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Effect of *Bt*-cottonseed meal feeding on performance, fermentation, ciliates population and microbial hydrolytic enzymes in lamb

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Effect of the *Bt*- or conventional cottonseed meal was assessed as well as the performance, fermentation, ciliate protozoa population and microbial enzyme of lambs. Three feed mixture (FM, forage: concentrate ration of 35: 65) contained groundnut oilmeal (GNM), insect protected *Bt*-cottonseed meal (*Bt*-CM) or conventional whole cottonseed (C-CM) as protein source, were fed for 123 days to the control, C-CM and *Bt*-CM group of lambs, respectively. Whole seed meal *Bt*- and C-CM had similar nutrient composition. The Ca was higher while Zn content was lower in C-CM. Both CM had similar metabolizable energy (MJ/kg DM). Lambs of three groups had similar daily gain, DM intake and nutrient digestibility. Rumen fluid pH and TVFA were similar, while ammonia-N (mg/l) was higher (p<0.001) in control lambs. Cottonseed feeding eliminated (p<0.001) rumen protozoa; protozoa were 101.1, 59.0 and 39.6 ×10^4/ ml in rumen fluid respectively in control, C-CM and *Bt*-CM diet fed lambs. Rumen enzymes activities of xyalanase, β-glucosidase and β-xylosidase were similar, while *Bt*-CM feeding reduced (p=0.010) carboxymethyl cellulase (CMCase) activity by 47% and increased proteases activity by 22%. The study concludes that inclusion of *Bt*-CM produced pronounced defaunation with reduced rumen ammonia concentrations, which improved daily gain. Therefore, *Bt*-CM can be incorporated at 180 g/kg in lamb diet.

Key words: Cottonseed, genetically modified feed, performance, fermentation, rumen enzymes.

INTRODUCTION

The groundnut oil meal (GNM) is a conventional protein supplement in animal feeding in India. However, during last several years, this feed resource has been diverted toward production of cheaper food supplements for human consumptions. The availability of GNM is reducing continuously for animal feeding, which in turn accelerated its prices.

There is need to explore feed resources that can replace GNM in ruminant feeing and cottonseed could be an alternative (Solomon et al., 2008; Solaiman et al., 2009; Dayani et al., 2007); because, use of whole cottonseed in animal feeding provides high fiber, energy and protein, and has considerable quantity of rumen escape proteins that support high requirement of amino acids for various productions.

However, cotton crop is highly susceptible to insect and pests infestation that causes significant economic losses to the producers, and high dosing of chemicals are required for successful crop production. Using advanced molecular techniques, cotton crop has been modified

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by induction of specific DNA sequences containing either the *Cry1A*c gene (Bollgard cotton) or both *Cry1Ac* and *Cry2Ab* gene to produce Bollgard II cotton, which provide protection against insect and pests. The cotton hybrids containing *Bt* gene produces its own toxin for bollworm attack thus significantly reducing chemical use and providing a major benefit to cotton growers and the environment.

Selective induction of herbicide tolerance and or insect pest resistance encoding genes enhanced the agronomic performance of genetically modified (GM) crops by reduced cost of production thus encouraging economic cultivation. The global area of GM crops at present is 84.0 million ha, which has increased over 50-fold from 1996 to 2009. FAO/WHO (2000) recommended evaluation of biotechnologies derived plant and an appropriate counterpart such as the parental line from which the GM plant derived by performing animal trials. The inclusion of 20% whole cottonseed in sheep was recommended (Dayani et al., 2007) for potential defaunation. A 300 g whole cottonseed meal supplementation is recommended (Solomon et al., 2008) per goat for better performance and carcass traits on grass hay based goat production system, while 15.7% whole cottonseed feeding improved intake and growth of growing kids (Solaiman et al., 2009) and higher levels of inclusion exhibited deleterious effects when soybean meal and concentrate was replaced. In dairy cattle feeding, only 150 g/kg diet whole cottonseed meal inclusion is recommended, while improved milk production was achieved (Singhal et al., 2011), when cattle received 2.9 kg per day genetically modified cottonseed.

India is the leading cotton (*Gossypium* spp.) growing country in the world and have 20% of the total area sown under cotton crop. However, with nine million hectare, the highest cotton cultivation area, India ranks 13th position with only 13% production of cotton. India's average cotton yield is 319 kg/ha lintas compared to world average 603 kg/ha. The *Bt*-cotton cultivation has been approved in several regions of India and production of this GM crop will increase in coming years.

Among the agro-industrial by-products, oilseed meals are the chief sources of protein supplements in animal feeding. The whole cottonseed meal also a good source of protein but gossypol content is the main anti-nutritional factor limiting its utilisation in animal feeding. However, gossypol of whole cottonseed meal could not induce deleterious effects on ruminants with a functional rumen (Wolf et al., 1980). The ruminants have ability to detoxify the gossypol by binding with soluble protein in rumen (Hawkins et al., 1985).

Further, genetic modification by inducing insect resistant gene does not change gossypol content of cottonseed (Singhal et al., 2011; Hawkins et al., 1985). Therefore, this study aimed to assess the influence of replacement of GNM with C-CM or *Bt*-CM in lamb diet on performance, nutrient utilisation, rumen fermentation, ciliate protozoa population and microbial enzyme status.

MATERIALS AND METHODS

The experiment was conducted at the Central Sheep and Wool Research Institute, Avikanagar (Rajasthan, India) located at 26° 17'N latitude and 75° 28'E longitude and 320 m above sea level. The climate is hot and semi-arid. The study was initiated in September and ended in January and, during the experiment, minimum and maximum ambient temperature ranged from 3 to 27°C and 20 to 35°C, respectively while relative humidity varied from 27 to 92%.

In-vitro fermentation study

Cottonseeds (*Bt* and its conventional isolines) were obtained from Central Institute for Cotton Research, Nagpur, India. Both cottonseeds were grown under identical agronomic and environmental conditions. The samples of cottonseeds were processed for chemical analyses and *in-vitro* gas production procedure of 100 ml glass syringe was used for *in-vitro* fermentation. In brief, the seed samples were ground to pass 1 mm screen and a 200 mg homogenised sample was placed in the bottom of glass syringe, mixed with 30 ml microbial inoculums and incubated at 39°C for 24 h. To maintain anaerobic conditions during *in-vitro* fermentation, the carbon dioxide was flux appropriately at several stages until the initiation of fermentation. Total gas production was measured by piston displacement method for 24 h; volume of gas was converted to mmol assuming 1 mol of gas is equivalent to 22.4 L of gas under the atmospheric pressure and temperature conditions of gas measurement in our laboratory. After the 24 h, the syringe contents were transferred in 100 ml spout less beaker with repeated washing of neutral detergent solution and neutral detergent fibre (NDF) was estimated to assess truly degradable dry matter (TDDM). From these samples, truly degradable organic matter (TDOM) was also estimated by ashing at 450°C for 4 h. The recommended (Mould et al., 2005) microbial inoculum contained distilled water (365 ml), buffer (183 ml) (NH_4HCO_3, 4.0 g; $NaHCO_3$, 35.0 g; dissolved in 1000 ml water), macro-mineral solution (183 ml) (Na_2HPO_4, 5.70 g; KH_2PO_4, 6.20 g, $MgSO_4.7H_2O$, 0.60 g; dissolved in 1000 ml water), micro-mineral solution 100 µl ($CaCl_2.2H_2O$, 13.2 g; $MnCl_2.4H_2O$, 10.0 g; $CoCl_2.6H_2O$, 1.0 g; $FeCl_3.6H_2O$, 8.0 g; dissolved in 100 ml water), strained rumen fluid (330 ml), rezasurie (0.01 mg) and reducing solution (38.8 ml) (1 N NaOH, 2.0 ml; $Na_2S.9H_2O$, 285 mg and water 47.5 ml).

Animals, housing and feeding

Thirty-three (33) lambs (90 ± 5 day of age; 15.5 ± 0.89 kg BW) were divided randomly into three equal groups and penned in well-ventilated enclosures, dewormed once using 'Albendazole' at 10-mg/kg BW (WOCKHARDT India Ltd. Bombay) and fed *ad libitum* a feed mixture individually for 123 days. The feed mixture (FM), had forage: concentrate (F:C) ration of 350: 650 (Table 2). Control lambs were fed a FM containing groundnut oil cake as source of crude protein while the other two diets contained either conventional cottonseed meal (C-CM) or *Bt*-cottonseed meal (*Bt*-CM). The FM contained essential constituents recommended (ICAR, 1998) for native growing lambs. Fresh feed was offered daily at 09:00 h in an excess of 10% of previous day's intake after discarding the residue. Chemical composition of C-CM and *Bt*-CM (Table 1) and the diets (FM; Table 2) are presented. Feed samples were collected at weekly intervals for dry matter (DM) determination and three or

Table 1. Chemical constituents in seed meal of *Bt* and non-*Bt* isomers (6 observation of each).

Chemical constituent	*Bt*- CM[a]		C-CM[b]		P- value
	Mean	SE	Mean	SE	
Composition (g/ kg dry matter)					
Dry matter	908.7	0.667	896.0	2.000	0.065
Organic matter	950.6	0.097	938.9	0.342	0.190
Crude protein	229.7	2.512	207.5	1.322	0.478
Total lipids	204.9	3.929	170.5	2.513	0.307
Crude fibre	171.3	6.286	275.9	8.915	0.410
Neutral detergent fiber	561.7	3.528	572.1	2.034	0.386
Acid detergent fiber	354.2	2.350	364.5	2.569	0.281
Hemicellulose	207.5	5.647	206.67	06.819	0.856
Total carbohydrates	385.3	5.005	457.0	2.314	0.226
Nitrogen free extract	305.3	8.251	285.1	7.919	0.913
Total ash	49.4	0.100	61.1	0.348	0.187
Acid insoluble ash	2.7	0.081	6.7	0.529	0.037
Mineral contents					
Ca (g/kg)	1.45	0.016	1.77	0.092	<0.001
P (g/kg)	0.129	0.002	0.133	0.004	0.492
Cu (mg/kg)	61.89	0.766	63.29	0.878	0.911
Zn (mg/kg)	65.43	2.384	54.27	4.013	<0.001
Mn (mg/kg)	29.44	0.836	29.03	0.868	0.705

[a]*Bt*-cottonseed meal, [b]Conventional cottonseed meal.

four-week samples were pooled for chemical analysis. Lambs were weighed at weekly intervals before feeding and watering for two consecutive days and mean live weights were used to determine growth pattern.

Nutrient utilisation and rumen fermentation

A digestibility trial was conducted near the end of experimental feeding (100^{th} day), on six randomly selected lambs from each treatment, for 10 days (that is, three days adaptation followed by seven days of sample collection) during which daily feed intake and output of faeces were collected and recorded. Samples of feed, orts and feces were collected every morning. Feces was collected into acidified containers containing 100 ml H_2SO_4 (100 ml concentrated H_2SO_4 diluted to 1000 ml with distilled water) using a total collection method. For chemical analysis samples of feeds, feces and orts were dried to a constant weight in a forced air oven at 70°C. Dried samples of 7 days collection were pooled and ground to pass a 1 mm screen and preserved for chemical analysis.

Rumen fluid samples were collected from intact animal using stomach tube at 0, 4, 8, 12, 18 and 24 h post feeding for pH determination, analysis of total volatile fatty acids (TVFA by Barnett and Reid, 1957) and ammonia nitrogen (NH_3-N by Conway, 1962) and enumeration of ciliate protozoa population (Kamra et al., 1991). Rumen contents (40 to 50 ml) were sampled and pH measured within 5 min of aspiration. Samples of rumen contents (10 ml) were placed into screw-capped vials for the VFA and NH_3-N analysis, to which two drops of saturated $HgCl_2$ had been added previously to arrest fermentation immediately. These samples were stored frozen at -20°C until analyzed.

Carcass traits evaluation

Animals were slaughtered after the termination of experiment at experimental abattoir. The lambs were fastened for 18 h with free access of water prior to slaughter and live weight was recorded before slaughter. The weight of carcass and non-carcass traits was recorded after slaughter. The empty body weight was determined by difference between live body weight at slaughter and gut content. Hot carcass weight was obtained by excluding the edible and non edible offals. Dressing percent was calculated as proportion of hot carcass weight to slaughter as well as empty body weight. The rib eye muscle area was measured by tracing the cross section area of the 12^{th} and 13^{th} vertebrae after cutting perpendicular (ISI, 1963). The depot fat percent was calculated to slaughter weight of different sited depositions.

Determination of microbial enzymes

Fibre and protein degrading enzymes were analysed in rumen fluid (RF) samples collected at 4 h post rumen fermentation as per the procedures of Kamra and Agarwal (2003) with slight modification (Raghuvansi, 2003). Fibre degrading enzymes viz. carboxymethyl cellulase (CMCase; endo-1, 4-β-glucanase; EC 3.2.1.4), xylanase (1, 4-β-xylan xylano hydrolase, endo-1, 4-β-xylanase; EC 3.2.1.8); cellobiose degrading enzyme β-glucosidase (β-D-glucoside gluco-hydrolase, EC 3.2.1.21) and protein degrading enzyme proteases were analysed. Enzyme activities were determined separately for individual animals and expressed as units/100 ml RF. In brief, 10 ml of fresh RF was centrifuged at 24,000 × *g* for 20 min and the supernatant was used as source of enzyme for extracellular fraction. The pellet containing microbial biomass (Bacteria, protozoa and fungi) was suspended in 5 ml of 0.1 M phosphate buffer (pH 6.8), 2 ml CCl_4 and 2 ml lysozyme solution (4 g/ l). The suspension was incubated for 3 h at 39°C and then centrifuged at 24,000 × *g* for 20 min. The supernatant collected was used as an enzyme source for the cellular portion.

Table 2. Ingredient and chemical composition of the feed mixture fed to lambs.

Chemical composition	Diets (g/kg as mixed)		
	Control	C-CM[a]	*Bt*-CM[b]
Ingredient composition			
Perl millet stover	350	350	350
Maize	150	132	132
Barley	150	126	132
Wheat bran	54	36	42
De-oiled rice bran	48	24	30
Groundnut cake	180	84	66
Cottonseed meal	-	180	-
Bt-Cottonseed meal	-	-	180
Salt (NaCl)	6	6	6
Mineral and vitamin premix[c]	12	12	12
Molasses	50	50	50
Chemical composition (g/kg DM)[d]			
Dry matter (g/kg)	927.6	944.6	941.5
Organic matter	900.0	912.6	890.5
Crude protein	146.7	145.5	139.4
Total lipids	30.4	49.2	54.0
Neutral detergent fiber[e]	515.3	591.0	561.9
Acid detergent fiber[e]	347.2	370.3	389.6
Hemicellulose[e]	168.1	220.7	172.3
Cellulose[e]	264.5	274.7	312.5

[a]C-CM, Conventional cottonseed meal. [b]*Bt*-CM, Insect protected *Bt*-cottonseed meal. [c]Mineral and vitamin premix contained (per kg), Ca 280, P 120, Co 0.2, Cu 1, I 1, Fe 6, Mn 1.2, Zn 2 g and Se 10 mg; Vit. A 625000, D_3 62500, E 250 IU and niacinamide 1 g (Nutrimilk, Pfizer Animal Health, Pfizer Limited, Pfizer center 5, Mumbai, India). [d]Mean of four observations. [e]Determined using a sequential procedure.

The activity of proteases was determined by measuring the amount of protein hydrolysed during incubation of the substrate (1% casein solution in 0.1 M phosphate buffer pH 6.8) with enzyme. The reaction mixture contained 1.5 ml 0.1 M phosphate buffer (pH 6.8), 0.25 ml substrate and 0.25 ml enzyme, which was incubated at 39°C for 120 min. The reaction was stopped by the addition of 2 ml 20% TCA and incubated overnight at room temperature. The amount of protein hydrolyzed was estimated (Lowry et al., 1951) in supernatant obtained by centrifugation at 5000 rpm for 10 min. A unit of enzyme activity was defined as the amount of enzyme, which hydrolyzed 1 µmol protein per min.

The substrate, 1% carboxymethyl cellulose and 0.25% xylan (Oat spent) solution was used to estimate the CMCase and xylanase enzyme activities respectively. The reaction mixture contained 0.1 M phosphate buffer (pH 6.8) (1 ml), substrate (0.5 ml), enzyme (0.5 ml), which was incubated at 39°C for 60 min. The reaction was stopped by the addition of dinitro-salicylic acid reagent.

The glucose thus produced was estimated using dinitro-salicylic acid method (Miller, 1959) for CMCase and a unit of enzyme activity was defined as the amount of enzyme, which produced 1 µmol glucose per hour from carboxymethyl cellulose. The amount of xylose released during incubation of reaction mixture was estimated for xyalanse and a unit of enzyme activity was defined as the amount of enzyme, which produced 1 µmol xylose per min from xylan.

For β-glucosidase estimation, the reaction mixture contained 0.9 ml p-nitrohpenol β-D-glucopyranoside and for β-xylosidase estimation, the reaction mixture contained 0.9 ml p-nitrohpenol β-D-xylopyranoside, 0.1% in 0.1 M-phosphate buffer (pH 6.8) and enzyme (0.1 ml). The reaction mixture was incubated for 10 min at 39°C. The reaction was terminated by adding 1 ml solution of 2% Na_2CO_3. The amount of p-nitrophenol released during incubation of reaction mixture was estimated (Sewale and Sadana, 1978). The unit of enzyme activity is defined as amount of enzyme that produced 1 µmol p-nitrophenol per min.

Chemical analysis

The DM of feed, orts and faeces were analyzed by drying at 100°C for 24 h or to constant weight. The AOAC (1995) analytical procedures were used for the OM determination by ashing at 550°C for 4 h and N estimation by a Kjeldahl technique. Total lipid was estimated using solvent extraction procedure with Soxhlet apparatus and crude fibre (CF) by refluxing moisture and fat free sample with weak acid (2.04N H_2SO_4) and weak alkali (2.50N NaOH) following the procedure (Sastry et al., 1999). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by a sequential procedure using the same sample. For NDF determination, the procedure of Van Soest et al. (1991) was followed without sodium sulphite or α-amylase, whereas the procedure described by Robertson and Van Soest (1981) was used for ADF and lignin determination. The NDF and ADF are expressed with residual ash.

Table 3. *In-vitro* fermentation characteristics of conventional and *Bt*-cottonseed meal (9 observation of each).

Parameter	*Bt*- CM[a]		C-CM[b]		P- value
	Mean	SE	Mean	SE	
Gas (ml/ g DM incubated)	113.16	3.037	105.67	3.700	0.192
mmol gas/ g DM incubated	5.05	0.135	4.72	0.167	0.193
mmol gas/ g DM fermented	7.29	0.118	8.15	0.141	0.010
ME (MJ/ kg DM)	5.41	0.083	5.19	0.101	0.877
TDDM (g/kg)[c]	692.6	11.20	578.6	10.45	0.002
TDOM (g/kg)[d]	662.9	10.52	550.5	9.76	0.001
Fermentation efficiency[e]	5.83	0.087	5.22	0.087	0.008
MBP (mg)[f]	85.2	1.14	65.8	0.96	<0.001

[a]*Bt*-cottonseed meal. [b]Conventional cottonseed meal. [c]Truly degradable dry matter. [d]Truly degradable organic matter. [e]Fermentation efficiency (mg DM digested/ ml gas). [f]Microbial biomass produced (mg/ 200 DM incubated = [Truly degradable OM/ 200 mg DM - (2.2 × actual gas, ml/ 200 mg DM)], metabolizable energy (ME, MJ/ kg DM) = 2.2 + (0.136 × actual gas, ml/ 200 mg DM) + (0.0057 × %CP).

Mathematical calculations and statistical analysis

The metabolizable energy (ME), microbial biomass production (MBP) and fermentation efficiency of cottonseeds were calculated following the procedure (Blummel et al., 1997) using total gas production as: ME (MJ/ kg DM) = 2.2 + (0.136 × actual gas, ml/ 200 mg DM) + (0.0057 × % CP); MBP (mg/ 200 DM) = [truly degradable OM/ 200 mg DM - (2.2 × actual gas, ml/ 200 mg DM)]; Fermentation efficiency = (mg DM digested/ ml gas produced). The ME content of diet was calculated following the equations (AAC, 1990): ME (MJ/kg DM) = (digestible OM (g/kg DM) / 1000) × 18.5 × 0.81. Subtracting crude protein, total lipids, crude fiber and total ash from sample DM, calculated the nitrogen free extract (Sastry et al., 1999).

Nutrient equivalence and *in-vitro* fermentation results of *Bt*-CM and C-CM were analysed using student "t" test, while *in-vivo* results of nutrient intake, digestibility, nutritive value of diets and rumen microbial hydrolytic enzymes were subjected to analysis of variance for statistical significance test using general linear mathematical model as: $Y_{ijk} = \mu + T_i + e_{ij;}$ where: Y_{ijk} = observation mean; μ = General mean, T_i = effect of i^{th} treatment (i = 1, 3), e_{ij} = random error. The effect of diet and post feeding periods 0, 4, 8, 12, 18 and 24 h on rumen fermentation metabolites was analyzed using mathematical model as: $Y_{ijkl} = \mu + T_i + P_j + (T_i \times P_j)_k + e_{ijk}$; where Y_{ijkl}= observation mean; μ = general mean, T_i = effect of i^{th} treatment (i = 1, 3), P_j = effect of j^{th} post feeding time (j = 1, 6), $(T_i \times P_j)_k$ = interaction between i^{th} dietary treatment and j^{th} post feeding time, e_{ijk}= Random error. The significance in term of linear and quadratic effects was also tested. The significant levels were determined among diets by Duncans multiple range tests (SPSS base 14, 2005).

RESULTS AND DISCUSSION

Chemical composition and *in-vitro* fermentation

Chemical composition of *Bt*-CM and C-CM estimated in terms of chemical composition was not different (Table 1). Ca content was higher ($p<0.001$) in C-CM than in *Bt*-CM, while Zn was higher ($p<0.001$) in *Bt*-CM compared to C-CM. However, P, Cu and Mn contents were similar in the *Bt*- and C-CM seed. The *in-vitro* fermentation characteristics show that ME content was not different between *Bt*- and C-CM, while gas production mmol/ g DM fermented was higher in C-CM seeds. The TDDM, TDOM, fermentation efficiency and MBP was higher ($p<0.05$) in *Bt*-CM (Table 3). The chemical composition of C-CM and *Bt*-CM seeds has minor differences. Both cottonseeds used in this study were grown in India under identical agronomic and environmental conditions, thereby eliminating nutrient differences. Transgenic cotton crop containing different genes or gene combinations did not differ in nutrient composition (Castillo et al., 2004). Differences in chemical constituents and anti-nutritional content were also not found (Hawkins et al., 1985; Kumar and Singhal, 2004) in whole seed and defatted meal between *Bt* and non-*Bt* cotton grown in India. Similar range of variations were reported (Kumar and Singhal, 2004) in chemical constituents but higher levels of CP and fat for both *Bt* and non-*Bt* cottonseed were 294.8 and 269.0, 296.0 and 192.2 g/kg, respectively, compared to the present observations. The author evaluated dehulled seed; hull contains fewer nutrients, while the present study evaluated whole cottonseeds, which caused lower levels of CP and lipids (Kumar and Singhal, 2004). Seed mineral contents of C-CM and *Bt*-CM of the present study were not consistent with the reported observations (Kumar and Singhal, 2004; Cooke et al., 2007). Soil fertility status might have caused variations in mineral content of seeds, as soil-plant relationship exists for several minerals. In general, minerals are not supplemented under Indian agronomic practices (Tripathi and Karim, 2008).

Further, Hamilton et al. (2004) reported comparable chemical, gossypol and mineral contents between GM and conventional commercial cotton varieties. Possibly minor differences attributed by higher CP, lipids, NFE and lower CF content in *Bt*-CM promoted *in-vitro* fermentation with reduced gas production. The gas production during fermentation is contributed by quality of feed and poor

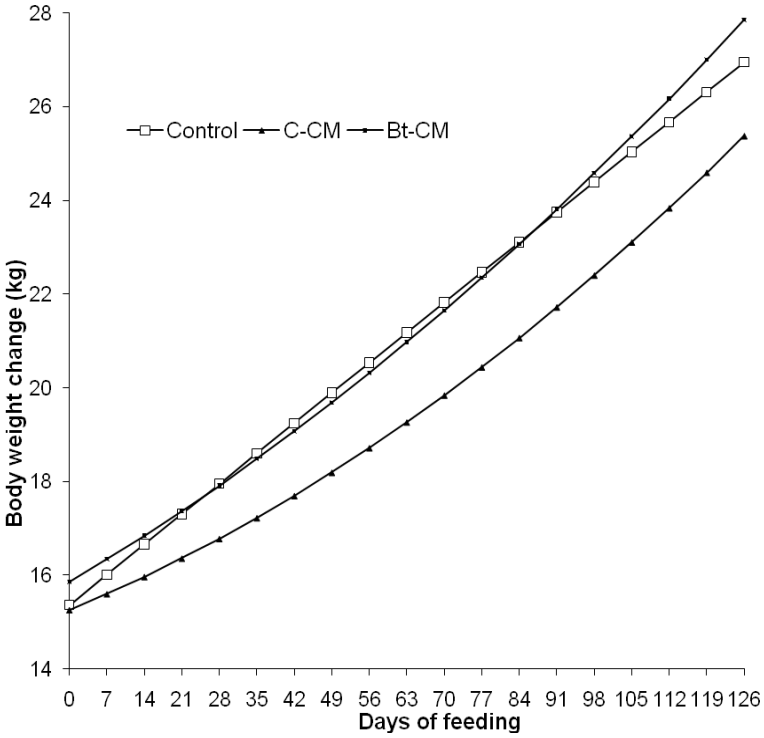

Figure 1. Body weight (BW) change of lambs fed diets containing groundnut cake (control), conventional (C-CM) or *Bt*- cottonseed meal as protein supplement. BW (kg, Control, lambs) = 15.361 + 0.649X - 0.0003X^2 (R^2 = 0.977). BW (kg, C-CM, lambs) = 15.260 + 0.328X + 0.0130X^2 (R^2 = 0.985). BW (kg, *Bt*-CM, lambs) = 15.860 + 0.470X + 0.0109X^2 (R^2 = 0.970).

quality feeds containing high crude fibre which produce high amount of gas per unit fermented substrate (Cooke et al., 2007; Dijkstra et al., 2005; Getachew, et al., 2005). Although gas production was similar between *Bt*-CM and C-CM but less gas production per g DM fermented in *Bt*-CM was due to higher fermentation efficiency contributed by higher protein, lipids and lower crude fibre content. Higher microbial biomass production improved fermentation efficiency and thus TDDM and TDOM content of *Bt*-CM were higher.

Possibly, higher CP content of *Bt*-CM provided added NH_3-N from protein catabolism in the medium that facilitated microbial growth. Increased rumen bacteria numbers are associated with ammonia nitrogen as substrate for cell synthesis and subsequently, there is an increase demand for ammonia (Seng et al., 2001). Inclusion of urea in whole cottonseed with cornstarch improved growth of mixed rumen micro-organism *in-vitro* (Bernard et al., 2001).

Rumen micro-organism utilize ammonia as source of N, which may be limiting when rapidly fermented carbohydrates are fed (Hoover, 1986), and increased NH_3-N improve fermentation because of promoted microbial growth in rumen (Hoover and Stokes, 1991; Bernard et al., 1999).

Performance, digestibility and nutritive value

The live weight change of the lambs (Figure 1) had an average daily gain (ADG) of 102, 89 and 111 g, respectively in the control, C-CM and *Bt*-CM diet fed lambs (Table 4) which was not different among three diets. The DM intake was similar among three lamb groups and digestibility coefficients of DM, OM, CP and NDF were not different, while ADF and cellulose digestibility was higher and hemicelluloses digestibility was lower in C-CM diet fed lambs. Possibly improved intake and better fermentation of *Bt*-CM enhanced growth of the lambs fed *Bt*-CM diet. Although, statistically non significant but a 20% increase in daily gain was attributed to 11% increase in feed intake in *Bt*-CM diet fed lambs which provided more ME and protein to support better growth. These are consistent with the observations of Singhal et al. (2011), who observed increased milk production in crossbred cows fed daily 2.9 kg Bollgard II cottonseed meal. Nutrient utilization and animal performance did not change in the present study upon replacement of GNM by C-CM or *Bt*-CM in lamb diets, which were within the reported range of variations for native lambs in semi arid regions of India (Tripathi et al., 2008; Tripathi and Karim, 2010; Bhatt et al., 2009, 2011).

Table 4. Nutrient intake and digestibility of diets containing C-CM or *Bt*-CM as protein supplement.

Diet	Dietary treatment			SEM	p-value
	Control	C-CM[a]	*Bt*-CM[b]		
Performance					
Initial BW (kg)	14.3	15.6	15.2	0.668	0.288
Finishing BW (kg)	26.9	25.4	28.8	1.140	0.633
Daily gain (g/day)	102.0	89.0	111.0	5.72	0.270
Nutrient utilisation trial					
Body weight (BW, kg)	24.3	22.08	24.52	0.794	0.407
Dry matter intake					
g/kg $W^{0.75}$	71.0	69.3	77.8	2.57	0.383
kg/ 100 kg BW	3.19	3.20	3.50	0.101	0.399
Nutrient digestibility coefficient					
Dry matter	60.0	58.1	59.1	0.063	0.268
Organic matter	63.4	62.6	63.0	0.062	0.902
Crude protein	62.1	60.7	60.6	0.151	0.779
Neutral detergent fibre	44.5	49.1	47.8	0.112	0.104
Acid detergent fibre	34.3b	46.1[a]	39.4[b]	0.190	0.022
Hemi-cellulose	64.0[a]	54.5[b]	64.0[a]	0.190	0.047
Cellulose	52.6[a]	63.0[b]	60.7[b]	0.153	0.002
Nutritive value of diets					
Digestible crude protein (g/ kg)	91.24	86.85	83.91	2.184	0.411
Metabolizable energy (MJ/ kg)	8.53	8.59	8.40	0.079	0.627

[a]C-CM, Conventional cottonseed meal. [b]*Bt*-CM, Insect protected *Bt*-cottonseed meal.

It has been recently reported that feeding of Bollgard II cottonseed did not have adverse effect on dairy cattle performance when compared with conventional cottonseed meal (Singhal et al., 2011), while, improved animal performance was reported (Solaiman et al., 2009) upon supplementation of whole cottonseed meal up to 300 g/ day, which accounted for 50% of total dry matter intake in Sidama goats.

Similar to the present findings, feeding of whole cottonseed at 15.7% of the diet DM was recommended (Solaiman et al., 2009) in growing goat kids without any adverse effect on intake, daily gain and health. A higher level of whole cottonseed was fed (Solomon et al., 2008) to growing kids maintained on range and this was the only supplement.

However, goat kid diets may have higher levels of whole cottonseed meal if maintained for meat production, whereas, reproductive problems may occur at higher level of cottonseed feeding (Solaiman et al., 2009). The replacement of GNM by C-CM or *Bt*-CM feeding in this study did not change intake, digestibility, and nutritional value of diet. The average daily gain, tended to improve with *Bt*-CM feeding in comparison to C-CM. Present findings support the recommendations (Hamilton et al., 2004 ; Flachowsky et al., 2005; Lutz et al., 2005 ; Alexander et al., 2007 ; Flachowsky et al., 2007; Trabalza-Marinucci et al., 2008) that inclusion of genetically modified feed in animal feeding did not influence animal performance, if compositionally and nutritionally genetically modified plants is equivalent to conventional varieties (Berberich et al., 1996; Bertrand et al., 2005; Nida et al., 1996; Castillo et al., 2006). Further, genetically modified plants without substantial change in their composition do not significantly differ in their nutritional value from those of the isogenic varieties (Singhal et al., 2011).

Rumen fermentation and microbial hydrolytic enzymes

Mean rumen fluid pH was not different among the three diets which ranged from 6.68 to 6.71 (Table 5). Rumen fluid pH was lowest at 4 h post feeding in C-CM and *Bt*-CM diet fed lambs whereas control diet fed lambs had lowest pH at 8 h post feeding (Figure 2). Rumen fluid pH increased linearly ($p<0.001$) after 4 h post feeding (Table 6), which had ($p<0.001$) a quadratic ($p<0.001$) decrease

Table 5. Rumen fermentation characteristics and ciliate protozoa population of lambs fed diets containing *Bt* or conventional cottonseed meal.

Parameter	Dietary treatment			SEM	p-value
	Control	C-CM[a]	*Bt*-CM[b]		
pH	6.68	6.71	6.68	0.020	0.687
TVFA (mmol/ l)[c]	80.67	86.94	82.49	2.572	0.534
NH_3-N (mg/ l)	97.54[a]	76.20[c]	88.74[b]	1.670	<0.001
Ciliate protozoa population					
Holotrichs ($\times10^4$/ml)					
Small (≤ 10 mm)	1.78[a]	1.30[a]	0.87[b]	0.156	0.039
Large (>10 mm)	0.04[a]	0.03[a]	0.01[b]	0.011	0.225
Total	1.82[a]	1.33[b]	0.87[c]	0.160	0.037
Spirotrichs ($\times10^4$/ml)					
Small (≤ 10 mm)	87.17[a]	51.45[b]	35.02[c]	3.521	0.001
Large (>10 mm)	12.12[a]	6.26[b]	3.76[c]	0.843	<0.001
Total	99.29[a]	57.75[b]	38.77[c]	3.725	<0.001
Total ciliates ($\times10^4$/ml)	101.11[a]	59.07[b]	39.64[c]	3.797	<0.001

[a]C-CM, Conventional cottonseed meal. [b]*Bt*-CM, Insect protected *Bt*-cottonseed meal. [c]TVFA, Total volatile fatty acids.

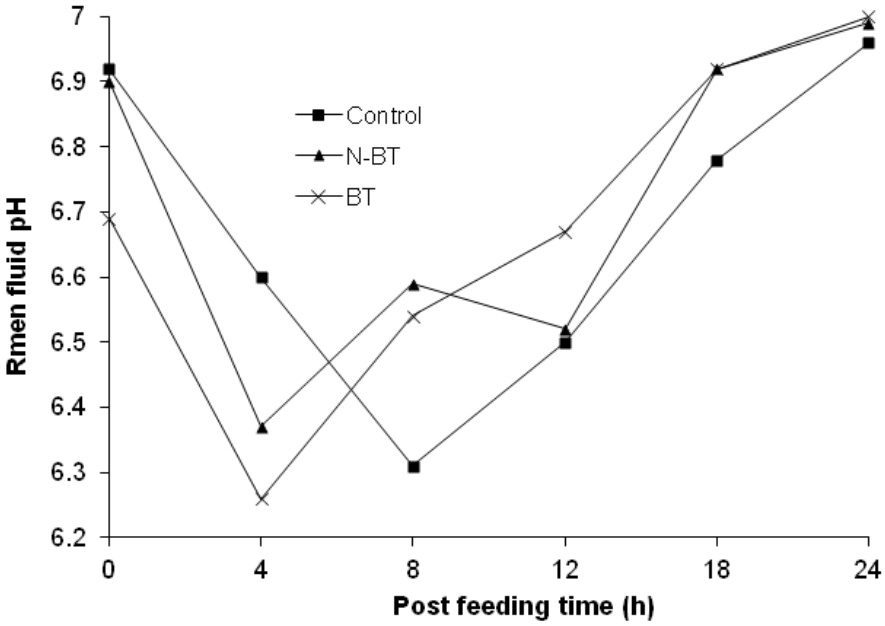

Figure 2. Rumen fluid pH of lambs at different post feeding time (h).

at 4 h post feeding; interactions between treatment and post feeding time were also significant (p= 0.008). Mean total volatile fatty acid concentrations (mmol/l) were also similar among the three diet fed lambs, while NH_3-N (mg/l) was lower (p<0.001) in C-CM diet fed lambs. Rumen NH_3-N was lowest at 4 h post feeding in C-CM and at 8 h in the control, while *Bt*-CM diet fed lamb had increased NH_3-N up to 8 h post feeding (Figure 3). The TVFA concentration were the highest at 4 h in C-CM and *Bt*-CM. The post feeding period has significant influence on NH_3-N and TVFA concentration, which increased linearly (p<0.001) with increasing post feeding time. Mean population of small and total holotrichs was lower (p<0.05) in *Bt*-CM diet fed lambs, similarly small, large

Table 6. Rumen fermentation characteristics and ciliate protozoa population at different hours of post feeding of lambs diets containing *Bt* or conventional cotton seed meal.

Parameter	Hours post feeding						SEM	p-value			
	0	4	8	12	18	24		Period	T×P[b]	Lin[c]	Quad[d]
pH	6.84	6.41	6.48	6.56	6.87	6.98	0.020	<0.001	0.008	<0.001	<0.001
TVFA (mmol/l)[a]	49.08	92.83	78.69	90.47	96.47	92.63	2.572	<0.001	0.222	<0.001	0.004
NH_3-N (mg/l)	79.91	76.67	76.33	97.59	94.18	100.29	1.678	<0.001	<0.001	<0.001	0.435
Ciliate protozoa population											
Holotrichs ($\times10^4$/ ml)											
Small (≤ 10 mm)	0.70	1.05	1.99	0.44	0.53	3.03	0.204	0.001	0.083	0.006	0.028
Large (>10 mm)	0.83	0.00	0.00	0.00	0.00	0.05	0.011	0.048	0.736	0.394	0.002
Total	0.79	1.05	1.99	0.44	0.53	3.08	0.160	<0.001	0.104	0.008	0.20
Spirotrichs ($\times10^4$/ ml)											
Small (≤ 10 mm)	20.04	35.72	60.40	63.91	77.20	76.59	3.521	<0.001	<0.001	<0.001	0.170
Large (>10 mm)	24.74	6.66	4.12	2.83	1.75	3.40	0.843	<0.001	0.002	<0.001	<0.001
Total	44.78	42.38	64.52	66.73	78.94	79.98	3.725	0.043	0.017	0.001	0.815
Total ciliates ($\times10^4$/ ml)	45.58	43.43	66.51	67.17	79.47	83.06	2.797	0.041	0.017	0.001	0.883

[a]TVFA, Total volatile fatty acids; [b]*T*xP, interaction between treatment and sampling period; [c]Lin, linear effect of sampling period; [d]Quad, quadratic effect of sampling period.

and total spirotrichs were lower (p<0.001) in *Bt*-CM diet fed lambs followed by C-CM and control lambs (Table 5). The lamb fed C-CM diet had 60% lower ciliates population than that in the control lambs and *Bt*-CM lambs had 39.0% lower population than in C-CM lambs. Cottonseed feeding had defaunation ability and *Bt*-CM had more profound defaunation ability than conventional cottonseed.

The population of holotrichs, spirotrichs (Figure 4) and total ciliates (Figure 5) remain the lowest in *Bt*-CM and the highest in control diet fed lambs at all post feeding time. Small and total holotrichs increased linearly (p<0.05) with increasing post feeding time, while large holotrichs were not present between 4 to 18 h post feeding. Diet and post feeding period had significant (p<0.05) effect on spirotrichs population which increased linearly (p=0.001) with increasing post feeding time. Rumen microbial enzymes showed that proteases, xyalanases, β-glucosidase and β-xylosidase activities were not different among lamb groups, except the activity of carboxymethyle cellulase which was higher (p=0.01) in C-CM diet fed lambs (Table 7). Possibly higher levels of slowly degradable protein of C-CM and *Bt*-CM containing diets linearly increased ruminal fluid pH after 4 h post feeding. Microbial degradation of protein in the rumen is known to produce ammonia, which act as rumen buffer on high concentrate feeding (Ivan et al., 2001). When animals are fed high concentrated diets there is an increase in acid production in the rumen resulting drop in rumen pH and additional ammonia produced by amino-acid catabolism may enhance rumen fluid pH (Bernard et al., 2001). In spite of higher rumen pH between 4 to 24 h posts feeding (Figure 2), mean ammonia-N concentration remained low in C-CM and *Bt*-CM diet fed lambs. This phenomenon suggested that ammonia-N was utilized by rumen microbes for their cells growth. It suggested that rumen micro-organism especially rumen bacteria utilize ammonia as their primary source of N for effective cell growth and activity (Hoover, 1986). Present observations corroborate favourably with the findings of Dayani et al. (2007) who reported reduced ammonia nitrogen with no effect on TVFA in sheep fed crushed cottonseed. Furthermore, defaunation is known to decrease ammonia N in rumen (Dayani et al., 2007; Seng et al., 2001). Feeding of cottonseed is reported to have defaunation ability because cottonseed oil is rich in linoleic acid, which exerts toxic effect to most susceptible group of protozoa holotrichs followed by cellulolytic protozoa (Ivan et al., 2000a,b; Ivan et al., 2001). We observed decrease in totals protozoan population 1.7 times by C-CM and 2.6 times by *Bt*-CM diet to that of control diet. The *Bt*-CM diet had a 50% less protozoan population in comparison to C-CM diet fed lambs.

Possibly, linoleic acid content reduced protozoan population in the C-CM and *Bt*-CM diet fed lambs, higher magnitude of reduction in *Bt*-CM diet was due to higher dietary level of total lipids. Feeding of diets containing 20% whole cottonseed (Dayani et al., 2007) obtained partial defaunation. Defaunation caused reduction in rumen ammonia-N and fibrolytic enzymes activities, while proteolytic activity improved. The period of feeding of cottonseed removed all holotrichs within 11 days of feeding and the *Entodinium* sp. are the only protozoa survived in sheep rumen, which were detrimental to the

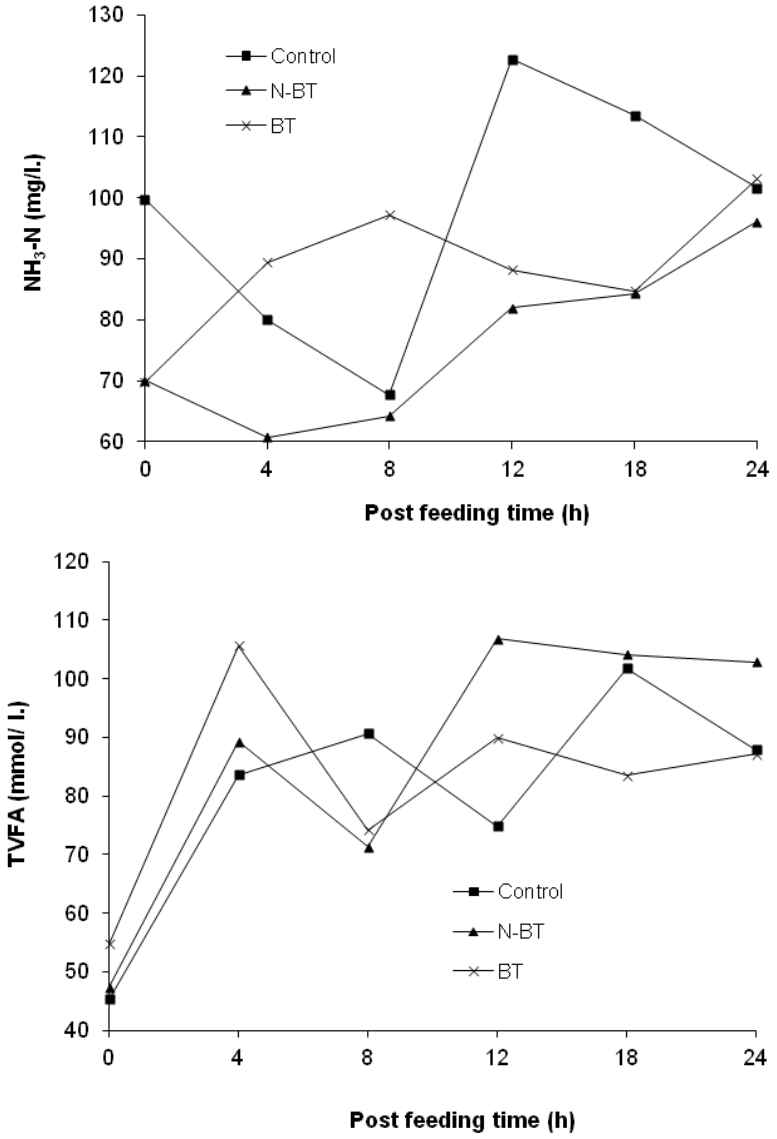

Figure 3. Rumen NH_3-N and TVFA concentrations of lambs at different post feeding time (h).

protein nutrition of the host (Dayani et al., 2007; Ivan et al., 2000a,b). In spite of a high level of defaunation in *Bt*-CM diet fed lambs, nutrient digestibility was not affected. This supports the hypothesis that defaunation is associated with decreased ammonia concentration by decreesing N recycling from engulfment of bacteria by protozoa and increased bacteria numbers utilize more ammonia as substrate for cell synthesis, which subsequently increase amino acid flow from the rumen to the intestinal absorption and utilisation (Seng et al., 2001; Veira et al., 1983).

The reduction in fibrolytic enzymes activities might be due to reduction in protozoan numbers, which are known to contribute significantly in rumen fibrolytic activities. Improved proteolytic enzymes activities in present study possibly contributed by increased numbers of bacteria due to defaunation, which is further supported by *in-vitro* finding of higher microbial biomass production and improved fermentation efficiency of *Bt*-CM fermentation, which contributed toward larger defaunation due to higher total lipids and therefore linoleic acid.

Carcass characteristics

The replacement of GNM by C-CM or *Bt*-CM in lamb diets did not change pre-slaughter weight (BSW), empty live weight (EBW), hot carcass weight and carcass yield (dressing, ranged 48.3 to 51.2% of BSW and 53.9 to 57.9% of EBW). These carcass traits are in agreement

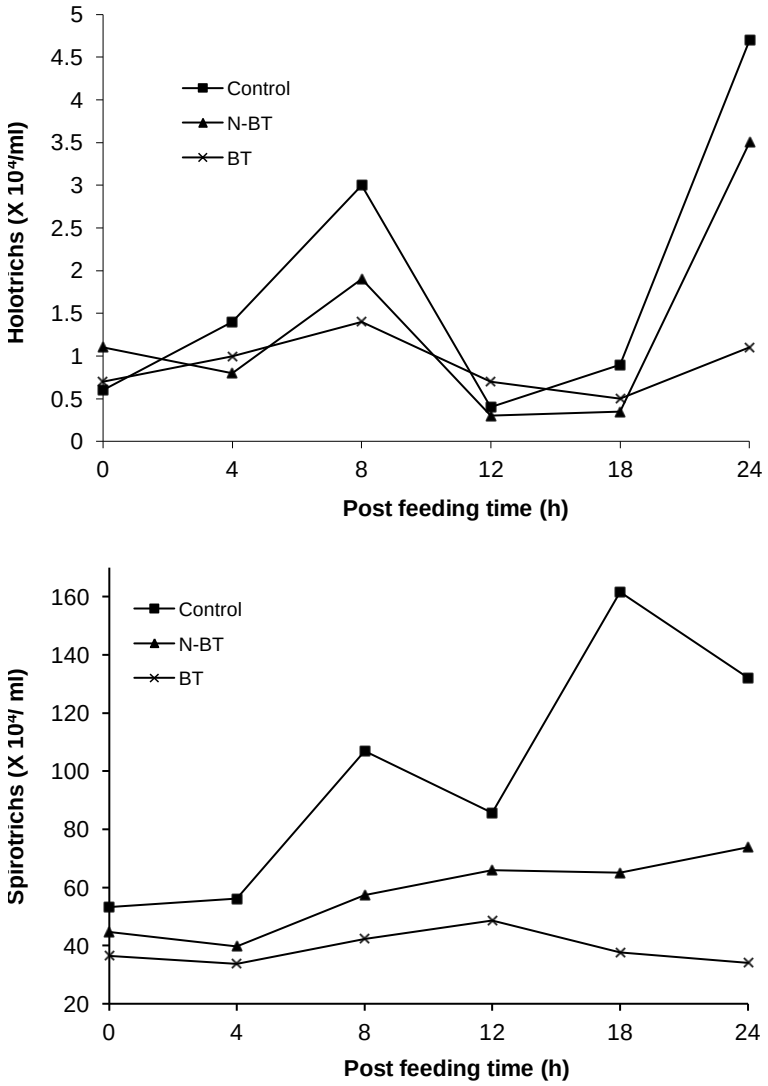

Figure 4. Holotrichs and spirotrichs population of ciliate protozoa of lambs at different post feeding time (h).

and are with in the normal range of variations reported for native lambs (Bhatt et al., 2011; Karim et al., 2007; Tripathi and Karim, 2011) on varying dietary regimens. The kidney fat was lower (p = 0.049) in control which increased by C-CM and *Bt*-CM diet fed lambs. Although, there was a trend of increased fat deposition at testes upon cottonseed feeding but total depot fat level remained similar (Table 8). The increased fat deposition in C-CM and *Bt*-CM diet fed lambs might be attributed by the higher levels of total lipids of cotton seed meals, which increased dietary lipid levels. Increased fat deposition was also reported in Sidama goats fed different levels of cottonseed meal (Solomon et al., 2008). Higher levels of dietary fat are reported (Bhatt et al., 2011) to favour fat deposition in essential organs in kid and lambs. Consistent to the present finding, goats (Bhuyan et al., 1996) and cattle (Zinn et al., 1997) were maintained at varying levels of dietary protein which had no effect on carcass traits and rib-eye muscle area.

Conclusions

The whole *Bt*-cottonseed meal in lamb diet did not deteriorate live weight change, nutrient utilisation and rumen fermentation in comparison to conventional cottonseed meal feeding. Whole seed meal of both *Bt*- and conventional cotton had similar nutrient composition and metabolizable energy content. Cottonseed feeding exhibited significant defaunation ability. The microbial hydrolytic activities of xyalanase, β-glucosidase and β-xylosidase did not change, while *Bt*-CM feeding reduced carboxymethyl cellulase (CMCase) activity but increased (22%) proteases activity in rumen. Feed DM intake,

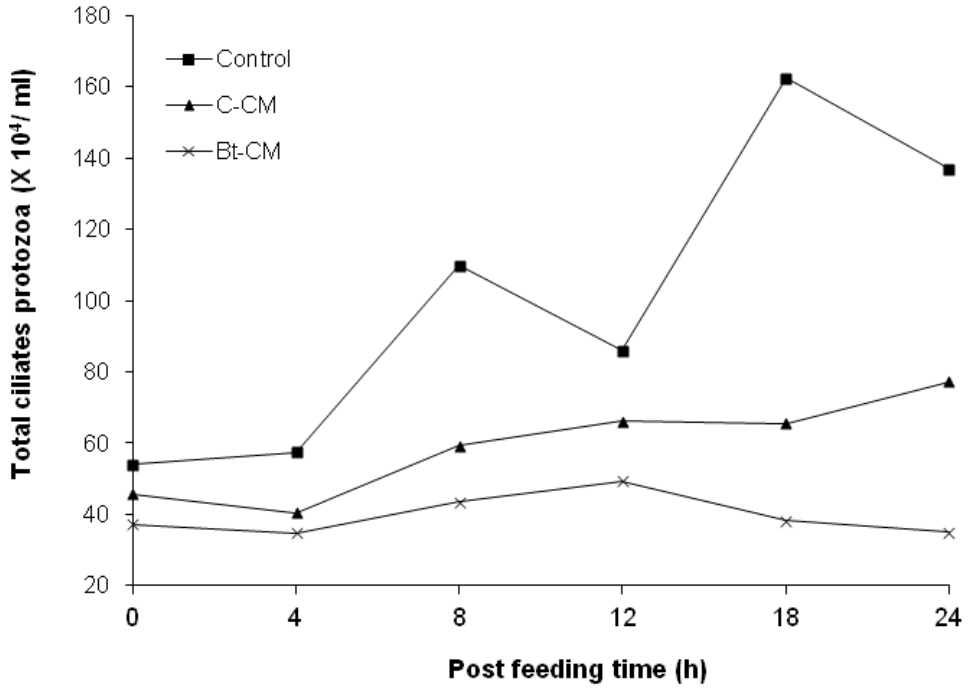

Figure 5. Total ciliate protozoa population of lambs at different post feeding time (h).

Table 7. Rumen microbial enzyme status of lambs (4 h post feeding) fed *Bt* or conventional cottonseed as protein supplement.

Parameter	Dietary treatment				
	Control	C-CM[a]	*Bt*-CM[b]	SEM	p- value
Proteases activity (μg protein hydrolysed/min/ml)					
Specific activity (U/ min)	4.96	5.86	7.07	0.540	0.289
Enzyme activity (U/h/100 ml)	821.95	751.27	1002.19	64.614	0.274
Carboxymethyle cellulase (μg glucose released/h/ml)					
Specific activity (U/ h)	3.29b	5.27a	3.18b	0.304	0.019
Enzyme activity (U/h/100 ml)	527.6b	674.7a	457.8b	26.189	0.010
Xyalanses (μg xylose releases/ min/ ml)					
Specific activity (U/ h)	9.77	11.28	9.29	0.621	0.555
Enzyme activity (U/h/100 ml)	1563.3	1448.6	1296.2	632.5	0.238
β-glucosidase (nmol *p*-nitrophenol/min/ml)					
Specific activity (U/ h)	9.77	11.28	9.29	0.788	0.371
Enzyme activity (U/h/100 ml)	58.66	47.12	34.43	5.063	0.169
β-xylosidase (nmol *p*-nitrophenol/min/ml)					
Specific activity (U/ h)	19.54	16.32	13.79	1.728	0.555
Enzyme activity (U/h/100 ml)	16.01	16.40	18.48	1.678	0.807

[a]C-CM, Conventional cottonseed meal. [b]*Bt*-CM, Insect protected *Bt*-cottonseed meal.

nutrient digestibility and nutritional value of diets remained unaffected upon replacement of GNM by whole cottonseed. Inclusion of 180 g *Bt*-CM produced pronounced defaunation effect with reduced rumen

Table 8. Carcass characteristics of lambs fed diets with control, C-CM or *Bt*-CM

Parameter	Dietary treatment			SEM	p- value
	Control	C-CM	*Bt*-CM		
BW at slaughter (BSW, kg)	25.52	23.87	28.60	1.338	0.449
Empty BW (EBW, kg)	23.56	21.39	25.84	1.207	0.465
Hot carcass weight (kg)	13.10	11.75	14.13	0.806	0.569
Dressing yield (%)					
BWS basis	51.24	48.34	49.44	0.991	0.490
EBW basis	57.92	53.93	55.51	0.970	0.275
Rib-eye area (cm^2)	10.96	10.88	12.90	1.047	0.714
Depot fat (% of BWS)					
Kidney fat	0.49	0.94	1.41	0.127	0.049
Caul fat	1.25	1.27	1.95	0.184	0.301
Testes fat	0.17	0.11	0.15	0.009	0.087
Total fat	1.90	2.31	3.51	0.315	0.169

[a]C-CM, Conventional cottonseed meal; [b]*Bt*-CM, insect protected *Bt*-cottonseed meal.

ammonia concentrations, which improved intake and daily gain by 10%. Therefore, whole cottonseed meal (conventional or *Bt* line) can be incorporated at 180 g/kg diet in replacement of conventional protein supplement in lamb feeding for mutton production. Further studies are required for recommending higher levels of inclusion in ruminant diets.

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