contrary, the serum glutamic-pyruvate transaminase activity was considerably higher in buffaloes fed non-transgenic cottonseed (102.25±31.85 units/l) than in buffaloes fed transgenic cottonseed (66.75±9.13 units/l) which may be attributed to the large variation within the group of buffaloes fed non-transgenic cottonseed but the values were in the normal range as reported for bovines (Chauhan, 1995). Higher values of aspartate amino transferase (glutamic-oxaloacetate transaminase), alanine amino transferase (glutamic-pyruvate transaminase) and alkaline phosphatase activities due to feeding of excess amount of cottonseed meal in the diet of cows have been reported (Allah and Fadali, 1996; Coppo et al., 1995). However, Colin-Negrete et al. (1996) did not observe any significant effect on the serum enzyme activities in growing Holestein heifers fed 30% whole cottonseed for 430 days.

The Bt. protein in the blood of lactating buffaloes fed ration containing transgenic cottonseed was not detected after 35 days of feeding which indicated that Bt. proteins were degraded in the rumen and not available for absorption through gastrointestinal tract and thus not detected in blood of buffaloes.

Transgenically modified cottonseed is nutritionally similar to non-transgenic cottonseed in lactating buffaloes and poses no health risk based on this study of intake and blood analyses.

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