Research Report

(Indian Council of Agricultural Research, New Delhi)

TITLE OF PROPOSAL

ANIMAL FEEDING TRIAL ON BIO-SAFETY STUDIES WITH BIOTECHNOLOGICALLY TRANSFORMED BT-COTTON CROP USING SEED MEAL

DIVISION OF ANIMAL NUTRITION
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INDIA

FUNDED BY:

Central Institute For Cotton Research
(ICAR)
Post Bag No.2, Shankar Nagar
NAGPUR- 440 010, Maharastra

Research Report of Inter Institution Project

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Collaboration Between

CENTRAL SHEEP & WOOL RESEARCH INSTITUTE, (ICAR)
AVIKANAGAR - 304 501, INDIA

&

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Contents

Title :	Animal feeding trial on bio-safety studies with of Bt-cotton crop using seed meal
Objective:	To asses the bio-safety of feeding genetically modified BT gene containing cottonseed in lamb feeding
Priority research area :	Bio-safety of transgenic crops
Sanction / Fund receipt No. :	AA/2-1/9-07/Trains-crop/10/37; dated: 05-03-07
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Executive Summary

Bt cotton is a transgenic plant, producing an insect controlling protein Cry1A(c), the gene which has been derived from the naturally occurring bacterium, *Bacillus thuringiensis*. The cotton hybrids containing Bt gene produces its own toxin for bollworm attack thus significantly reducing chemical insecticide use and providing a major benefit to cotton growers and the environment. Bt cotton contains the three genes inserted via genetic engineering techniques, wherein, the *Cry1Ac* gene, encodes an insecticidal protein, Cry1Ac, derived from the common soil microbe *Bacillus thuringiensis* which has concern on food and feed safety issues. With this background, a project entitled "***Animal feeding trial on bio-safety studies with of Bt-cotton crop using seed meal"*** was initiated at Division of Animal Nutrition, Central Sheep and wool Research Institute (ICAR) Avikanagar, in collaboration with Central Institute For Cotton Research (ICAR), Nagpur. The project aim was to asses the bio-safety of BT-cottonseed in lamb feeding, which was initiated during August 07 and continued for 120 days on weaner lambs at a high plane of nutrition.

Nutrient (OM, CP and fiber fractions) and mineral (Ca, P. Mn, Co and Zn) contents were identical in BT-cotton and non-BT cotton seeds. The growth performance of lambs was similar on control, non- BT cotton seed and BT-cotton seed included diets. The growing lambs consumed 168 g BT-cotton seed per day and did not have apparent adverse effect on dry matter intake, nutrient utilization and nitrogen balance. Similarly BT-cotton seed intake of 0.681 % of body weight or 19.5 % of dry matter intake did not produce deleterious effect on performance and dry matter intake, thus palatability and growth performance was not a problem for BT-cotton seed feeding in lambs even under high plane of nutrition. Rumen fermentation characteristics viz, pH, TVFA and NH_3-N concentrations was not influenced by feeding of GNC, non- BT cotton seed or BT-cotton seed in lamb diets. Heamatological observations did not change due to BT-cotton seed feeding compared to non-BT cottonseed or GNC feeding. Intrestigly feeding of BT-cotton seed increased RBC and decreased WBC in blood. Serum IgG level

did not change due to BT and non-BT cotton seed feeding. Thus feeding of BT-cottonseed to lambs did not alter immunity and allergen status.

Internal organs weights as g per kg empty live weight (ELW) indicated precise effect of BT- cottonseed feeding on internal organ changes. The weights of kidney, spleen, pancrease, heart, lung, penis, kidney fat, cole fat, GI tract, ingest and empty GI tract were not different among BT cotton seed and non-BT cotton seed fed lambs. However BT cotton seed feeding increased liver weight, testicle weight and testicles fat g / kg empty live weight.

Chemical composition of Non-BT and BT-cotton seeds

The chemical composition (% DM basis) of cotton seed is presented in table1. The Non BT cotton seed (N-BT) and BT cotton seed (BT) had 92.76 % and 94.46 % dry matter (DM); 20.35 % and 22.97 % crude protein (CP); 95.1 % and 93.9 % organic matter (OM); 57.22 % and 56.16% NDF; 36.44 % and 29.42 % ADF and 20.77 % and 26.74 % hemicellulose respectively. The CP and hemicellulose contents were higher in BT than N-BT cotton seed. However, the OM, NDF and ADF content showed a reverse trend being high in NBT than BT.

Mineral contents viz. Calcium, Phosphorus, Manganese and Copper were identical in N-BT and BT cottonseed, while Zinc content was higher in BT-cotton seeds by 10 ppm.

Table 1
Chemical Composition of cottonseed

	Type of Cottonseed	
Chemical constituents (*g/kg DM*)	**Non-BT**	**BT**
Dry matter	896.0	908.7
Organic matter	951.4	938.9
Crude protein	203.5	229.7
Neutral detergent fiber	572.2	561.6
Acid detergent fiber	364.4	294.2
Hemicellulose	207.7	267.4
Mineral contents		
Calcium (*g %*)	0.193	0.144
Phosphorus (*mg %*)	11.270	12.839
Manganese (*ppm*)	27.829	28.018
Copper (*ppm*)	18.517	17.272
Zinc (*ppm*)	36.544	43.996

Composition of Diets

Diets were prepared to provide adequate essential and non-essential nutrients to support optimum growth of growing lambs as per ICAR recommendations. Ingredient composition and constituents of concentrate are presented in table-2. Bajra Kadbi (Perl Millet Stover) was used as major roughage source. Complete feed mixture (premixes) of total mixed ration (TMR) contained 35 part Bajra Kadbi, 60 parts concentrate mixture and 5 part molasses. Thus TMR had roughage: concentrate ratio of 35: 65. A total of three test diets were prepared. The concentrate mixture was prepared using various grinded concentrate ingredients (Table-2). Diet of control animals contained groundnut cake (GNC), as conventional protein supplement, which was replaced by Non-BT cotton seeds and BT-cotton seed in diets fed to animals allocated in Non-BT and BT group of test diets. Diets were balanced for total protein content with the addition of GNC.

The total mixed rations were iso-nitrogenous that contained crude protein 14.7, 14.6 and 13.9 % respectively in control, N-BT and BT diets. Fiber fractions viz. NDF, ADF, hemicellulose and cellulose were slightly higher in N-BT diet compared to control and BT diets.

Animals and Dietary Treatment

Weaner Malpura lambs (90 $\pm$ 5 day of age; 15.5 $\pm$ 0.89 kg) were used for the present experiment. The animals were randomly allocated to three dietary treatments, maintaining a sex ratio of 50:50; male and female lambs.

Keeping in view the quantity of BT-cottonseed received three of each male and female lambs were randomly allocated to each treatment II and III, while control group had ten lambs of similar sex ratio.

Treatment I: Control; Groundnut cake was used as major protein supplement

Treatment II: N-BT; Non-BT cottonseed was used to replace groundnut cake (g/g)

Treatment III: BT ; BT cottonseed was used to replace groundnut cake (g/g)

Table 2

Composition of diet (total mixed ration), concentrate mixture and cottonseed

	Diets		
Ingredient composition (g/kg)	Control	N-BT	BT
Total mixed ration			
Perl millet stover	350	350	350
Concentrate	600	600	600
Molasses	50	50	50
Concentrate mixture			
Maize	250	220	220
Barley	250	210	220
Wheat bran	90	60	70
De-oiled rice bran	80	40	50
Groundnut cake	300	140	110
Cottonseed non-BT	-	300	-
Cottonseed-BT	-	-	300
Common Salt (NaCl)	10	10	10
Mineral mixture	15	15	15
Supplivit-M	5	5	5
Chemical composition (*g/kg DM*)			
Dry matter	927.6	944.6	941.5
Organic matter	900.0	912.6	890.5
Crude protein	146.7	145.5	139.4
Neutral detergent fiber	515.3	591.0	561.9
Acid detergent fiber	347.2	370.3	389.6
Hemicellulose	168.1	220.7	172.3
Cellulose	264.5	274.7	312.5

Diet and Feeding Regimen

Three test diets were prepared to provide adequate nutrient to support active growth phase of weaner lambs. The total mixed ration contained 60 % concentrate that had 30 % groundnut cake (GNC) fed to control group of lambs, whereas lambs of non- BT (Cottonseed) and BT (BT-Cottonseed) group had 30 % respective cottonseed. Total mixed rations were prepared separately, which had 18 % either of GNC, non-BT Cottonseed and BT-Cottonseed.

Experimental animals were fed individually in separate enclosures, feed offered and residue left were recorded daily to determine daily feed intake. Feed residues were discarded before offering the feed of the day. The feed of the day was offered once in the morning at 10:00 h, to an excess of 10 % of previous days intake. Animals were allowed loitering in an open yard, without vegetation for two hrs daily. Water was available free choice twice in day at 09: 00 h and 16:00 h.

Growth Performance of Lambs

To asses the growth performance lambs, body weight changes were recorded by weighing lambs every week before offering the feed and water for two consecutive days, these means live weights were used to monitor growth profile of the lambs. The growth performance of lambs is presented in table 3. The pattern of live weight change is given in Figure 1.

Initial ($p= 0.228$) and finishing ($p= 0.633$) live weight of lambs were similar, which were respectively 14.3 and 26.9; 15.6 and 25.4; and 16.3 and 27.8 kg of control, N-BT and BT groups. Total live weight gain of 12.6, 10.6 and 12.9 kg; and average daily weight gains were 102; 89 and 111 g were also similar among three groups. Similarly, feed conversion ratio (kg feed/ kg gain) and feed efficiency (%) were also similar among three groups of lamb which ranged from 8.2 to 9.1 kg and 11.1 to 12.3 % respectively.

Table 3
Growth performance of weaner lambs on diets containing BT or non-BT cottonseed.

	Diets*			SEM	P
	C	N-BT	BT		
Initial live weight (kg)	14.3	15.6	16.3	0.529	0.228
Finishing live weight (kg)	26.9	25.4	27.8	1.008	0.633
Total live weight gain (kg)	12.6	10.9	12.9	0.666	0.496
Average daily gain (g)	102	89	111	5.349	0.270
Total feed intake (kg)	102.5	96.3	108.2	4.298	0.591
Feed conversion ratio (kg feed/ kg gain)	8.2	9.1	8.6	0.230	0.320
Feed conversion efficiency (%)	12.3	11.1	11.8	0.338	0.390

*Diets: Total mixed ration contained roughage, concentrate and molasses in ration of 60:35:5, concentrate contained groundnut cake (Control-C), non-Bt cottonseed (N-BT) or Bt-cottonseed (BT) as major protein source, which fed to lambs.

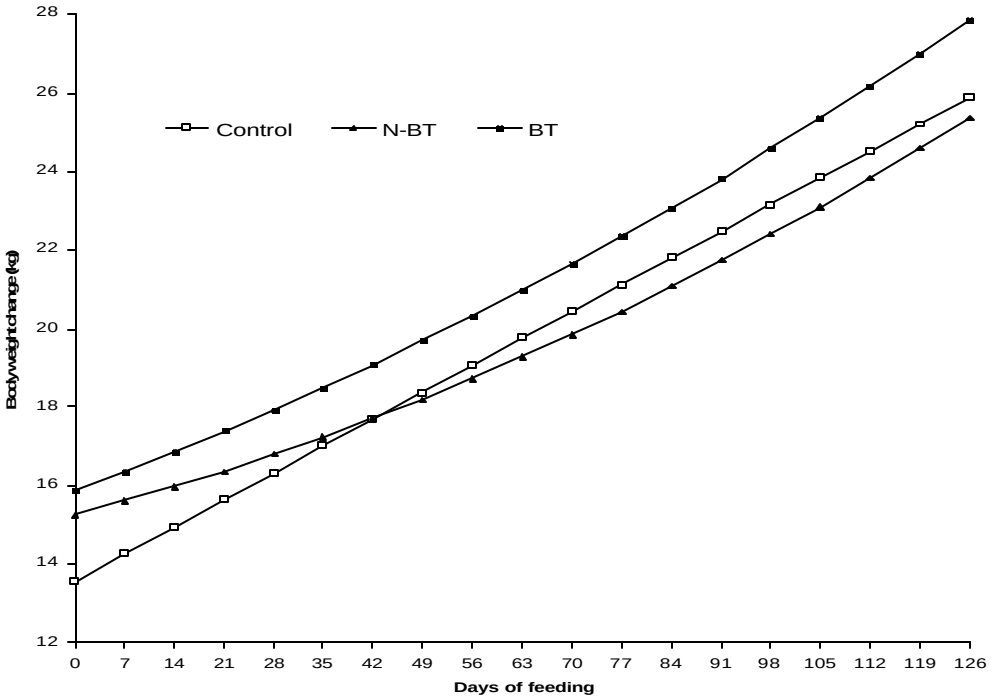


Fig. 1: Body weight (BW) change of lambs fed diets containing groundnut cake (control), BT or Non-BT cotton seed as protein supplement.
BW (kg, Control lambs) = $13.561 + 0.689X - 0.0003X^2$ ($R^2 = 0.977$)
BW (kg, N-BT lambs) = $15.260 + 0.328X + 0.0130X^2$ ($R^2 = 0.985$)
BW (kg, BT lambs) = $15.860 + 0.470X + 0.0109X^2$ ($R^2 = 0.970$)

Nutrient Intake, Utilization and Balance

A metabolic trail was carried out toward the end of experiment on all animals of treatment II and III, whereas on six representative animals of treatment I. During metabolic trial animals were allowed three days acclimatizing to metabolic cages followed by seven days collection of faeces and urine voided. Daily records of feed intake, faeces and urine voided were maintained to determine nutrient intake, utilization and balance.

Urine was collected under 10 % sulphuric acid, maintaining a pH between 2-3 to avoid degradation of urinary purine derivatives. Urine purine derivates estimates the microbial protein synthesis in rumen.

Dry matter intake

Daily dry matter intake varied from 706 g to 861 g among three dietary treatment groups and was not statistically (p= 0.373) different. The lambs in present experiment consumed feed dry matter 71, 69, and 78 g/ kg $W^{0.75}$ (P= 0.383), and 3.1, 3.2 and 3.5 % of body weight (P= 0.399), respectively in control, N-BT and BT group of experimental lambs (Table 4). Similarly intake of roughage, concentrate and protein source (g/day) were also not statistically different among three lamb groups. Apparently, compared to control and N-BT group of lambs, the lambs fed BT-cotton seed had higher dry matter intake in terms of g/day, g/kg $W^{0.75}$ and % of body weight. The growing lambs consumed 168 g BT-cotton per day did not have apparent adverse effect on dry matter intake. Similarly BT-cotton seed intake of 0.681 % of body weight or 19.5 % of dry matter intake did not produce deleterious effect on dry matter intake, thus palatability is not a problem for BT-cotton seed feeding in lambs.

Nutrient intake and digestibility

Intake of organic matter (OM; 711, 647 and 766g), crude protein (CP; 116, 103 and 120 g), NDF (408, 417 and 483 g), ADF (274,261 and 335 g) and hemicellulose (133, 156 and 148 g), respectively of control, N-BT and BT lamb

groups, was not statistically different among three feeding regimen, whereas intake of cellulose was higher ($p = 0.053$) in BT group of lambs (269g) compared to N-BT (194g) lamb group, however control and BT- group of lambs had similar cellulose intake.

Digestibility of dry matter, 60.3 57.8 and 58.9 %; OM, 63.2, 62.6 and 62.9 %; CP, 62.2, 59.7 and 60.2 % and NDF 43.5, 49.1 and 46.7 % and were respectively in control, N-BT and BT diet fed lambs, which were similar among the three feeding regimens. However, digestibility of ADF was significantly ($p = 0.022$) lower by 7 % units, while hemicellulose digestibility was higher ($p = 0.047$) by 10 % units in BT lambs compared to N-BT lamb group, whereas cellulose digestibility was similar between BT and Non-BT lambs. Interestingly, the digestibility of DM, OM and CP were slightly higher but NDF, ADF, and cellulose were lower in control lambs.

Nutritive Value of Diet

Three diets prepared using GNC, N-BT cotton seed and BT-cotton seed did not significantly influence nutritive value of diet in terms of digestible crude protein (DCP, g/ kg) and metabolizable energy (ME, MJ/ kg) content of diet. The DCP % was 9.1, 8.7 and 8.4 while ME content was 8.5, 8.6 and 8.4 MJ/ kg diet respectively in control, N-BT and BT diets (Table 5).

Digestible Nutrient and Energy Intake

Intake of digestible nutrient and metabolizable energy were also not different among three dietary treatments. Digestible DM intake (g/ day) was 483, 407 and 509 g; digestible OM intake (g/ day) 456, 403, and 484 g and (g / kg diet) 569, 573 and 560 g; digestible CP intake (g/ day) 72, 61 and 73 g and ME intake (MJ/ day) was 6.8, 6.0 and 7.3, respectively in control, N-BT and BT lamb group.

Table 4

Nutrient intake and utilization of weaner lambs on diets containing BT or non-BT cottonseed.

	Diets*			SEM	*P*
	C	N-BT	BT		
Body weight (BW, kg)	24.3	22.08	24.52	0.794	0.407
Body weight $W^{0.75}$ (kg)	10.93	10.16	11.00	0.272	0.403
Dry matter intake					
g/day	790.32	705.93	860.53	43.685	0.373
g/kg $W^{0.75}$	71.04	69.25	77.79	2.571	0.383
kg/ 100 kg BW	3.19	3.20	3.50	0.101	0.399
Roughage intake (g/day)	276.61	247.08	301.19	15.290	0.373
Concentrate intake (g/day)	513.70	458.86	559.35	28.395	0.373
Protein source intake					
g/day	154.11	137.66	167.80	8.519	0.373
Kg/100 kg BW	0.623	0.624	0.681	0.197	0.399
Intake and digestibility coefficient					
Dry matter					
Digestibility coefficient	0.603	0.578	0.589	0.006	0.268
Organic matter					
Intake (g/day)	711.28	646.63	766.30	38.701	0.477
Digestibility coefficient	0.632	0.626	0.629	0.006	0.902
Crude protein					
Intake (g/day)	115.94	102.71	119.96	6.192	0.521
Digestibility coefficient	0.622	0.5969	0.6019	0.0145	0.779
Neutral detergent fiber					
Intake (g/day)	407.49	417.21	483.53	23.520	0.379
Digestibility coefficient	0.435	0.491	0.467	0.011	0.104
Acid detergent fiber					
Intake (g/day)	274.40	261.41	335.26	16.700	0.157
Digestibility coefficient	0.338	0.460	0.392	0.019	0.022
Hemi-cellulose					
Intake (g/day)	133.09	155.80	148.27	7.663	0.496
Digestibility coefficient	0.636	0.542	0.637	0.019	0.047
Cellulose					
Intake (g/day)	209.04	193.92	268.92	13.803	0.053
Digestibility coefficient	0.520	0.629	0.598	0.015	0.002

*Diets: Total mixed ration contained roughage, concentrate and molasses in ration of 60:35:5, concentrate contained groundnut cake (Control-C), non-Bt cottonseed (N-BT) or Bt-cottonseed (BT) as major protein source, which fed to lambs

Table 5

Nutritive value of diets, digestible nutrient intake and N-balance of weaner lambs on diets containing BT or non-BT cottonseed.

	Diets*				
	C	N-BT	BT	SEM	P
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
Digestible nutrient and energy intake					
Digestible DM intake (g/ day)	482.55	407.08	509.18	29.634	0.367
Digestible OM intake (g/ day)	455.79	403.40	483.77	27.213	0.501
Digestible OM intake (g/ kg diet)	569.19	573.12	560.26	5.304	0.627
DCP intake (g/ day)	71.72	61.18	72.55	4.079	0.474
ME intake (MJ/ day)	6.83	6.05	7.25	0.408	0.501
DOMR (g/ day)	296.27	262.21	314.45	17.689	0.501
DOMR (g/ kg diet)	369.98	372.52	364.17	3.448	0.627
N-utilization and balance					
N-intake (g/day)	18.55	16.43	19.19	0.991	0.521
N-excretion					
Faeces (g/day)	7.08	5.75	7.59	0.537	0.375
Urine (g/day)	5.19	4.28	5.66	0.326	0.225
Total (g/day)	12.27	10.86	13.24	0.648	0.340
N- balance					
g/day	6.28	5.57	5.95	0.478	0.848
% of intake	33.36	33.58	30.61	1.678	0.748
% of absorbed	53.67	52.41	51.11	2.787	0.939

*Diets: Total mixed ration contained roughage, concentrate and molasses in ration of 60:35:5, concentrate contained groundnut cake (Control-C), non-Bt cottonseed (N-BT) or Bt-cottonseed (BT) as major protein source, which fed to lambs.

Similarly, digestible organic matter apparently fermented in rumen (DOMR; g/ day) and DOMR in term of g/ kg diet were also similar among control, N-BT and BT diets, DOMR (g/ kg diet) was respectively amounting to 369, 372 and 364 g among three dietary regimens (Table 5).

Nitrogen Utilization and Balance

Nitrogen utilization in terms of N-intake, N-excretion in faeces and urine and balance were not statistically different among control, N-BT and BT diets. N- intake was 18.6, 16.4 and 19.2 g; N-excretion in faeces was 7.1, 5.8, 7.6g and in urine was 5.2, 4.3 and 5.7 g and total-N excretion was 12.3, 10.9 and 13.2 g respectively in control, N-BT and BT diets.

Similar to N-utilisation, N-balance was also not different among three dietary treatments. N- balance in term of g/ day was slightly higher (6.3 g) in control lambs compared to N-BT (5.6 g) and BT (6.0 g) diet fed lambs (Table 5). N-balance as percent of intake ranged between 31 to 34 % and as per cent of absorbed 51 to 54 % in control, N-BT and BT diets.

Table 6

Rumen fermentation and microbial protein synthesis of weaner lambs on diets containing BT or non-BT cottonseed.

	Diets*				*P-values***		
	C	N-BT	BT	SEM	T	P	T*P
pH	6.68	6.71	6.68	0.0287	0.867	0.001	0.008
TVFA (mEq/l)	80.66	86.94	82.49	2.807	0.638	0.004	0.222
NH_3-N (mg/l)	97.54	76.20	88.74	2.145	0.62	0.168	<0.001

*Diets: Total mixed ration contained roughage, concentrate and molasses in ration of 60:35:5, concentrate contained groundnut cake (Control-C), non-Bt cottonseed (N-BT) or Bt-cottonseed (BT) as major protein source, which fed to lambs.
** Significance level (Diet effect-T, Period effect-P, Interaction T×P).

Rumen Fermentation Characteristics

Samples of rumen liquor (50 ml) were withdrawn from intact lambs at 0, 4, 8, 12, 18 and 24 h post-feeding using a stomach tube. Each sample was placed in a 100 ml glass jar and the pH determined using a portable pH meter within 4 to 5 min of sampling. After pH measurement rumen fluid was strained with four layer of muslin cloth, a few drops of saturated mercuric chloride were added to stop microbial activity and stored –20ºC pending analysis. Rumen fluid samples were also processed to monitor the ciliate protozoa population. A 10 ml rumen fluid was processed to separate cellular and extra cellular fractions to estimate microbial enzymes activity in rumen.

Rumen fermentation characteristics are presented in Table 6. The rumen fluid pH was almost similar in control, N-BT and BT group of lambs, which was respectively 6.68, 6.71 and 6.68. Sampling period had significant ($p<0.001$) influence on rumen fluid pH and was stable between 4 to 18 hr of post feeding (Fig 2). Interactions among post feeding hours and treatment were also significant ($p<0.008$).

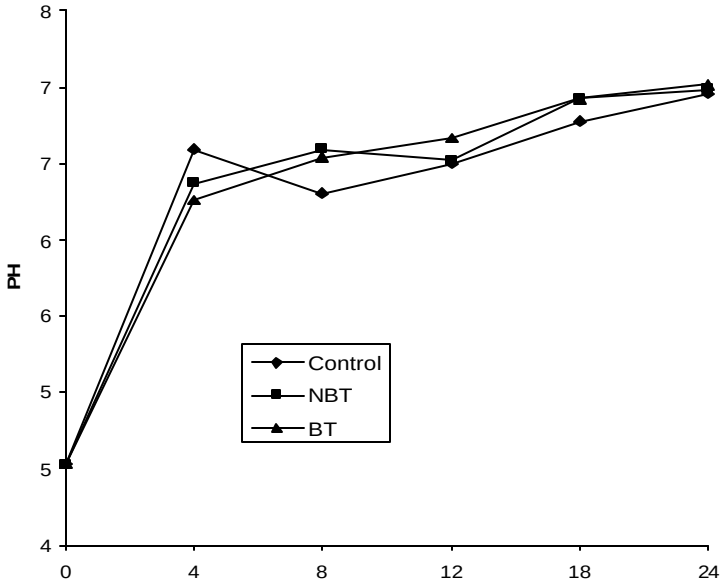


Fig 2: Rumen pH at different post feeding Hours

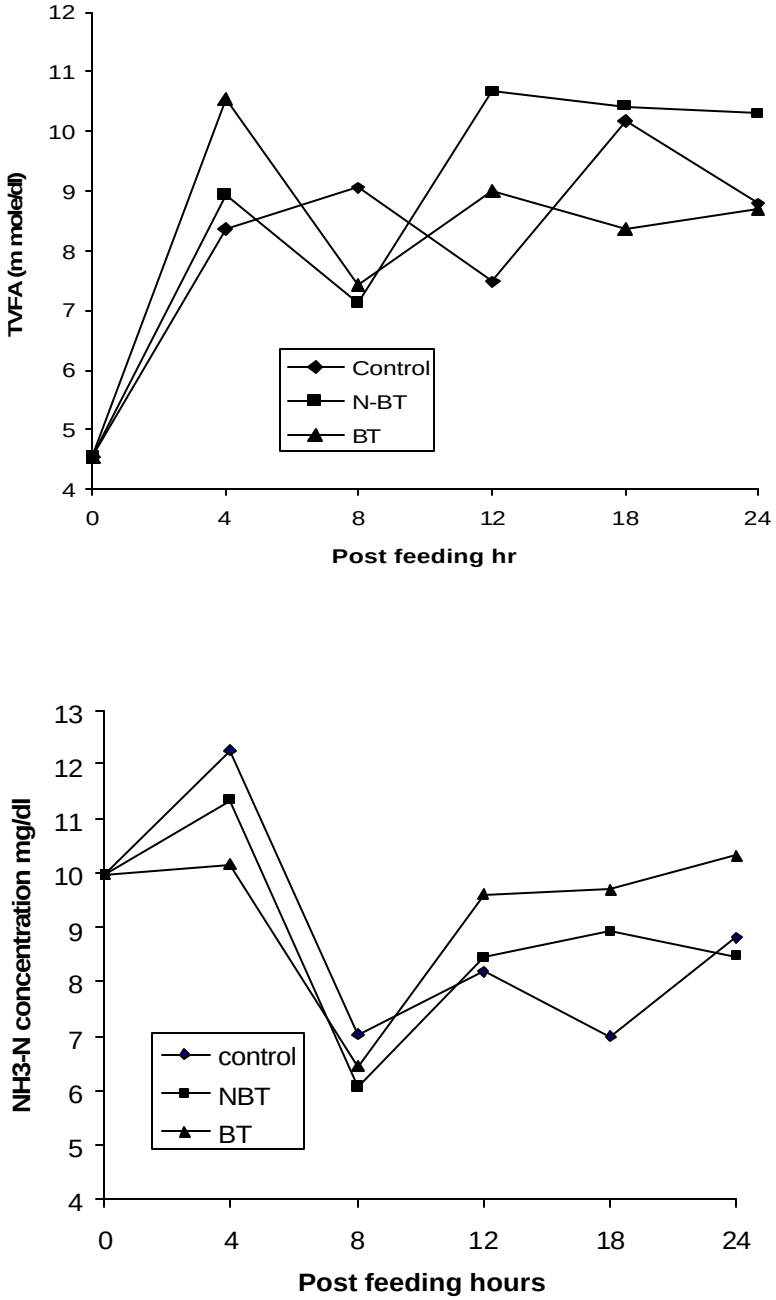


Fig 3: Rumen TVFA and NH_3-N at different post feeding Hours

Rumen TVFA Concentration

The ruminal TVFA (mmol/ l) were also similar in control, N-BT and BT group of lambs, which were respectively 80.7, 86.9 and 82.5 m mol. Sampling period had significant ($p<0.004$) influence on TVFA concentration, a sharp decline in TVFA concentration was between 4-8 hr post feeding for BT and N-BT diet fed lambs, where this decline was between 8-12 hrs post feeding in control lambs (Fig 3). Interactions among post feeding hours and treatment were not significant ($p = 0.222$).

Rumen NH_3-N Concentration

The rumen NH_3-N (mg/ l) concentration were similar in control, N-BT and BT group of lambs, which were respectively 97.5, 76.2 and 88.7 mg. Sampling period also did not influence NH_3-N concentration, a sharp decline in NH_3-N concentration was between 4-8 hr post feeding, while it increased till 12 hr post feeding, thereafter established (Fig 3). Interactions among post feeding hours and treatment were significant ($p < 0.001$).

Blood Composition and Hematology attributes

Blood samples were collected through jugular vein puncture at start of the experiment and at every 30^{th} day of the experiment to study blood bio-chemical and hematological changes.

Results of hematology are presented in table 7. Hemoglobin (Hb %) concentration was almost similar (11.1%), whereas packed cell volume (PCV, %) ranged from 11.7 to 12.9 %; ESR at 2 hr ranged from 25.6 to 29.5 and white blood cells ranged from 6.8 to $8.3\times 10^3/\mu l$ which were not statistically different among control, N-BT and BT group of lambs. While red blood cells concentration was significantly ($p <0.001$) higher ($18.41\times10^6/\mu l$) in BT diet fed lambs compared to control ($15.03\times 10^6/\mu l$) and N-BT ($14.85\times10^6/\mu l$) lambs.

Table 7
Hematology of weaner lambs on diets containing BT or non-BT cottonseed.

	Diet*				*P-values***		
	C	N-BT	BT	SEM	T	P	T×P
Haemoglobin (Hb %)	11.17	11.07	11.09	0.119	0.915	0.202	0.016
Packed cell volume (%)	12.92	11.72	11.70	1.561	0.199	<0.001	0.830
ESR 1hr	24.75	25.43	39.82	4.315	0.497	0.042	0.310
ESR 2hr	29.2	25.6	29.5	1.980	0.707	0.009	<0.001
Red blood cells ($\times 10^6$/µl)	15.03**b**	14.85**b**	18.41**a**	0.482	<0.001	0.494	0.986
White blood cells ($\times 10^3$/µl)	6.82	7.8	8.25	0.445	0.220	0.033	0.603
Lymphocytes (%)	58.73	62.00	59.92	0.839	0.347	0.355	0.057
Neutrophils (%)	35.78	33.22	35.80	0.780	0.308	0.269	0.076
Monocytes (%)	3.81	3.56	3.68	0.273	0.943	0.777	0.008
Eosinophils (%)	1.75	1.86	1.18	0.193	0.251	0.093	0.544
Serum IgG (U/dl)	35.10	36.29	37.08	0.533	0.190	0.690	0.420

*Diets: Total mixed ration contained roughage, concentrate and molasses in ration of 60:35:5, concentrate contained groundnut cake (Control-C), non-Bt cottonseed (N-BT) or Bt-cottonseed (BT) as major protein source, which fed to lambs.
** Significance level (Diet effect-T, Period effect-P, Interaction T×P).

Hemoglobin concentration was lower in BT lambs at 60th day of sampling thereafter increased but control lambs shown a steady increase in Hb level with progress of experiment, however N-BT lambs had highest Hb level at 90th day of blood collection (Fig 4). But these differences were not statistically different (p= 0.202), however interaction between treatment and period of collection were significant (p = 0.016).

ESR concentrations incrased throughout experiment in control and N-BT lambs, but BT lambs had fluctuation levels, which increased from 0 to 30 and 60 to 120 days of feeding but decreased sharply between 30 to 60 days of sampling (Fig 4), and were significantly (p = 0.042) different among the sampling whereas interactions were not significant.

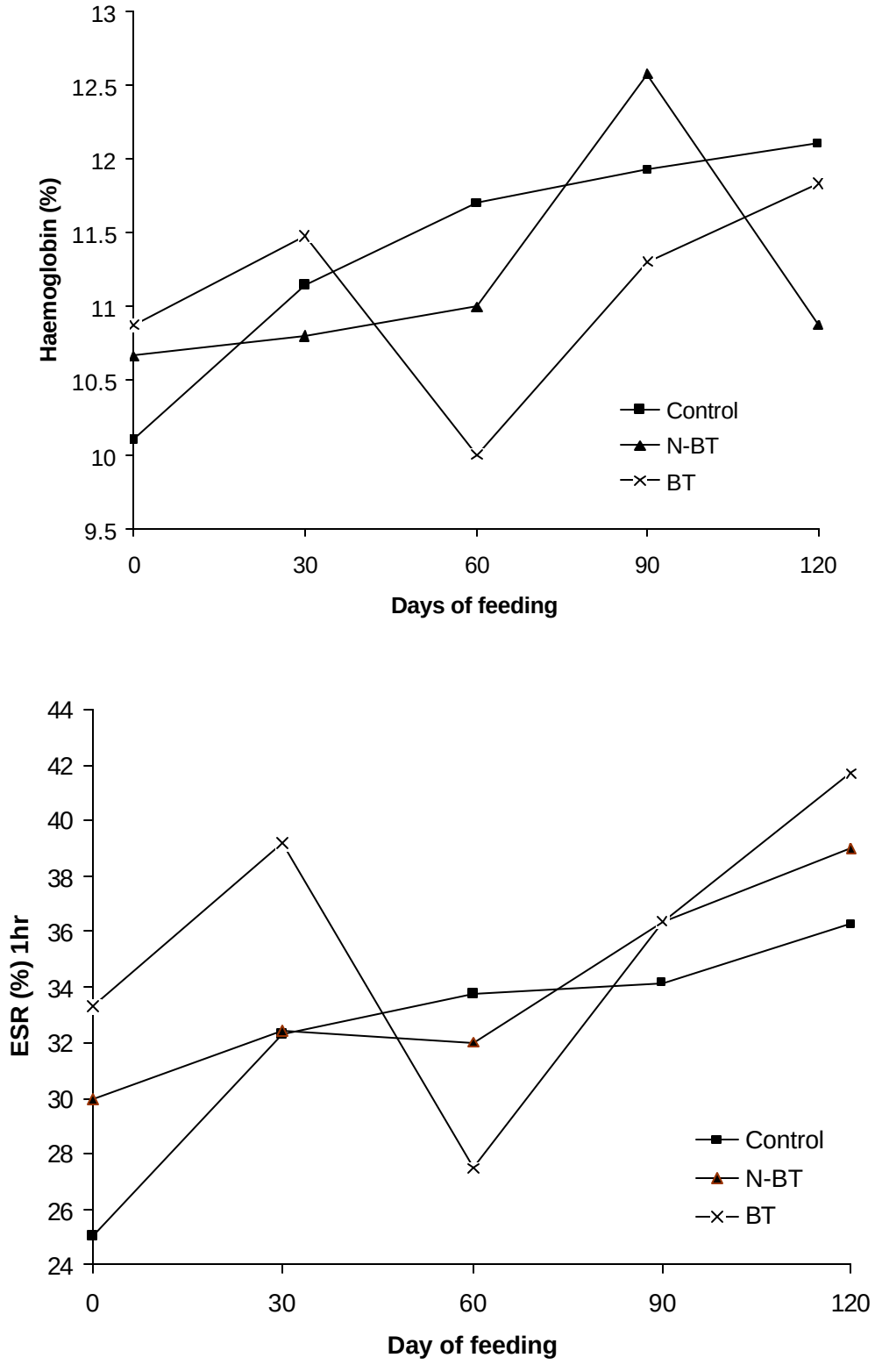


Fig 4: Hemoglobin (%) and ESR (%) concentration of lambs during experimental feeding

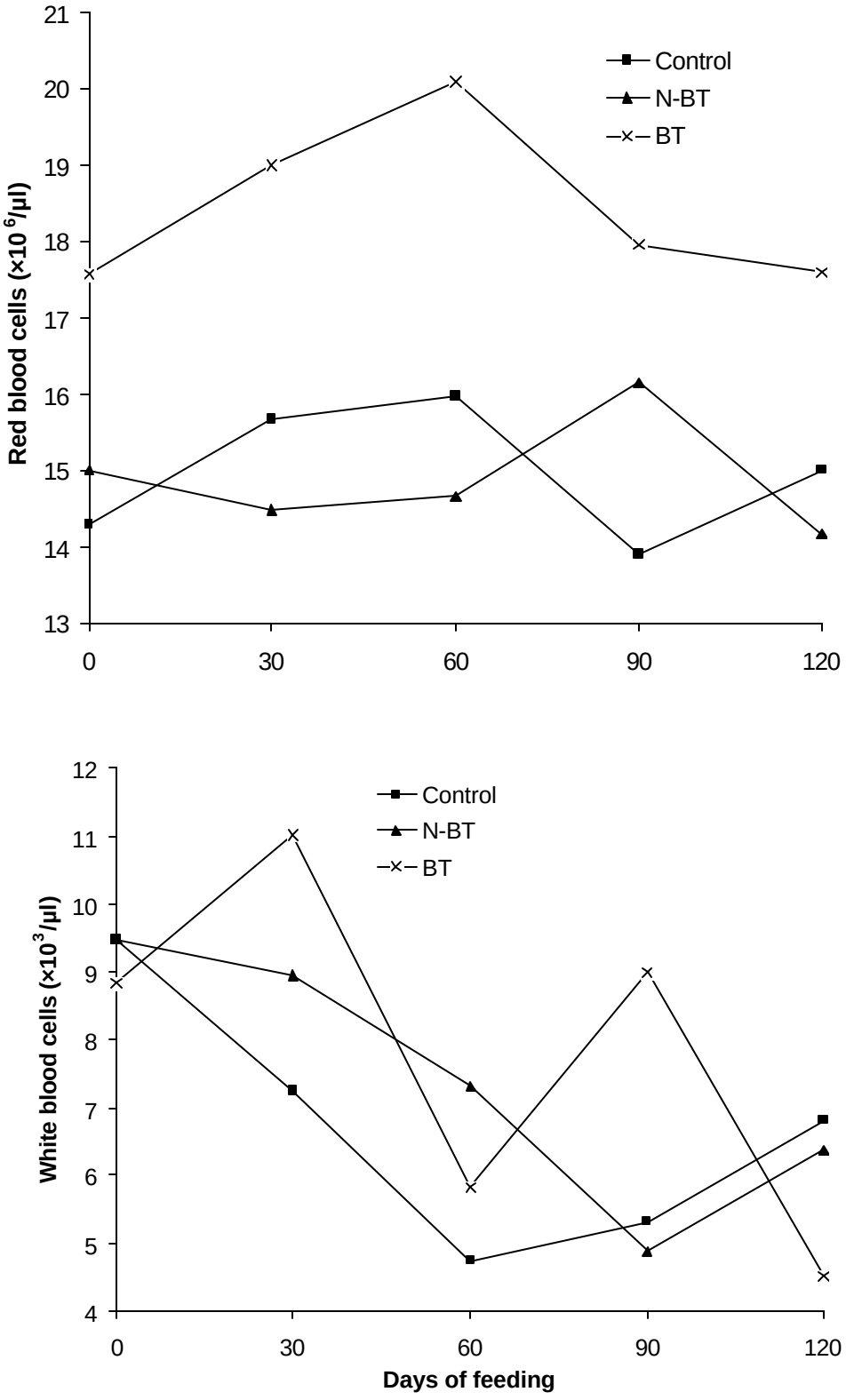


Fig 5: RBC and WBC concentration of lambs during experimental feeding

Pattern of Red blood cells and white blood cells concentration are presented in fig 5. The RBCs were higher throughout experiment in BT lambs compared to control and N-BT lambs. Neither sampling period nor treatment and period interactions were statistically significant.

White blood cells concentration decreased significantly ($p = 0.033$) with increase in experimental period in three group of lambs. Interactions among treatment and period of sampling were not statistically different.

Serum IgG level

Serum IgG determination assesses the immunity and allergens status of animals. Increased levels are the indicative of immunity depression and induction of allergens. The IgG levels were not different ($p = 0.190$) among in control, N-BT and BT diet fed lamb groups. The pattern of IgG change during experimental feeding of BT and N-BT diet to lambs is presented in Table 7 and Fig. 6. Neither period of blood sampling nor interactions between dietary treatments and period of blood collection had significant influence on IgG level of lambs. Thus feeding of BT-cottonseed to lambs did not alter immunity and allergens status of animals.

Slaughter Study to asses Changes of Internal Organs

Animals were slaughtered using standard procedure under the supervision of institute veterinarian, internal organs were weight and collected for histopathological examinations. Carcass of BT-cotton fed animals were not sold to consumer and disposed off using stands producers.

Internal organs weights as gross and in terms of per kg empty live weight (ELW) were estimated to assess a precise effect of BT- cottonseed feeding on internal organ changes. The gross weights of liver and testicles, gross weight and per kg ELW

of kidney, spleen, pancrease, heart, lung, penis, kidney fat, cole fat, GI tract, ingest and empty GI tract were not different among three feeding regimes.

However, liver weight (g / kg ELW) were different (p = 0.04), and were 16.99 g in control lambs, 17.57 g in BT lamb group and 14.95 in N-BT lamb group. The N-BT lamb group had lowest and BT lamb group had highest liver weight, However control and BT lambs had statistically similar liver weights.

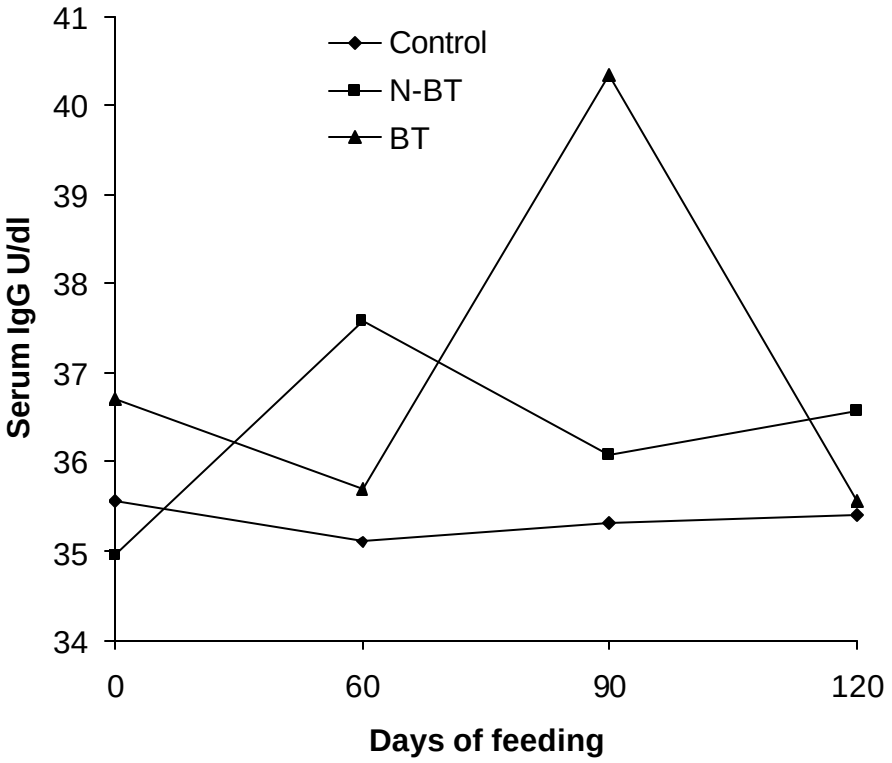


Fig 6: Serum IgG level of lambs during experimental feeding

Table 8

Slaughter study of weaner lambs on diets containing BT or non-BT cottonseed

Attributes	**Diets***			**SEM**	***P***
	Control	**N-BT**	**BT**		
Empty Live weight kg	23.52	25.06	25.48	0.721	0.517
Liver weight g	398.8	375.0	448.3	16.415	0.239
Liver weight g/kg ELW	16.99**b**	14.95**a**	17.57**b**	0.429	0.040
Kidney weight g	60.00	65.00	61.67	2.357	0.770
Kidney weight g/kg ELW	2.56	2.60	2.46	0.131	0.929
Spleen weight g	45.00	45.00	43.33	1.757	0.927
Spleen weight g/kg ELW	1.93	1.79	1.70	0.088	0.566
Lung weight g	515.00	520.00	583.33	20.713	0.360
Lung wt g/ kg ELW	22.02	20.74	22.94	0.763	0.641
Kidney fat g	195.00	360.00	405.00	45.315	0.079
kidney fat g/kg ELW	8.39	14.48	15.83	1.7056	0.114
Cole fat g	316.25	500.00	563.33	57.291	0.138
Cole fat g/ kg ELW	13.56	20.06	21.85	2.066	0.188
Dressing % of ELW	58.17	56.79	55.51	0.789	0.386
Pancreas weight g	37.50	25.00	40.00	4.120	0.433
Pancreas weight g/kg ELW	1.57	0.99	1.56	0.142	0.271
Heart weight g	108.75	110.00	100.00	3.310	0.483
Heart wt g/ kg ELW	4.63	4.38	3.96	0.156	0.177
Testicles weight g	155.0**b**	202.5**b**	266.7**a**	19.041	0.006
Testicles weight g/kg ELW	6.57**b**	8.11**ab**	10.58**a**	0.732	0.023
Testicles fat g	47.50	35.00	43.33	2.357	0.110
Testicles fat g/kg ELW	2.03**a**	1.40**b**	1.70**ab**	0.110	0.049
Penis weight g	52.50	50.00	43.33	3.093	0.486
Penis weight g/kg BW	2.22	2.00	1.69	0.121	0.155

GI tract weight kg	5.13	5.15	5.69	0.257	0.661
GI tract weight g/kg ELW	217.28	205.11	224.37	8.457	0.762
Empty GIT weight kg	2.14	2.31	2.57	0.101	0.188
Empty GIT weight g/kg ELW	91.10	92.20	100.70	2.573	0.261
Ingesta weight kg	2.99	2.84	3.12	0.213	0.915
Ingesta weight g/kg ELW	126.18	112.91	123.67	8.156	0.856

*Diets: Total mixed ration contained roughage, concentrate and molasses in ration of 60:35:5, concentrate contained groundnut cake (Control-C), non-Bt cottonseed (N-BT) or Bt-cottonseed (BT) as major protein source, which fed to lambs

Gross testicle weight was higher ($p = 0.006$) in BT lambs compared to control and N-BT lambs, similarly testicles weights in terms of g/ kg ELW was also significantly ($p = 0.023$) higher in BT lambs compared to Control lambs but were not statististically different to N-BT lambs. However testicles fat g / kg ELW was lowest in N-BT lambs compared to control and BT-lambs.

Findings of the Project

- The CP and hemicellulose contents were higher and ADF content was lower in BT-cotton seed compared to than non-BT cotton seed,

- Mineral contents viz. Calcium, Phosphorus, Manganese and Copper were identical in N-BT and BT cottonseed, while Zinc content was higher in BT-cotton seeds by 10 ppm,

- The growing lambs consumed 168 g BT-cotton seed per day and did not have apparent adverse effect on dry matter intake. Similarly BT-cotton seed intake of 0.681 % of body weight or 19.5 % of dry matter intake did not produce deleterious effect on dry matter intake, thus palatability is not a problem for BT-cotton seed feeding in lambs.
- BT-cotton seed feeding @ 20 % of total dry matter intake improved growth, nutrient utilization, rumen fermentation and N balance compared to non-BT cotton seed feeding,

- BT-cotton seed feeding did not alter haematological attributes of lambs and were with in the normal range of variations,

- BT-cotton seed feeding increased RBC and decreased WBC in blood,

- Serum IgG level did not change due to BT and non-BT cotton seed feeding. Thus feeding of BT-cottonseed to lambs did not alter immunity and allergens status,

- Internal organs weights as g per kg empty live weight (ELW) estimated precise effect of BT- cottonseed feeding on internal organ changes. The weights of kidney, spleen, pancrease, heart, lung, penis, kidney fat, cole fat, GI tract, ingest and empty GI tract were not different among between BT cotton seed and non-BT cotton seed fed lambs. However BT cotton seed feeding increased liver weight, testicle weight and testicles fat g / kg empty live weight.

Funds Utilization

Total funds received: Rs. 4,45,000=00

Total expenditure incurred: Rs. 4,88,000=00

Deficit of funds due to over expenditure : Rs. 43,000=00

Reasons for over expenditure:

The total emoluments of the Research Fellow have been revised and applicable with effect from 01.04.2007. Hence, fellowship arrears are to be paid to SRF worked under the project.

CERTIFICATE

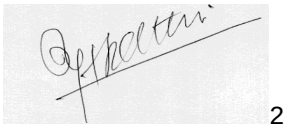
Institute and investigators of the project in principle agree with the research results reported in present report

Dr. S. A. Karim
Principal Investigator

28.03.2008

Signature with date

Dr M. K. Tripathi
Co- Principal Investigator


28.03.2008

Signature with date

Director