Comparison of chicken performance when fed with diets containing Bt cotton, parental non Bt line or commercial cotton

Chapter V

Annexure 3

FINAL REPORT

Comparison of Chicken Performance When Fed With Diets containing Bt Cotton, Parental Non-Bt Line or Commercial Cotton

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Final Report

Project Title: Comparison of Chicken Performance When Fed With Diets containing Bt Cotton, Parental Non-Bt Line or Commercial Cotton

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Study Dates :

Chicks received	: 05-02-2001-09-14
Biossay period	: 5th Feb ï 19th Mar. 2001
Summary	: 21st Mar. 2001
Final Report	: 25th Mar. 2001

Objectives: Comparing performance and carcass yield of broiler chicks fed with Bt cottonseed, Parental Non-Bt Cottonseed, and commercial cottonseed.

Summary

This study was conducted to assess comparative nutrient utilization and carcass quality in broiler chickens fed diets containing solvent extracted cottonseed meal (CSM) processed from **Bt & parental non-Bt line cottonseeds.** For effective comparison similarly processed meal of national check and commercially produced cottonseeds were also used in the broiler diets and fed separately. The free gossypol contents were 0.02, 0.02, 0.44 and 0.03% in solvent extracted meals of Bt, parental Non-Bt line, national check and commercially produced cottonseeds, respectively. Day-old unsexed broiler chicks (n=243) were divided to 27 groups of 9 each. Nine dietary treatments (isonitrogenour, 23% crude protein and isocaloric, 2800 kcal ME/kg) were formulated viz., DI (control, soybean meal-SBM based), D2 and D3 (Bt CSM included at 10% of diet with and without additional iron, respectively), D4 and D5 (Non-Bt CSM with and without additional iron, respectively), D6 and D7 (national check CSM with or without additional iron, Respectively). The additional iron (Fe) was included at the rate of 2 ppm for every 1 ppm of free gossypol. Each dietary treatment was offered to 27 broiler chicks in three replicated groups up to 6 weeks of age. All the birds were housed in battery cages under uniform housing, management and feeding except the variations in the dietary treatments as envisaged above. The body weight change and feed intake were recorded weekly. At the end of 6 weeks of age, 10 birds were taken out randomly from each treatment and were sacrificed to study carcass and giblet yield and weight of organs.

The date of 0-6 weekôs period revealed that the body weight from **Bt (D2, 1753g & D3, 1638g) & parental non-Bt (D4, 1653g & D5, 1687g) Line cottonseeds** with or without additional Fe were similar to those of broilers fed the soybean meal **(solvent ext.) based diet (D1, 1676g).** The feed intake and feed conversion efficiency (feed intake : weight gain) in these dietary treatments (Bt, non-Bt line based diets) also did not differ significantly ($P>0.05$) from control diet. Similar observation with also observed in dietary treatments (D8 & D9) containing solvent extracted cottonseed meal of commercially produced origin. However, a significant decrease ($P>0.01$) in

body weight gain and feed intake was observed in D6 containing national check CSM with high gossypol content. Addition of Fe in the diet (D7) improved (P>0.01) feed intake and weight gain but not to the extent as observed in diets

Containing Bt, Parental non-Bt, commercially produced CSM or control. However, CSM, regardless of type, did not affect feed conversion efficiency when fed with or without addition iron.

The carcass characteristics in terms of dressing percentage and giblet yield were similar (P>0.05) between the Bt, non-Bt, commercially produced, or control soybean meal treatments. The eviscerated yields emanated from diets containing either Bt, non-Bt or commercially produced CSM were statistically similar to that of the control. However, eviscerated yield of broilers fed National check CSM with or without iron supplementation was significantly (P>0.05) lower than the control soybean meal.

Survival, growth rate, feed intake, feed conversion and carcass characteristics were not statistically different between boiler chicks fed Bt cottonseed meal compared to broiler chicks fed non-Bt or conventional cottonseed meal. Furthermore, based on the extensive research experiences at the Central Avian Research Institute, the survival, growth rate, feed intake, feed conversion and carcass characteristics measured in the current study for broiler checks fed Bt cottonseed meal were typical of those observed for broiler chicks in other studies. Based on the data from the current study, Bt cottonseed meal is as safe and nutritious as non-Bt cottonseed meal for performance of broiler chicks.

Growth rate, feed conversion and carcass characteristics of broiler chicks fed diets containing Bt or non-Bt cottonseed meal were not statistically different from broiler chicks fed diet containing soybean meal. Therefore, the results of this study suggest that solvent-extracted cottonseed meal could be used in place of soyabean meal in broiler chick feed up to 6 weeks of age (market age)

A significant decrease in body weight gain and feed intake was observed broiler chicks fed diets containing National check CSM with high gossypol content. Addition of Fe in the diet improved weight gain and feed intake but not to the extent as observed in diets containing low gossypol levels (Bt, parental non-Bt, commercially produced CSM or soybean control). Lower body weight gain and feed intake in the National check might be attributed to higher free gossypol content in the National check variety (0.44% flee gossypol). Addition of iron at the rate of 2 ppm per 1 ppm gossypol partially ameliorated the toxic effect of gossypol but was unable to curb its toxicity completely.

Introduction

Proteinic ingredients are costlier components in poultry mash. Identification and evaluation of effective levels of proteinic agro-industrial by-products has been the priorities amongst the nutritionists. Cottonseed and cottonseed meal (expeller pressed) are popular amongst the dauy farmers because these help in increasing the butter fat content and separation of fat from milk However, with the installation of solvent extraction plant, it is likely that large quantity of solvent extracted cottonseed meal will be available in market in near future, which may have little value in the diet of milch animal for milk production and separation of milk fat. Therefore, it may be used in poultry mash as a diversified source of protein. There are certain factors viz. high fibre content, gossypol content and lysine deficiency are limiting its *use* in poultry feed. Bt and non-Bt cottonseeds meals (solvent extracted) are low in fibre and free gossypol contents. therefore, the present experiment was conducted to find out the efficacy of Bt cotton non-Bt cotton and commercial cottonseed meals at 10% levels in soybean meal (a commonly used proteinic by-product rich in lysine) based diets for optimum broiler performance.

Materials and Methods

The research work was conducted at Avian Nutrition and Feed Technology Division of Central Avian *Researc*h Institute, Izatnagar, India The biological trial involving broiler chicks was conducted from 5" Feb. to 19Lh Mar. 2001. The detailed materials and methods employed in the study are presented as follows:

Selection of chicks and experimental design

Three hundred day-old unsexed broiler chicks (ARBOR ACRESCRES strain) were procured from local hatchery at Haldwani, Uttaranchal state. They were vaccinated against Ranikhet (day 1) and Infectious Bursal Disease (14 and 35 days of age). Two hundred and *forty* three (243) thrifty and bright looking unsexed, day-old broiler chicks were selected, wing banded, weighed and randomly distributed into 27 groups of 9 chicks each. The experiment was conducted following completely randomized design having 9· *dietary* treatments with 3 observations (replicates) in each.

Housing and Brooding

Chicks from day-old were housed in battery brooder cages up to 3 weeks of age. and thereafter shifted to battery cages fitted with feeder, waterer and dropping trays.

Selection of leed ingredients

The feed ingredients were procured once for all in one lot from feed store of Central Avian Research Institute. They were analysed for proximate composition, calcium and phosphorus. The four cottonseed (Bt cotton, non-Bt cotton, National check .cotton and commercially produced of cotton) samples, supplied by Maharashtra Hybrid Seeds company Ltd. (Mahyco), New Delhi, were processed (defatted and ground) in the laboratory (Central Avian Research Institute, Izatnagar,

India) for preparation of their respective solvent extracted meals (undecorticated). The proximate composition of different cottonseed meals is presented in Table 1. The gossypol contents of each cottonseed meal are presented in Table 2.

Feeds and feeding

During the entire growth period extra care was exercised to ensure efficient feeding and watering of chicks. They were supplied fresh drinking water *ad lib.* Ninedietary treatments (isonitrogenous, 23% crude protein and isocaloric, 2800 kcal ME/kg) were formulated following standard specifications (ISI, 1992) viz., D1 (control, soybean meal-SBM based), D2 and D3 (Bt CSM included at 10% of diet with and without additional iron, respectively), D4 and 13;5 (non-Bt CSM included at 10% of the diet with and without additional iron, respectively), D6 and D7 (national check CSM included at 10% of the diet with or without additional iron, respectively) and T8 and T9 (commercially produced CSM included at 10% of the diet with or without additional iron). Additional iron(as ferrous sulphate) was included at the rate of2 ppm for every 1ppm of free gossypol. The details of identification of dietary treatments, their ingredient and chemical composition are given in Tables 3 and 4. The ingredients were ground (2.4 mm, screen size) if required, weighed as per feed formulae and added successively, by and by, in the whole lot The mineral and vitamin supplements were premixed in maize before mixing in the whole lot to ensure uniform mixing. All diets had more or less similar metabolisable energy, crude protein, lysine, methionine, calcium, phosphorus and crude fibre concentration, and met the requirements as specified by ISI (1992). No medications or feed additives *I* growth promoters was added to the diet. The mixing was done in vertical *feed* mixer. Each dietary treatment was offered to 27 broiler chicks in three replicated groups of 9 each up to 6 weeks of age. Body weight of individual broiler chicks was recorded weekly. The feed intake of chicks was recorded at weekly intervals from 0-6 weeks of age by offering weighed quantity of feed and weighing their residues The mortality of birds was recorded as and when it occurred, weighed and sent for postmortem examination. The feed conversion ratio was calculated on the basis of unit feed consumed to unit

body weight *gain*. The body weight of dead birds were recorded as and when it occurred and the gain was also taken into consideration for calculation of feed conversion ratio.

Carcass traits

At the end of 6th week of age, 10 broiler chicks picked up randomly from each treatment groups'(3 birds each from two pens & 4 from the third pen). They were starved for 12-hr. (drinking water was supplied *ad lib),* and were sacrificed as per standard procedure (Mead, 1989) for evaluation of carcass characteristics including thedefeathered yield, eviscerated carcass yield, weight of the liver, heart and gizzard of chicks.

Laboratory Analyses

The representative samples of feed ingredients were analysed for proximate composition (moisture, crude protein, ether extract, crude fibre, total ash and nitrogen free extract), calcium and phosphorus following standard techniques (AOAC, 1990). The processed cotton seed meal samples were analysed for total and ~ee gossypol content following standard techniques (AOCS, 1989).

Statistical analysis

Data were subjected to analyses of variance following one way classification of completely randomized design (Snedecor and Cochran, 1989). The means of different dietary treatments were tested for statistical significance using Duncan's multiple range tests (Duncan, 1969)

Results

The results.obtained in the present study are presented in this chapter under different headings.

Body weight gain

The body weight gains at 0-4 weeks and 0-6 weeks of age are given in Tables 5 and 6, respectively. The live weight changes have also been depicted in fig. 1 and fig. 2. The six weeks body weight gain of broiler chickens receiving solvent extracted cottonseed meal (CSM) processed from **Bt (D2, 1753g & D3, 16388) and parental non-Bt D4, 16538 & M, 16878) line cottonseeds** with or without additional Fe were similar to those observed for broilers fed **soybean meal (solvent ext.) based diet (D1, 16768).**

The rapidly growing chicks are highly sensitive to any toxic effect of the feed which may manifest itself by growth depression. As Bt, non-Bt and commercially produced CSM did not depress body weight gain either at 0-4 or 0-6 weeks of age, one can conclude the incorporation of CSM at 10% level did not exert any toxic effect. However, a significant decrease CP<O:OI) in body weight gain was observed in D6 containing National check CSM with high gossypol content. Addition of Fe in the diet (D7) improved (e<0.01) weight gain but not to the extent as observed in diets containing low levels of gossypol (Bf parental non-Bt, commercially produced CSM or soybean control). Lower body weight gain in D6 might be attributed to higher free gossypol content in National check variety (0.44% free gossypol). Addition of iron at the rate of 2 ppm per 1 ppm of free gossypol partially ameliorated the toxic effect of gossypol but was unable to curb its toxicity completely.

Feed intake and Feed conversion ratio

The feed intaice and body weight gain (Tables 5 and 6) for dietary treatments (Bt, non-Bt line based diets) were similar (P>O.O5) to the soybean meal control diet. Feed intake recorded in *D8* and D9 (with commercially produced CSM) was also similar to that in the soybean meal control. These results indicate that the cottonseed meal from Bt, non-Bt or commercially produced CSM were equally palatable and nutritious for broiler chick growth. However, a signi6cant (P~0.05) depression in feed intake was observed in D6 containing CSM of National check, which might account for the lower body weight gain of birds in this group. Supplementation of Fe improved feed intake (D7). Lower feed intake in D6 might be attributed to higher free gossypol content in National check variety (0.44% free gossypol).

There was no significant difference in feed conversion efficiency (feed intake: body weight gain) among any of the CSM and control soybean meal treatments when fed with or without additional iron both at 0-4 (Table 5) or 0-6 (Table 6) weeks of age. Therefore, feed conversion efficiency was not affected by the level of free gossypol (up to 0.44%) in this study.

Carcass characteristics

The carcass characteristics (Table 7) in terms of dressing percentage and giblet yield were not significantly (P>0.05) different between the Bt, non-Bt, ·commercially produced CSM, or control soybean meal treatments. The eviscerated yields emanated from diets containing either Bt, non-Bt or commercially produced were statistically similar to that of control. However, eviscerated yield of broilers fed National check CSM with or without iron supplementation was significantly (P<0.05) lower than the control soybean meal. (Table 7 shows no statistical difference between the National Check and the other CSMs, but difference from control soy). The yield of different organs when expressed on unit live weight did not differ significantly among the treatments (P>0.05).

Mortality

Total of seven birds died (Table 9) during the whole experimental period which is equivalent to 2.89%, an insignificant event. The mortality of birds were 2, 2, 2, and 1 in dietary treatments D2, D5, D6 & D9, respectively. However, the pathological changes observed on post-mortem examination of dead birds during the experimental period did not attribute to the dietary treatments.

Conclusion

Survival, growth rate, feed intake, feed conversion and carcass characteristics were not statistically different between broiler chicks fed Bt cottonseed meal compared to broiler chicks fed non-Bt or conventional cottonseed meal. Furthermore, based on the extensive research experiences at the Central Avian Research Institute, Izatnagar, the survival, growth rate, feed intake, feed conversion and carcass characteristics measured in the current study for broiler chicks fed Bt cottonseed meal were typical of those observed for broiler chicks in other studies. Based on the data from the current study, Bt cottonseed meal .is as safe and nutritious as non-Bt cottonseed meal for performance of broiler chicks.

Growth rate, feed intake, feed conversion and carcass characteristics of broiler chicks fed diet containing Bt or non-Bt cottonseed meal were not statistically different from broiler chicks fed diet containing soybean meal. Therefore, the results of this study suggest that solvent-extracted cottonseed meal could be used at 10% level (w/w) in place of soybean meal in broiler chick feed up to 6 weeks of age (market age).

A significant decrease in body, weight gain and feed intake was observed in broiler chicks fed diets containing National check CSM with high gossypol content. Addition of Fe iii the diet improved weight gain and feed intake but not to the extent

as observed in diets containing low levels of free gossypol. Addition of iron at the rate of 2 ppm per 1 ppm of 6~ee gossypol partially ameliorated the toxic effect of gossypol but was unable to curb its toxicity completely.

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Table 1. Proximate composition (% on dry matter basis) of cottonseed meals

CSM	Moisture	CP	EE	CF	TA	NFE
BT CSM	7.22	34.40	0.91	11.12	5.32	48.25
Non BT CSM	3.09	33.18	0.50	12.65	5.06	48.61
National check	5.48	35.20	0.85	15.57	5.10	43.28
Comm. CSM	7.43	35.17	0.77	16.65	5.07	42.34

Table 2. Protein & Gossypol Content (% on dry weight basis) in CSM

	Protein		Gossypol, tot.		Gossypol, free	
	Seeds	**CSM**	**Seeds**	**CSM**	**Seeds**	**CSM**
BT CSM	**27.1**	**34.40**	**1.23**	**0.74**	**1.14**	**0.02**
Non BT CSM	**25.2**	**33.18**	**1.52**	**0.58**	**1.39**	**0.02**
National check	**25.4**	**35.20**	**1.59**	**0.70**	**1.35**	**0.44**
Comm. CSM	**-**	**35.17**	**-**	**0.72**	**-**	**0.03**

Table 3. Treatment identification and distribution of chicks in different experimental diets.

Diet No.	Treatment	Chicks taken	Removed
1	**Control (SBM)**	**27**	**Nil**
2	**BT**	**27**	**Nil**
3	**BT Fe**	**27**	**Nil**
4	**Non BT**	**27**	**Nil**
5	**Non BT Fe**	**27**	**Nil**
6	**National**	**27**	**Nil**
7	**National Fe**	**27**	**1 (weakling)**
8	**Comm.**	**27**	**Nil**
9	**Comm. Fe**	**27**	**1 (weakling)**

Table 4. Ingredient (% as such basis) and chemical composition of experimental diets.

	Treatment								
Ingredient	D1 Control	D2 Untreated	D3 Treated	D4 Untreated	D5 Treated	D6 Untreated	D7 Treated	D8 Untreated	D9 Treated
Maize	54.5	56	56	56	56	56	56	56	
Soyabean	29	23.7	23.7	23.7	23.7	23.7	23.7	23.7	2
Rice Bran	6.2	0	0	0	0	0	0	0	
CSM, A	0	10	10						
CSM, B				10	10				
CSM, C						10	10		
CSM, D								10	
Fish meal	8	8	8	8	8	8	8	8	
LS	1	1	1	1	1	1	1	1	
DCP	1	1	1	1	1	1	1	1	
T. min.[a]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
Vot. Prem.[b]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
Salt	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	
Fe So_4	0	0	0.02	0	0.02	0	0.04	0	C
Total	100	100	100	100	100	100	100	100	
Chemical composition (dry matter basis)									
ME* kcal/kg	28.02	28.02	28.02	28.02	28.02	28.02	28.02	28.02	2
CP, %	23.02	23.02	23.02	23.02	23.02	23.02	23.02	23.02	23
Lys*, %	1.21	1.18	1.18	1.18	1.18	1.18	1.18	1.18	1
Met.* %	0.52	0.53	0.53	0.53	0.53	0.53	0.53	0.53	C
Ca%	1.25	1.30	1.30	1.29	1.29	1.29	1.29	1.29	1
P(total)	0.89	0.91	0.91	0.90	0.90	0.90	0.90	0.90	C
P(Av. P)	0.48	0.49	0.49	0.48	0.48	0.48	0.48	0.48	C
CF	4.20	4.35	4.35	4.50	4.50	4.80	4.80	4.90	2

(a) supplied mg / kf diet: mg, 300; Mn,55;I, 0.4; Fe, 56; Xen, 30; Cu, 4

(b) supplied per kg diet: Vit.A, 8250 IU; vit.D_3,, 1200 ICU; Vit.K, Img; VitE, 40 IU; Vit. B_1, 2 mg; Vit. B2 4 mg; Vit.B12, 10mcg; miacin, 60mg; pantothenic acid, 10mg; choline, 500mg.

*** Calculated Values**

Table 5. Production Performance of broilers from 0-4 wk of age

Diet	Treatment	Weight gain (grams/bird)	Feed intake (grams/bird)	PCR
1.	Control (SBM)	848.3[a]	1637[a]	1.93
2.	BT	881.6 [a]	1648.5 [a]	1.87
3.	BT Fe	815.3 [a]	1550.5 [b]	1.90
4.	Non BT	835.7 [a]	1615.9 [ab]	1.93
5.	Non BT Fe	829.7 [a]	1628.1 [ab]	1.97
6.	National	573.9 [c]	1158.0 [d]	2.00
7.	National Fe	715.7 [b]	1413.5 [c]	1.98
8.	Comm.	829.0 [a]	1646.7 [a]	1.99
9.	Comm. Fe	834.0 [a]	1616.7 [ab]	1.94
	SEM	18.89	31.04	0.015

SEM Standard error (±) of means
A,b,c Means with the same superscript in the column are not significantly different (P>0.05).

Table 6. Production Performance of broilers from 0-6 wk of age

Diet	Treatment	Weight gain (grams/bird)	Feed intake (grams/bird)	FCR
1	Control (SBM)	1676.1[a]	3472.4 [a]	2.07
2	BT	1753.0 [a]	3472.3 [a]	1.98
3	BT Fe	1637.8 [a]	3277.9 [a]	2.05
4	Non BT	1653.1 [a]	3444.8 [a]	2.08
5	Non BT Fe	1686.9 [a]	3463.9 [a]	2.05
6	National	1149.0 [c]	2370.5 [c]	2.06
7	National Fe	1456.1 [b]	2830.0 [b]	1.95
8	Comm.	1620.6 [a]	3475.4 [a]	2.15
9	Comm Fe	1683.9 [a]	3381.4 [a]	2.01
	SEM	36.02	75.62	0.02

SEM Standard error (±) of means
Feed conversion ratio = feed consumed g/body weight gain, g
Abc Means with the same superscript in the colmn are not significantly different (P>0.05)

Table 7. Carcass yield (% of live weight) of broilers at 6 weeks of age

Diet	Treatment	Defeathered Yield (%)	Eviscerated Yield (%)	Giblet (g/100g)
1	Control (SBM)	89.57	64.93[ab]	21.08
2	BT	90.21	65.09 [ab]	18.98
3	BT Fe	89.24	65.89 [a]	19.14
4	Non BT	90.04	64.73 [ab]	21.37
5.	Non BT Fe	89.58	65.05 [ab]	22.06
6	Nantional	90.38	63.83 [b]	20.82
7	National Fe	90.45	63.63 [b]	23.90
8	Comm.	90.94	66.31 [a]	21.78
9	Comm. Fe	90.73	66.26 [a]	21.84
	SEM	0.16	0.22	0.49

SEM Standard error (±) of means

Abc Means with the same superscript in the column are not significantly different (P>0.05).

Table 8. Carcass yield (% of live weight) of broilers at 6 weeks of age

Diet	Treatment	Gizzard (g/100g)	Heart (g/1000g)	Liver (g/1000g)
1	Control (SBM)	21.08	4.55	21.52
2	BT	18.98	4.6	21.99
3	BT Fe	19.14	4.83	21.43
4	Non BT	21.37	5.07	23.50
5	Non BT Fe	22.06	4.68	21.76
6	National	20.82	4.79	22.20
7	National Fe	23.90	5.12	23.20
8	Comm.	21.78	5.12	23.62
9	Comm. Fe	21.84	4.62	21.46
	SEM	0.33	0.079	0.32

SEM Standard error (±) of means

Table 9. Mortality of birds at different experimental weeks

Diet	Treatment	1st	2nd	3rd	4th	5th	6th	Total	Total (%)
1	Control (SBM							Nil	
2	BT		1	1				2	7.4
3	BT Fe							Nil	
4	Non BT							Nil	
5	Non BT Fe		2					2	7.4
6	National		2					2	7.4
7	National Fe							Nil	
8	Comm.							Nil	
9	Comm Fe		1					1	3.7

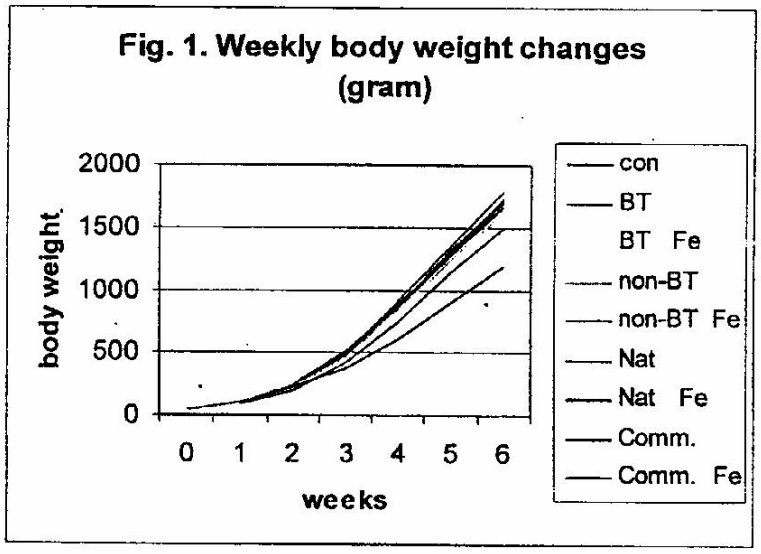
Fig. 1. Weekly body weight changes (gram)
2000
1500
1000
500
0
body weight
0 1 2 3 4 5 6
weeks
con
BT
BT Fe
non-BT
non-BT Fe
Nat
Nat Fe
Comm.
Comm. Fe

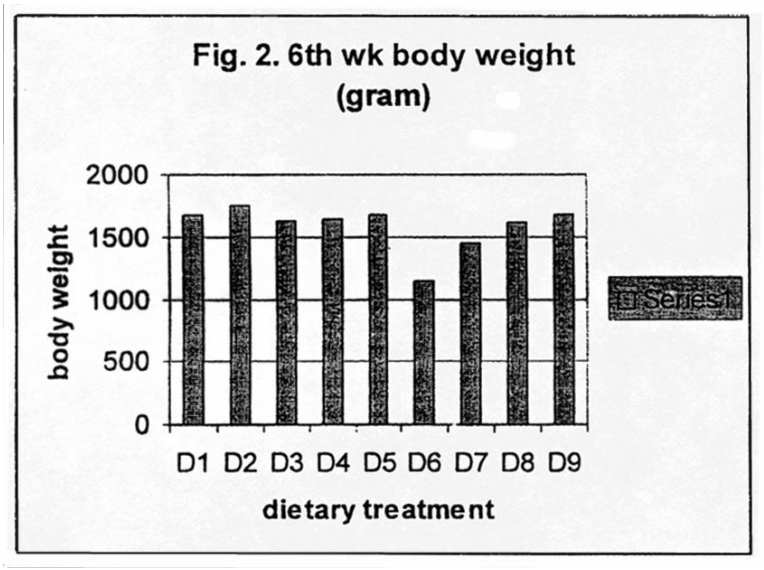
Fig. 2. 6th wk body weight (gram)
2000
1500
1000
500
0
body weight
D1 D2 D3 D4 D5 D6 D7 D8 D9
dietary treatment
Series1

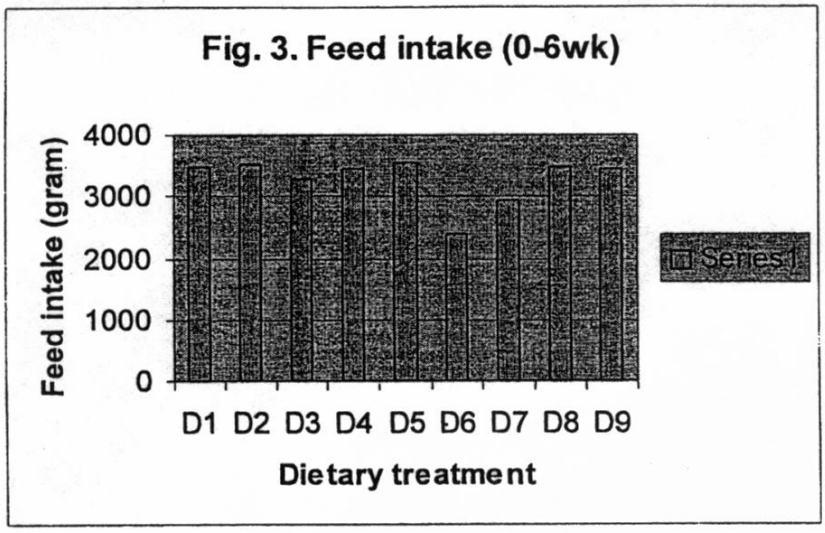
Fig. 3. Feed intake (0-6wk)
Feed intake (gram)
4000
3000
2000
1000
0
D1 D2 D3 D4 D5 D6 D7 D8 D9
Dietary treatment
Series1

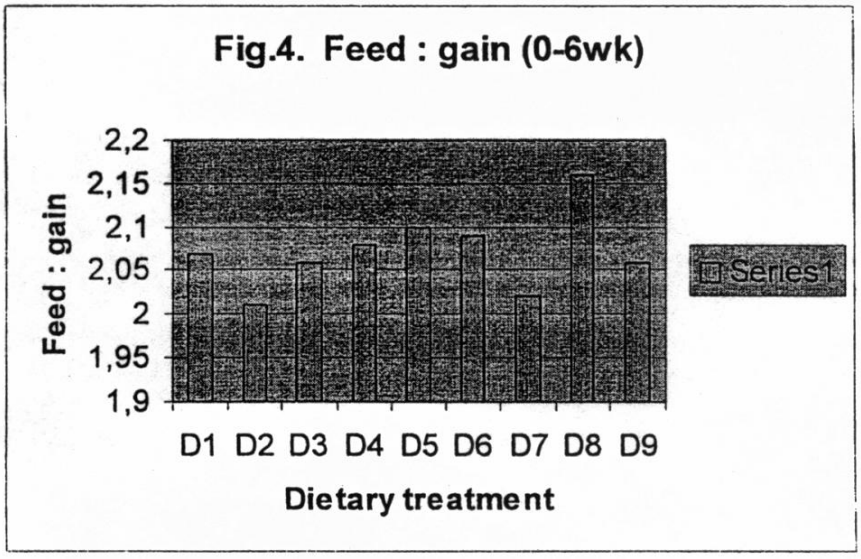
Fig.4. Feed : gain (0-6wk)
Feed : gain
2,2
2,15
2,1
2,05
2
1,95
1,9
D1 D2 D3 D4 D5 D6 D7 D8 D9
Dietary treatment
Series1

Effect of feeding cottonseed produced from Bt cotton on feed intake milk production and composition in lactating cows in India

Chapter V

Annexure 6

FINAL REPORT

Effects of Feeding Cottonseed Produced from Bt Cotton for Four Weeks on Feed Intake, Milk Production and Composition in Lactating Cows in India

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Dairy Cattle Nutrition Division
NATIONAL DAIRY RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
KARNAL 132 001, Haryana

Final Report

April, 2001

Study Title

Effects of Feeding Cottonseed Produced from Bt Cotton for Four Weeks on Feed Intake, Milk Production and Composition in Lactating Cows in India

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Statement of No Data Confidentiality Claims

This is to certify that the performing laboratory is not making any claim of confidentiality for any information in this study.

This performing laboratory also states that the issues related to raw data and materials will be stored in the laboratory and will be accessible to the authorized scientists of the laboratory and/or persons authorized by the sponsors. The workers on the project are free to publish the data in the form of research paper or in any other form.

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Principal Investigator	Dr. K.K. Singhal
Designation	Sr. Scientist

Signature

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Date 18.4.2001

Statement of Particular of Study

Title	Effects of feeding cottonseed produced from Bt cotton for four weeks on feed intake, milk production and composition in lactating cows in India
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Study Initiation Data	22.01.2001
Experimentation termination Date	10.03.2001
Record Retention	Raw data of the study will be retained by the Study Director and this will be made available to the authorized personal from the sponsors

Signature of Approval

Study Director	**Date** 18/4/2001
Co study Director	**Date** 18/4/2001
Co study Director	**Date** 18/4/2001
Incharge, Consultancy Cell	**Date** 18/4/2001

Abbreviations

ADF	Acid detergent fibre
Ad lib.	Ad libitum
Bt	*Bacillus thuringiensis*
^{o}C	degree Celsius
DM	Dry matter
g	gram
l	litres
kg	Kilogram
KF	Karn Friasian
KS	Karn Swiss
MAHYCO	Maharashtra Hybrid Seed Company
mg	milligram
NDF	Neutral detergent fibre
NDRI	National Dairy Research Institute
NBt	Non *Bacillus thuringiensis*
µg	microgram
SCC	Somatic cell counts

ABSTRACT

Twenty crossbred (KS and KF) multiparous cows, divided in two groups of 10 each on basis of milk yield and stage of lactation were housed in two separate well ventilated sheds, and fed on berseem fodder *ad lib.*, wheat straw (1kg/cow/day) and a concentrate mixture consisting of crushed cottonseed (Non Bt) 40 parts, groundnut cake 15 parts according to their nutritional requirements for 13 days to adapt them to the cottonseed based diet. Group 1 was continued on the same ration and designated as NBt group while in the concentrate mixture of group 2 cottonseed was replaced with Bt cottonseed and designated as Bt group. Milk yield and voluntary feed intake were recorded daily. Average milk yield in Bt and NBt group during adaptation period of 13 days was 7.47 and 7.99 kg, respectively. Milk samples were collected at weekly intervals during the four week experimental period for the analysis of milk composition and to test for the presence of Bt protein. At the end, a blood sample from each cow was collected and plasma was separated to test for the presence of Bt protein. Bt protein in cottonseed, milk and blood samples was measured by ELISA method.

The amount of Bt protein in BT cottonseed was 52 µg/g cottonseed. Mean voluntary dry matter intake /100 kg body weight varied in the range of 2.61 to 4.07 kg (Av 3.16 ± 0.16) in Bt group and 2,.61 to 3.71 kg (Av 3.32 ± 0.13) in NBt group and cows maintained their body weight during the study. Average milk yield in corresponding groups was 8.15 ± 0.31 and 9.04 ± 0.66 kg/cow/day. The average 4% FCM yield in Bt and NBt groups was 8.87 ± 1.09 and 9.65 ± 0.76 kg, respectively. Average milk yield as well as dry matter intake/100 kg body weight was similar ($P>0.05$) between the groups. Mean and Standard Error (SE) for fat, protein, and lactose content in yield of Bt group was 4.55 ± 0.18, 2.39 ± 0.08 and 4.64 ± 0.09 % and the value in NBt group was 4.41 ± 0.16, 2.43 ± 0.03 and 4.58 ± 0.18 %, respectively. The analysis of milk did not exhibit the presence of Bt protein in any of the samples in milk of both groups at any stage of sampling in lactating cows. Similarly, Bt protein was not detected in blood plasma of both groups. Lactating dairy cows performed in a similar fashion when fed Bt and non Bt cottonseed during the flour week experimental period.

INTRODUCTION

Cotton (*Gossypium hirsutum*) has been genetically engineered to be resistant to be resistant to selected insect pests and this resistance has been accomplished by the insertion of gene from *Bacillus thuringiensis* var. *kurstaki* which encodes for the production of a protein insecticidal to Lepidopteran insect/pests of cotton but is safe to mammals, birds, fish and beneficial insects. Monsanto Co. has developed transgenic cotton containing cry-1Ac gene encoding a specific insecticidal protein from Bacillus

thuringiensis (Bt protein). Monsanto Co, USA and Maharashtra Hybrid Seeds Co. Ltd. (MAHYCO) has crossed genetically modified cotton developed at USA with Indian cotton on incorporate the *cry-1Ac* gene into Indian cotton hybrid to minimize the chemical sprays on the cotton frop and to economize cotton production. Prior to the commercialization of newly developmed varieties of cotton in India it is essential to investigate all aspects of cotton producting including the safety of proteins expressed by the inserted genes by assessing effects on domestic animals and beneficial insects. Traditionally, cottonseed is used in the ration of lactating animals as a source of energy and protein (Singhal and Sharma, 1995). Newly developed Indian varieties of transgenic cottonseed containing the Bt protein were investigated for their feeding value taking goats as a model of ruminants. It was concluded that Bt cottonseed and parent cottonseed (Non-Bt) were similar in nutrient composition and there was no meaningful difference in nutrient and toxicant content between Bt and Non Bt cottonseed. The growth performance of goats fed on Bt and Non Bt cottonseed was similar during 90 days of a feeding trial at the Industrial Toxicology Research Centre, Lucknow (Dutta and Dogra, 1998). These results indicated that the feeding of Bt cottonseed to ruminants did not cause any harmful effect on the performance of ruminants. The purpose of present study was to confirm that Bt cottonseed is nutritionally equivalent to non Bt cottonseed as a feed for lactating cows and to determine if the Bt protein is detectable in the milk of cows fed Bt cottonseed. Therefore, the objective of the present study is to investigate the effect of feeding transgenic cottonseed (Bt) feeding on milk yield and milk composition in lactating crossbred cows.

MATERIALS AND METHODS:

Twenty multiparous crossbred cows (KS) were selected from the general herd of National Dairy Research Institute, Karnal, Calving date and milk yield details of these animals are given in Table 1. The animals were maintained under loose housing system and fed a groundnut cake based concentrate mixture as per their milk yield and green fodder (berseem) *ad lib*.

Housing of Animals:

All the cows were housed in two well ventilated sheds having *pacca* floor and tied with ropes during pretreatment as well as during treatment period of four weeks. The sheds were equipped to individually feed the cows. Each animalôs manger was divided into two, one for green fodder and another for concentrate mixture and wheat straw. All the cows had their identification number tattooed on the hip region as well as in their ears.

Feeding of Animals:

Initially, all the cows were maintained on the concentrate mixture having groundnut cake as protein supplement for three days so that they can adjust to the changed housing system. Therefore, the cows were distributed in two groups of 10 each in a manner that each group was having similar milk yield. Concentrate mixture was replaced gradually with that containing crushed cottonseed (Non Bt, Control). After three days cows were stitched over to the new concentrate mixture completely. In addition to the green fodder (Berseem, *Trifolium alexandrinum*) fed *ad libitum,* each cow was supplied with one kg wheat straw/day to fulfill their dry matter requirement and to maintain good faecal consistency. Feeding of crushed cottonseed based concentrate mixture continued for 13 days for the adaptation of rumen microbes for the changed nutrient supplement. Both the groups were housed in separate shed. Control group, designated as NBt group, continued receiving the experimental cottonseed (Bt, MRC-162), crushed similarly as control cottonseed and designated as Bt group. Composition of concentrate mixtures containing Bt and NBt cottonseeds is given in Table 2.

Preparation of Concentrate Mixture:

Consignments of crushed cottonseeds (Non-Bt and Bt) received from Mahyco were stored in a godown (well ventilated store room) safely. The other ingredients were received from Central Store of NDRI and crushed using hammer mill in feed unit. Experimental cottonseeds, both Bt and Non Bt, were checked fro the presence of Bt protein using an adaptation of the ELISA described by Sims and Berberich (1996) test prior to the preparation of concentrate mixtures. The ingredients of concentrate mixture were mixed homogeneously using a mixer and both types of concentrate were stored safelyu in a godown. Fresh green fodder (Berseem) was supplied by Farm section of the institute, daily.

Table 1. Particulars of the crossbred cows in both the groups

Bt group			**N Bt group**		
Animal No.	**Date of calving**	**Milk yield (kg) at the time of distribution**	**Animal No**	**Date of calving**	**Milk yield (kg) at the time of distribution**
5782	22.12.1999	8.0	5229	26.09.2000	7.3
5920	29.02.2000	9.1	5094	21.05.2000	5.5
5909	30.03.2000	7.9	5922	15.09.2000	7.8
5663	11.04.2000	5.3	5934	21.02.2000	9.1
5816	28.06.2000	7.0	5908	19.03.2000	7.9
5342	01.07.2000	9.2	5956	03.04.2000	8.1
5797	24.07.2000	5.0	5919	14.07.2000	7.7
5519	18.08.2000	16.1	5898	31.08.2000	11.1
5822	14.09.2000	6.6	5675	06.09.2000	8.2
5786	25.10.2000	4.4	6049	20.09.2000	11.0

Table 2 Ingredient composition of concentrate mixture

Name of ingredient	Parts
Maize grain	20
Cottonseed (Bt/NBt)	40
Groundnut cake	15
Wheat bran	22
Common salt	1
Mineral mixture	2

Feeding schedule:

Cow in both groups were offered weighed amount of berseem three times a day i.e. 10 am, 10 noon and 5 pm. One kg wheat straw was offered at 10am to each cow daily. Respective concentrate mixture was provided to each cow in weighed amount at the time of milking. Total amount of concentrate mixture was according to NRC (1978) to fulfill their nutritional requirements. Left over green fodder of individual cow was weighed daily. Dry matter content of fresh fodder as well as left over material was determined daily to calculate the dry matter intake. Fresh and clean water was provided free choice to each cow two times a day.

Milking Schedule:

Cows was weighed daily. Dry matter content of fresh fodder as well as left over material was determined daily to calculate the dry matter intake. Fresh and clean water was provided free choice to each cow two times a day.

Milking Schedule:

Cows were milked manually three times a day i.e. 5 am, 12 noon and 6 pm daily and milk yield for individual cow was recorded. Milk sample from each cow were collected on 3, 7, 14, 21 and 28^{th} day of initiating the experimental feeding. Milk samples consist the milk of each milking in proportion of milk yield of individual cow. Milk samples collected at each milking were stored in a refrigerator prior to their pooling and analyzed for their composition.

Health Status:

Health of all the experimental cows were monitored daily by a veterinarian during the course of study. Body weight of each cow was recorded prior to their feeding and watering in the morning before starting the experimental feeding and again after completion of four week experimental feeding.

Disposal of Milk:

Total milk collected at each milking from the cows of experimental group was discarded in a pit which was inaccessible to the animals including birds and dogs.

Blood Sampling:

Blood sample from each cow were collected on 26^{th} day of experimental feeding by puncturing the jugular vein in a heparinized tube. Plasma was separated by centrifugation of blood samples at 3000g for 15 min. and analyzed for the presence of Bt protein on the same day.

Sampling and analysis of feed and milk:

Sample of each type of cottonseed was taken from the supply made by Mahyco and preserved in polythene bags till analysis.

(i) Feed: Representative samples: of both types of crushed cottonseed and the respective concentrate mixture were analyzed for dry matter, crude protein, ether extract, total ash (AOAC, 1990) neutral detergent fibre (NDF) and acid detergent fibre (ADF) as per Goering and Van Soast (1970). Pooled dry samples (28 days) of green berseem and left over feeds were also analyzed for above mentioned proximate and fibre fractions. Bt and Non Bt cotton seeds were also analyzed for the presence of Bt-protein. The amount of Bt protein in Bt-cotton seed was also measured using the ELISA method of Sims and Berberich(l996).

(ii) Milk and blood plasma samples: Milk samples, collected on3, 7,14,21 and *28th* day of *experimental* feeding, were analyzed for fat OSI, 1958), protein (Singhal and Desraj, 1989), lactose (Ferry and Doon, 1950) and somatic cell counts (SCC) using standard procedure (IDF, 1989) immediately after collection. All the milk samples were frozen in liquid nitrogen and stored at 200C for the analysis of Bt protein. Blood plasma samples, collected on 26th day of experimental feeding were analyzed on the same day. Following procedure was used for this purpose:

ELISA based sandwich was used for estimation of Bt-protein in Bt cotton seed, milk and blood samples. The protocol provided by MAHYCO was followed, and is an adaptation of method described by Sims and Berberich (1996). Blood samples were centrifuged and plasma was collected. For preparing standard curve for Bt- protein, either pure Bt-protein or Bt-protein-added-milk/blood samples were used. These samples were also subjected to the same *freaing* and thawing steps as used for analysis

of milk and plasma samples collected from lactating cows. Every ELISA plate contained standard samples. The ELISA procedure was as follows:

ELISA plates, supplied by MAHYCO were pre-coated with monoclonal antibody against Bt-protein. To each well, 50 µl polyclonal antiserum (rabbit antiserum against Bt protein) and 150 µl PBSTO (Phosphate buffer saline-Tween 20 ovalbumin) were added. Samples prepared from milk, plasma and cottonseed or milk and plasma with added Bt- protein were then added in 50 µl volumes. The ELISA plates were then washed two times with PEST, Then 250 µl goat anti-rabbit IgG alkaline phosphatase conjugate (1:2500 diluted with PBSTO) was added and ELISA plates were incubated at room temperature for 2 h. Each well was washed once quickly and followed by two 5 min. washes with PEST. Then, 250 ~1 of substrate (25 mg para-nitrophenyl phosphate dissolved in 25 mi ethanolamine-HCI buffer, pH 9.8) was added. After incubating ELISA plate at room temperature for 30 to 60 min,absorbance at 405 nm was recorded.

Following protocol was used for extraction of Bt- protein from milk of blood plasma.:

Milk or serum samples were thawed and diluted in 1:4 ratio with extraction buffer. The extraction buffer was prepared by mixing 1 mi Sigma cocktail I #P2850), 1 mi Sigma cocktail I (#P5726), 1 mi of 100 mM PMSF (dissolved in isopropanol) to IxPBS containing O.9% NaCI. Then milk and blood plasma samples were thoroughly mixed and incubated at 50C overnight. Next day, samples were *centrifuged* at 10,000 x g for 15 min, at 50C. Supernatant, avoiding fat was removed and used for analysis.

For testing presence of Bt- protein in Bt- cotton seed, dip-stick test was also used. The sticks were provided by MAHYCO. This is very quick test but relatively less sensitive as compare to ELISA *method* Bt- cottonseed gave colored band at defined position, which is characteristic of Bt- protein in sample. Non Bt-cotton seed gave negative response.

Statistical Analysis:

Statistical analysis of the data on daily dry matter intake , daily milk yield as well as milk composition determined at various intervals were considered while using the student's paired "t' test(Snedecor and Cochran, 1967).

RESULTS

Chemical composition of Bt and Non-Bt cottonseed indicated that the nutritive value of both the protein supplements was similar, however, Bt cottonseed contained higher oil, protein and lower ADF contents than that in Non-Bt cottonseed. The Bt cottonseed contained 52 µg/g Bt protein whereas this protein was not detected in Non-Bt cottonseed (Table 3).

Lactating cows in Bt and NBt groups were fed according to their nutritional requirements , however, the *dry* matter intake was higher by 36 per cent and crude protein intake was higher by 47 per *cent* than the recommended requirement given by Kearl (1982). The TDN intake was as per the recommended level (Table 5). The concentrate mixtures were formulated to ensure about 2 kg cottonseed intakes as per the protocol agreed upon by the sponsor. The concentrate mixtures were palatable as evident from their rapid consumption in both the groups. Concentrate mixtures, mixed with wheatstraw was consumed selectively, however, wheat straw was consumed completely by all the cows daily. Green fodder contained about·88 percent moisture and the faecal consistency was loose in spite Of feeding dry roughage i.e. wheat straw.

Feed intake:

Intake of berseem, wheat straw and concentrate mixture was recorded daily and the dry matter intake was calculated based on moisture determinations The total dry matter consumed per day *of* the concentrate mix, berseem and wheat straw during adaptation period as well as during experimental period is presented in Table 5. Mean total dry matter intake during *adaptation* period was 12.15 ± 0.08 and 12.27 ± 0.16 kg

in cows allotted to Bt and NBt group, respectively. During the experimental period of four week the value in Bt and NBt group was 12.13 ± 0.11 and 12.39 ± 0.18 kg and the variation *betwee*n groups was non- significant In addition, the consumption of berseem, wheat straw and concentrate mix were similar between the two groups. Total DM intake /1M)kg body weight in Bt and NBt group was 3.16 ± 0.16 and 3.32 ± 0.13 kg, respectively. These values were higher by 36 per cent than that recommended by feeding standard (*Kearl* 1982). Concentrate to roughage ratio i.e. 37:63 (on dry matter basis) was similar in both the groups and this ratio is considered optimum for medium yielding cows.

Milk production performance:

The milk yield improved beyond the initial milk yield during feeding of Non-Bt cottonseed as a part of concentrate mixture during the adaptation period, The average milk yield at the time of selection i.e. before starting the feeding of cottonseed based diet of animals was similar in both the groups (Figure 1). Mean ± SE milk yield in cows allotted to Bt and NBt groups was 7.47 ± 0.81 and 7.99 ± 0.45 kg, respectively and the variation between the groups was non-significant ~0.05). When these groups were fed their respective treatment diet the milk yield further improved and the average (meant SE) milk yield in Bt and NBt groups was 8.15 + 0.31 and 9.04 *c* 0.66 kg, respectively, The application of students 't' test indicated non-significant difference in milk yield between the groups. When the milk yield was calculated as 4% FCM, the milk yield in Bt and NBt group was 8.87 ± 1.09 and 9.65 ± 0.76 kg, respectively and again variations for milk yield and 4% FCM yield between the groups were non-significant.

Health Status:

In general, the animals maintained good health during the entire experimental period None of the animal exhibited any symptom of *sickness,* howevs, one animal in Pt group showed reluctance towards the Bt concentrate for few days and during that period she was given Himalayan Batisa, an Aauervedic appetizer. Cows maintained

their body weight. Before starting the experimental feeding the average body weight in Bt and NBt group was 411.7 ± 55.2 and 385.6 ± 56.5 kg, respectively and the final body weight (after terminating the experimental feeding) was 432.7 ~ 18.9 and 401.2 i 17.6 kg, respectively table 5). These results showed an average improvement in body weight was about 22 kg in Bt group and 16 kg in NBt group during four weeks of experimental feeding. Mastitis or any other disease was not detected in any of the cows of both the groups during the experimental period.

Milk composition:

Milk composition in terms of fat protein lactose, SCC and Bt protein wee determined on 3, 7, 14, 21 and 28th days of experimental feeding to check the influence of dietary treatments on milk *composition* Data on milk composition is presented in Table 6.

Fat: Average (mean ± SE) milk fat content was highs in Bt group than in NBt group irrespective of day of milk sampling. Overall fat content in Bt and NBt groups was 4.55 ± 0.18 and 4.41 ± 0.16 percent respectively. The variation in fat content between the groups was non- significant

Protein: Average (mean ± SE) milk protein content in Bt and NBt groups at various intervals was similar and the difference in milk protein content between groups was non- significant. The overall average milk protein content in Bt and NBt groups was 2.39 ± 0.08 and 2.39 ± 0.08 percent, respectively. These results showed that feeding cottonseed, irrespective of the variety, did not influence the milk protein content

Lactose: Average (mean ± SE) milk lactose content varied in the range of 3.87 to 4.25 percent irrespective of group or sampling day. The milk lactose did not show any pattern at different sampling days. The average milk lactose content in Bt and NBt groups was similar (P>0.05) (4.6.4 + 0.09 and 4.58 + 0.18 percent in Bt and NBt group, respectively).

Bt protein in milk: ELISA method, used for the estimation of Bt protein in milk samples, has a sensitivity to detect 0.002 ppm Bt protein in milk samples. Bt protein was not detected in any of milk sample drawn on various sampling intervals, both the Bt and NBt groups.

Bt-protein in blood: ELISA method used for estimation of Bt protein in blood plasma samples has a sensitivity to detect 0.004 ppm Bt protein in blood plasma samples. No Bt protein was detected in any of the blood plasma samples in both the Bt and NBt groups.

DISCUSSION

Nutrient composition of Bt cottonseed as well as non-Bt cottonseed (Table 3) indicated that both the seeds had similar composition. Bt cottonseed contained slightly higher oil and protein contents than non-Bt cottonseed, but the differences may not be statistically significant Similar variation in proximate composition in both varieties of cottonseed was reported by Subramaian *at al.* (personal communication). The values for proximate and fiber fractions in both types of cottonseed were within the normal range reported by Berberich, ei *a1.*(1996) who also concluded that the insect protected cottonseed was equivalent to that of conventional cottonseed. Similar proximate composition of cottonseed was reported by Learl (earl (1982). ADF content was lower and NDF content was higher in Bt cottonseed than in non-Bt cottonseed which may be attronited tp ots jogjer hamicellulose content Nutrient composition of concentrate mixture reflected the variation in Bt and Non Bt cottonseeds while the composition of berseem and wheat straw was similar to the reported values in the literature (Sen and Ray, 1971).

The dry matter intake in both the Bt and NBt groups of cows was similar during the adaptation period as well as during the experimental period The TDN intake in both the groups was similar to the recommendations of Kearl (1982),

however, crude protein intake was about *4T* per cent higher than the recommended values which may be attributed to the *ad lib* feeding of berseem fodder (Table 5). These results suggested that the inclusion of Bt cottonseed in the diet of dairy cows did not affect the feed intake during four weeks of feeding trial. Similar findings were reported by Dutta and Dogra (1998) after the dietary inclusion of Bt and non- Bt cottonseeds in the ration of goats.

Average milk yield as well as 40/0 FCM yield increased following the shifting of cows from normal farm ration to cottonseed (Non-Bt) based ration during adaptation period which may be attributed to the higher oil content in cottonseed based ration than in normal farm ration The magnitude of increase was maintained during the four week experimental period as both the rations were having almost similar composition. Based on the adaptation period milk yields, cows in the Bt group had stabilized their production whereas cows in the NBt group were still increasing so at the start of the treatment period, cows in NBt group already had higher production than those in the Bt groups. Increase in milk yield was higher in NBt group than in Bt group but this difference existed even at the time of allotment of treatment diets and persisted throughout the experimental period The non-significant difference between the groups indicated that dietary inclusion of Bt cottonseed did not influence the milk yield in crossbred dairy cows.

Body weight of *experimental* cows in both the groups was improved during the experimental period ~able 5) and there was no symptom of any disease during four weeks of experimental feeding. The improvement in body weight may be attributed to the high caloric value of cottonseed due to its higher ether extract content than that of normal farm ration based on expeller pressed groundnut cake as a source of protein.

Data on milk composition revealed that the milk fat, protein and lactosecontent were similar (pz0.05) in Bt and NBt groups (Table 2).

Bt protein was not detected in milk samples, collected during the course of study. From the data it was calculated that average Bt prdtein intake was 93.6 mg/day in Bt group, which got destroyed in the digestive tract of dairy cows. Ream (1994) studied in *vitro* digestive fate of Bt protein (*Bacillus thuringiensis* var. *kurstaki* HD 73) and reported that the Bt protein and its associated functional activity readily degraded upon exposure to gastric nuid in the mammalian digestive tract. Dutta and Dogra (1998) recorded the growth performance of ruminants, taking goat as ruminant model, fed on Bt and non-Bt cottonseeds based diets and did not find any significant effect on their growth performance without any *toxicity* symptom and they concluded that cottonseed containing the Bt protein was as wholesome and safe for feeding ruminants as cottonseed without Bt protein. Bt protein was not detected in the blood plasma samples of Bt and NBt groups on 26m day of feeding the experimental diets. These results further confirmed that dietary Bt protein was destroyed in the digestive tract of dairy cows.

In the present study, there were no statistical differences in feed intake, weight gain, milk yield and milk composition between cows fed Bt cottonseed compared to cows fed non-Bt cottonseed. Therefore, it is concluded that Bt cottonseed is as nutritious and wholesome as non-Bt cottonseed as a feed for *dairy* cows. Furthermore, no Bt protein was detected in milk or blood plasma samples of cows fed either Bt or non-8t cottonseed.

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Table 3. Chemical composition of Bt and Non-Bt cottonseed (% Dry matter basis)

Parameter	Bt cottonseed	Non Bt cottonseed
Organic matter	95.06	95.31
Crude protein	29.48	26.90
Ether extract	21.12	19.22
Crude fibre	18.24	21.73
Total ash	4.94	4.69
Neutral detergent fibre (NDF)	54.88	48.59
Acid detergent fibre (ADF)	21.61	27.67
Hemicellulose	33.26	20.92
Bt Protein (µg/g)	52.00	0.0

Table 4. Nutrient compositon of concentrate mixtures and roughages

Parameter	Bt Concentrate	NBT concentrate	Berseem	Wheat straw
Organic mater	91.8	92.34	81.85	84.18
Crude protein	24.82	24.23	20.10	3.08
Ether extract	10.71	8.64	3.56	0.96
Crude fibre	10.94	11.90	15.59	40.00
Total ash	8.20	7.66	18.15	15.82
NDF	68.91	55.61	48.37	84.42
ADF	19.98	19.74	41.15	55.22
Hemicellulose	48.93	35.87	7.22	29.20

Table 5. Performance of crossbred cows on Bt and non-Bt cottonseed based rations

Parameter	BT group	NBt group
Av. Initial body weight (Kg)*	411.7 ± 55.24	385 ± 56.5
Av. Final body weight (Kg)	432.7 ± 18.93	401.3 ± 7.55
Av. Body weight gain (Kg) During 4 weeks	21.0	15.6
DM intake during adaptation period (Kg/day)**		
Berseem	6.69 ± 0.13	6.78 ± 0.19
Wheat straw	0.9 ± 0.03	0.9 ± 0.03
Concentrate mixture	4.5 ± 0.05	4.5 ± 0.05
Total DM intake	12.15 ± 0.08	12.27 ± 0.16
DM intake/100kg b. wt.	3.11 ± 0.31	3.27 ± 0.29
Milk production during adaptation period (Kg/day)**		
Milk yield	7.47 ± 0.81	7.99 ± 0.45
4% FCM yield	7.90 ± 0.95	8.20 ± 0.23
DM intake /kg milk yield	1.62	1.53
DM intake during experimental period (Kg/day)***		
Berseem	6.73 ± 0.12	6.99 ± 0.17
Wheat straw	0.9 ± 0.03	0.9 ± 0.03
Concentrate mixture	4.5 ± 0.05	4.5 ± 0.05
Total DM intake	12.13 ± 0.11	12.39 ± 0.18
Crude protein intake	2.46	2.47
TDN intake	7.33	7.76
DM intake/100 kg b. wt.	3.16 ± 0.16	3.32 ± 0.13
Milk production during experimental period (Kg/day)***		
Milk yield	8.15 ± 0.31	9.04 ± 0.66
4% FCM yield	8.87 ± 109	9.65 ± 0.76
DM intake/kg milk yield	1.48	1.37

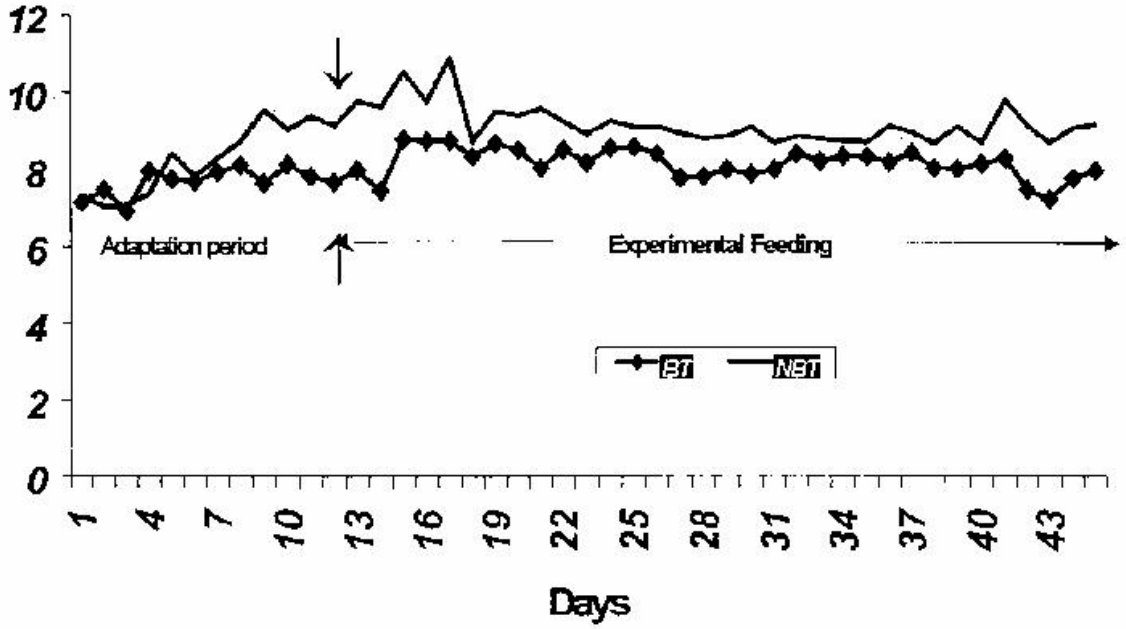
* Each value is an average of 10 observations
** Each value is an average of 130 observations
*** Each value is an average of 280 observations
Each value represents the mean and standard error.

Table 6 Milk composition as influenced by feeding Bt and Non Bt cottonseed based ration*

Day of sampling	Parameter	BT group	NBT group
3	Fat (%)	4.34 ± 0.24	4.13 ± 0.23
	Protein (%)	2.19 ± 0.05	2.23 ±0.06
	Lactose (%)	3.10 ± 0.12	3.42 ±0.22
	SCC (x 10^5/ml)	1.24 ±0.05	1.18 ±0.06
	Bt protein	ND	ND
7	Fat (%)	4.38 ±016	4.36 ±0.20
	Protein (%)	2.01 ±0.06	2.21 ±0.06
	Lactose (%)	3.87 ±0.17	4.08 ±0.29
	SCC (x 10^5/ml)	1.29 ±0.07	1.22 ±0.06
	Bt protein	ND	ND
14	Fat (%)	4.43 ±0.18	4.36 ±0.24
	Protein (%)	2.24 ±0.10	2.23 ±0.06
	Lactose (%)	4.11 ±0.21	4.04 ±0.20
	SCC (x 10^5/ml)	1.24 ±0.05	1.20 ±0.04
	Bt protein	ND	ND
21	Fat (%)	4.83 ±0.20	4.53 ±0.15
	Protein (%)	2.07 ±0.16	2.26 ±0.10
	Lactose (%)	3.41 ±0.17	3.98 ±0.30
	SCC (x 10^5/ml)	1.33 ±0.08	1.22 ±0.05
	Bt protein	ND	ND
28	Fat (%)	4.79 ±0.16	4.69 ±0.19
	Protein (%)	3.03 ±2.20	3.32 ±0.14
	Lactose (%)	4.25 ±0.19	4.20 ±0.23
	SCC (x 10^5/ml)	1.37 ±0.06	1.33 ±0.06
	Bt protein	ND	ND

*Each value is an average of 10 observations and represents the mean and standard error. No differences detected at the $P<0.05$ level of significance.
ND-Not detected

Fig. 1. Average milk yield (kg/head/day) in BT and NBT groups



Effect of feeding Bt cottonseed on fed intake milk production and compostion in lactating water buffaloes

Chapter V

Annexure 5

REPORT

Effect of Feeding Cottonseed Produced from Bt Cotton on Feed Intake, Milk Production and Composition in Lactating Water Buffalo in India

Study Sponsor
MAHARASHTRA HYBRID SEEDS COMPANY LIMITED
RESHAM BHAVAM, 4th Floor,
78, VEER NARIMAN ROAD
MUMBAI 400 020

2001

Department of Animal Nutrition
College of Veterinary Sciences
G.B. Pant University of Agriculture and Technology
Pantnager – 260 145, Uttranchal

Report

April, 2001

Study Title

Effects of Feeding Cottonseed Produced from Bt Cotton on Feed Intake, Milk Production and Composition in Lactating Water Buffalo in India

Principal Investigator — Dr. D/P/ Tiwari
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Statement of No Data Confidentiality Claims

This is to certify that the performing laboratory is not making any claim of confidentiality for any information in this study.

This performing laboratory also states that the issues related to raw data and materials will be stored in the laboratory and will be accessible to the authorized scientists of the laboratory and/or persons authorized by the sponsors. The workers on the project are free to publish the data in the form of research paper or in any other form.

Principal Investigator	Dr. D.P. Tiwari
Designation	Professor and Head Department of Animal Nutrition
Signature	Dr. D.P.Tiwari
Date	21/4/2020

Statement of Particulars of Study

Title	Effects of feeding cottonseed produced from Bt cotton on feed intake, milk production and composition in lactating water buffalo in India.
Facility	Department of Animal Nutrition College of Veterinary Sciences G.B. Pant Univ. of Agriculture & Technology Pantnagar. Fax: 05944-333473
Study Director	Dr. D.P. Tiwari
Co-Study Director	Dr. B.D. Lakhchaura
Investigators	Dr. Maha Singh & Dr. Seema Chavan
Study Initiation Date	22.02.2001
Experimentation Termination Date	16.04.2001
Record retention	Raw data of the study will be retained by the Study Director and this will be made available to the authorized personal from the sponsors

Signature of Approval

Study Director	Date 21/4/2001
Co study Director	Date 21.4.2001
Dean ,College of Veterinary Sciences	Date 21.4.2001

Abbreviations

ADF	Acid Detergent Fibre
ad lib.	Ad libitum
Bt	*Bacillus thuringiensis*
^{o}C	degree Celsius
FCM	Fat Corrected Milk
DM	Dry Matter
g	gram
l	litres
kg	Kilogram
MAHYCO	Maharashtra Hybid seed company
mg	milligram
NDF	Natural Detergent Fibre
NBt	Non *Bascillus thuringiensis*
µg	microgram
SCC	Somatic Cell Count

ABSTRACT

Twenty lactating Murrah buffaloes in mid lactation were housed in a we8ll ventilated shed with an arrangement for individual feeding. Buffaloes were fed on a concentrate mixture consisting of 39.5 parts crushed cotton seed (Non-Bt.), 59.5 parts milk feed and 1 part mineral mixture as per nutritional requirements along with 20 kg. Mixed green fodder (Oats + Berseem) and wheat straw old lib for 18 days to adapt the animals to a cotton-seed based diet. Thereafter, buffaloes were divided into 2 groups of 10 each on the basis of their milk yield and lactation number. Buffaloes in group 1 were continued on the same concentrate misture (Non-Bt. group), whereas buffaloes in group 11 were fed concentrate mixture containing 39.5 parts crushed Bt. cotton seed (Bt. group) instead of cotton seed in accordance with the nutritional requirements along with 20 kg. Mixed green fodder (Oats + Berseem) and wheat straw adlib. For 35 days during experimental feeding period. The allowance of concentrate mixture was offered two times to each buffalo at the time of milking at 4.00 a.m. and 4.00 p.m. daily. The buffaloes were offered green fodder and wheat straw twice daily. All the buffaloes were also offered adlib.clean drinking water thrice in a day. The average initial milk yield of buffaloes was yield, voluntary feed intake (concentrate mixture, green folder and wheat straw) were maintained during adaptation and experimental feeding periods. The representative samples of milk from each buffalo were collected on 7^{th} to 14^{th} and 21^{st} to 28^{th} day and 35^{th} day of the experimental feeding for analysis of milk for its chemical composition and also for testing for the presence of the Bt. Protein. After 35 days of experimental feeding, blood samples were also collected for testing the presence of Bt. protein. Bt. protein *Cry 1 Ac* in crushed non Bt. cotton-seed and crushed Bt. cotton seed, milk samples and blood samples was estimated by an ELISA method specific to the Cry 1 Ac protein.

The presence of the Bt. protein in both non-Bt and Bt. Cotton seed was also tested by a strip test method in which Bt. cotton seed gave colored band whereas non-Bt. cotton seed gave negative (no band) results. The Bt. cotton seed contained 20 µg/Bt protein/g.

The average initial body weight of experimental buffaloes was 442.1 and 468.9 kg. In non-Bt. and Bt. groups, respectively. The voluntary dry matter intake per 100 kg body weight ranged from 3.32 to 4.37 kg. In non Bt. group and from 3.18 to 4.52 kg in Bt. group. The average DM intake per 100 kg body weight was 3.98 and 3.75 kg in non-Bt and Bt. groups, respectively. The quantity of non-Bt. Cotton seed and Bt Cotton seed as percent of total dry matter intake was 10.75 and 10.83% in buffaloes fed non-Bt. and Bt. groups, respectively. The quantity of non-Bt. Cotton seed and Bt. Cotton seed as percent of total dry matter intake was 10.75 and 10.83% in buffaloes fed non-Bt. and Bt. cotton seed, respectively. During the experimental feeding period, the average daily milk yield was 4.88 and 4.87 kg, respectively in non-Bt. and Bt. groups of buffaloes. The 4% FCM yield was 8.47 kg, and 8.04 kg., respectively in non-Bt. and Bt. cotton seed was 8.99 and 9.61% respectively. The voluntary DM intake, milk yield, 4% FCM yield and fat % values were comparable in both the groups of buffaloes.

The milk samples tested on 7^{th}, 14^{th} and 21^{st} to 28^{th} day and also on 35^{th} day of sampling in buffaloes fed Bt. Cotton seed did not show the presence of Bt. protein at any stage of sampling. Similarly, the blood samples collected after 35 days of feeding did not show the presence of Bt. protein. The present study shows that voluntary DM intake, milk yield, FCM yield and fat % in milk were comparable between buffaloes fed Bt. cotton seed and non-Bt cotton seed. It is concluded from this study that Bt. cottonseed is as nutritious and sholesome as non-Bt. cottonseed to support the milk yield and development of buffaloes.

However the study is in progress for determining the digestibility of different nutrients in buffaloes fed non-Bt, and Bt. cotton seed. The analysis of milk for its composition and statistical analysis are in progress.

INTRODUCTION

Cotton (Gossypium hirsutum) has been genetically modified to provide resistance against certain insects and pests specially against bollwonn comples. This resistance has been produced by insertion of insect tolerance trait with gene coding for *Cry 1 Ac* protein derived from *Bacillus thuringiensis* (Bt.) Var. kurstaki which encodes for the production of a protein insecticidal to lepidopteran insects/pests of cotton but is safe to animals, birds, fish and beneficial insects. Monsanto Co. has developed a transgenic cotton containing *Cry 1 Ac* gene encoding a specific insecticidal protein from Bascillus thuringiensis (Bt. Protein). Maharashtra Hybrid Seeds Co. Ltd. (MAHYCO) has crossed genetically engineered cotton developed by Monsanto in USA with Indian cotton to insert the *Cry 1 Ac* gene into Indian cotton hybrid to minimize the chemical sprays on the cotton crop and has given crop producers an alternative strategy for managing insect/pest pressure with low cost. Prior to the commercialization of newly developed variety of cotton (Bt. cotton) in India, it is essential to investigate all aspects of cotton production including the safely of proteins expressed by the inserted genes by assessing the effects on domestic animals and beneficial insects.

Traditionally cotton seed is utilized in the ration of lactating crows and buffaloes as a source of energy and protein. Newly developed transgenic cotton seed in India by Mahyco which contains Bt. protein has been investigated for its feeding value in goats as a model of ruminants at Industrial Toxicology Research Centre, Lucknow and it is reported that Bt. cotton seed and parental cotton seed (Non-Bt.) were similar in nutrient composition with no significant difference in nutrient and toxicant contents between Bt. and non-Bt. cotton seed. The growth performance of goats fed rations containing Bt. and non-Bt cotton seed was similar during 90 days of a feeding trail (Dutta and Dorga, 1998). These findings indicated that the feeding of Bt. cotton seed to ruminants did not cause any harmful effect on their performance. The primary purpose of the present study was to assess the nutritional value of Bt. cotton seed in comparison to non-Bt. cotton seed in lactating water buffaloes and also

to determine if the Bt. protein is detectable in milk and blood of the lactating buffaloes fed ration containing Bt. cotton seed. In view to genrate the information, the present study was undertaken to investigate the effect of feeding transgenic cotton seed (Bt. cotton) on milk yield, milk composition and nutrient utilization in lactating murrah buffaloes.

Materials and Methods:

Twenty multiparous lactating Murrah buffaloes in mid lactating were selected from herd of the Livestock Research Centre, G.B. Pant University of Agriculture and Technology, Pantnager. All the animals were maintained under stall feeding on milk l (concentrate) as per their milk yield and green folder (Oats + Berseem) ab lib.

Housing of Buffaloes:

All the buffaloes were housed in a well ventilated shed having pucca floor and tied with iron chain having tail to tail arrangement during control (adaptation) period of 18 days as well as during experimental feeding period of 35 days. The manger was partitioned for individual feeding of buffaloes and they were tied with such a distance so as to avoid access to the manger of other buffaloes. All the buffaloes included under the study had their identification number uniquely tattooed on the hip region.

Feeding of Animals:

Initially at the buffaloes maintained on milk feed (concentrate) and green fodder (Oats+berseem) *ad lib.* Were fed concentrate misture consisting of 39.5 parts crushed cotton seed. Milk feed 59.5 parts ad miniral mixture 1 part as per nutritional requirements (Kearl. 1982) alongwith 20 kg green fodder (Oats+berseem_ and wheat straw *ad lib.* For 18 days as adaptation period to accustom the rumen microbes for changed feed supplement. After adaptation period, the buffaloes were divided into 2 groups of 10 each on the basis of their milk yield and lactation number so that each groups was having similar milk yield. The details the buffaloes are given in Tble 1. The buffaloes in group I were continued on the same concentrate mixture (non-Bt. group). Whereas the buffaloes in group II were fed concentrate misture containg 19.5 parts crushed Bt. cotton seed (Bt. group. MRC-162) in place of cotton seed as per their nutritional requirements (Kearl, 1982) alongwith 20 kg. Green fodder (Oats+berseem) and wheat straw *adlib.* to fulfill their dry matter requirement and to maintain good

faecal consistency. The experimental feeding period latest for 35 days. The composition of concentrate mixtures containing non-Bt. cotton seeds is given in Table 2.

Table1. Details of lactating Murrah buffaloes in Non-Bt. groups.

Non – Bt. group				Bt. group		
S. No.	**Animal No.**	**Date of Calving**	**Average milk Yiedl (kg./day) at the time of distribution**	**Animal No.**	**Date of Calving**	**Average milk Yield (kg./day) at the time of distriution**
1.	374	3.12.2000	7.2	388	10.12.2000	7.2
2.	404	27.9.2000	6.8	390	26.6.2000	7.0
3.	411	-	6.5	400	9.9.2000	6.3
4.	355	15.9.2000	5.5	384	28.9.2000	5.7
5.	407	30.6.2000	4.5	393	6.8.2000	5.6
6.	408	20.7.2000	5.2	397	15.8.2000	5.3
7.	363	21.8.2000	4.1	357	7.9.2000	3.8
8.	405	26.6.2000	4.2	394	3.9.2000	4.4
9.	406	4.7.2000	3.6	395	26.6.2000	3.4
10.	409	-	2.9	410	-	2.0

Table 2 : Composition of concentrate mixtures fed to buffaloes during experimental feeding period.

Ingredients	Parts
Cotton seed (Non-Bt./Bt.)	39.5
Milk feed (concentrate)	59.5
Mineral Mixture	1.0

Preparation of concentrate Mixture.

Consignments of crushed cotton seeds (non-Bt. and Bt.) received from Mahyco were stored in a well ventilated store room safely. The milk feed (concentrate) was received from Livestock Research Centre. The experimental cotton seeds both non-Bt, and Bt. were tested for the presence of Bt. protein using ELISA by the method as described by Sims and Berberich (1996). The ingredients of concentrate mixture were mixed homogenously and both types of concentrate mixtures were stored safely in the storeroom. The fresh mixed green fodder (Oats+Berseem) after chffing and wheat straw were supplied by the Livestock Research Centre of the university daily.

Feeding Schedule:

The buffaloes of both the groups were offered the weighed quantity of respective concentrate mixturate mixture in two instalments half an hour before milking i.e. at 4.00 a.m. and 4.00 a.m. daily according to their nutritional requirements (kearl, 1982). 20 kg. Mixed green fodder (Oars+ Berseem) was offered to each buffalo twice a day at 9.00 a.m. and 3.00 p.m. in equal instalments and wheat straw was offered *ad lib.* To fulfill the dry matter requirement. Left over wheat straw along with little green fodder of individual burraloes was weighed and recorded daily. The dry matter content of the feeds offered and left over was determined weekly and during digestion trial daily to calculate the dry matter intake. All the buffaloes were also offered fresh clean drinking water thrice in a day.

Milking Schedule:

All the buffaloes were miled twice daily at 4.30 a.m. and 4.30 p.m. the milk yield of individual buffalo was recorded daily. Milk samples from each buffalo were collected on 7^{th} , 14^{th} , 21^{st} and 28^{th} and 35^{th} day of the experimental feeding period for determing the chemical composition and for testing the presence of Bt. protein. The milk samples collected in the evening milking were stored in a refrigerator and then pooled with the samples of morning milking prior to analyze for chemical composition.

Health Status:

The Veterinary Officer did proper monitoring for health status of all the experimental buffaloes during the course of study. All the buffaloes were weighed for 3 consecutive days prior to the start also at the end of experimental feeding period of 35 days before morning feeding and watering.

Disposal of Milk:

The total milk of the experimental buffaloes fed ration containing Bt. cotton seed (Group II) at each milking was collected in a milk can and discarded in a soaking pit which was not accessible to other animals including dogs and birds.

Blood Sampling:

Blood samples from Murrah Buffalo were after 35^{th} day of experimental feeding period by puncturing the jugular vein in test tubes containing anticoagulant and analyzed for the presence of Bt. protein on the same day.

Sampling and analysis of feed and milk:

Feed:

Sampling of non Bt. cotton seed and Bt. cotton seed both supplied by Mahyco were taken in the polythene bags for proximate analysis and for testing of Bt. protein in Bt. cotton seed. Representative samples of feed offered and residue left were also collected for analysis of any matter, crude protein, mater extract, crude fibre and total ash (AOAC, 1990), neutral detergent fibre (NDF) and acid detergent fibre (ADF) as per Goering and Van Soast (1970). The amount of Bt. protein in Bt. cotton seed was measured using ELISA method (Sims and Berberich, 1996).

Milk:

Representative milk samples were collected from individual buffalo of both the groups during experimental feeding period from morning and evening milking and pooled on 7^{th}, 14^{th} , 21^{st} to 28^{th} and 35^{th} day of experimental feeding. The pooled samples were analyzed for fat (ISI, 1958), protein, ash (AOAC, 1990), lactose and for somatic cell counts (SCC) using standard procedure (IDF, 1989). All samples of milk were analyzed for Bt. protein on the same day following the ELISA method as described by Sims and Berberich (1996).

Blood:

Blood samples from each buffalo were collected on 35^{th} day of experimental feeding and analyzed for the presence of Bt. protein (Sims and berberich, 1996).

The following procedure was used for the Bt. or Bt. cotton seed, milk and blood samples. The protocol provided by Mahyco was followed which is an adaptation of method described by Sims and Berberich (1996).

Blood samples were centrifuged at 3000 rpm an plasma was separated and collected. For preparing the standard curve for Bt. protein, either pure Bt. protein or Bt. protein added milk or blood samples were used. These samples were also subjected to freexing and thawing steps as used for analysis of milk and plasma samples collected from lactating buffaloes. Every ELISA plate contained standard samples. The ELISA procedure was as follows:

ELISA plats supplied by Mahyco were pre-coated with monoclonal antibody against Bt. protein. To each well, 50 µl polyclonal antiserum (rabbit anti serum against Bt. protein) and 150 µl good anti-rabbit lg G alkaline phophatase conjugate (1:2500 diluted with PBSTO) was added and ELISA plates were incubated at room temperatures for 2 hours. Each well was washed once again quickly, followed by two 5 minutes washes with PBSTO. Thereafter, 250 µl of substrate (25 mg paranitrophenyl phosphate dissolved in 25 ml ethanol amine ï HCL buffer, Ph 9.8) was added. After incubating ELISA plate at room temperature for 20 to 60 minutes, absorbance at 405 nm was recorded.

Following protocol was used for extraction of Bt. protein from milk and blood plasma samples:

Milk or serum samples were thawed and diluted in 1:4 ratios with extraction buffer. The extraction buffer was prepared by mixing 1 ml Sigma cocktail I (#P2850), 1 ml Sigma cocktail II (#5726); 1 ml of 100Mm pmsf (dissolved in isopropanol) to 1xPBS containing 0.9% NaCl. Then milk and plasma samples were thoroughly mixed and incubated at 5^{o} C over night. Next day, samples were centrifuged at 10,000xg for 15 minutes at 5^{o} C Supernatant avoiding fat was removed and used for analysis.

For testing the presence of Bt. protein in Bt. cotton feed, strip test method was also used. The strips were provided by Mahyco. This is a very quick test but relatively less sensitive as compared to ELISA method. Bt, cotton seed gave colored bank at

defined position, which is characteristic of Bt. protein whereas, non Bt. cotton seed gave (no band) no response.

Statistical analysis:

Statistical analysis of the data on dry matter intake, milk yield as well as milk composition determined at different intervals during experimental feeding period is to be done using studentôs paired ótô test (Snedecor and Cochran, 1967).

Results:

The chemical composition of non-Bt. cotton seed alongwith gossypol and Bt. protein contents in presented in Table-3. The nutrients concentration s are more or less similar in both types of cotton seeds. The Bt. cotton seed contained 20 µg Bt protein/g whereas, non-Bt cotton seed did not contain any Bt. protein.

The analysis for chemical composition of other feed ingredients fed to buffaloes during experimental feeding period is in progress.

The average voluntary dry matter intake in buffaloes of non-Bt. (Group-I) and Bt. cotton seed (Group II) led groups was 3.98 kg per 100 kg body weight, (Table 4). The daily dry matter intake in buffaloes fed ration containing non Bt. cotton seed varied from 3.32 to 4.37 kg per 100 kg body weight whereas daily dry matter intake in buffaloes fed ration containing Bt. cotton seed varied from 3.18 to 4.52 kg per 100 kg body weight. Each buffalo in group II (Bt. cotton seed group) consumed 1.81 kg Bt. cotton seed/day on dry matter basis which varied from 1.23 to 2.18 kg/day. The quantity of non Bt cotton seed and Bt. cotton seed as percent of total dry mater intake was 10.83% in buffaloes fed non-Bt. (group1) and Bt. cotton seed (groupII), respectively.

Milk yield:

The average daily milk yield in buffaloes during experimental feeding period was 4.88 and 4.87 kg in group I (non-Bt. cottonseed) and group II (Bt. cotton seed), respectively whereas, 4% FCM yield was 8.47 and 8.04 kg, respectively in group I and group II.

Milk compstion:

Fat % : The average fat content in milk of buffaloes fed non-Bt. (groupI) and Bt. cotton seed (group II) was 8.99 and 8.61% respectively.

The voluntary dry matter intake, milk yield, 4% FCM, yield and fat% values in the buffaloes fed ration containing either non-Bt. cotton seed or Bt. cotton seed were comparable.

Health Status:

In general all the buffaloes maintained good health during the entire experimental feeding period. Not of the animal showed any symptoms of sickness. The buffaloes in both the groups gained body weight. The body weight gain/day was 488.6 and 414.3 in buffaloes of group I (non-Bt.) and group II (Bt.), respectively. At the start of experimental feeding period the average body weight of buffaloes in group I (non-Bt. cotton seed) and group II (Bt. cotton seed) was 442.1 and 468.9 kg, respectively, whereas, at the end of experimental feeding period of 35 days the body weight of buffaloes was increased to 459.2 and 483.4 kg, respectively, in group I (non-Bt. cotton seed) and group II (Bt. cotton seed).

Bt. Protein in milk:

The Bt. Protein was not detected in the milk of buffaloes collected on7^{th},14^{th}, 21^{st} to 28^{th} and 35^{th} day of feeding ration containing either Bt. cotton seed or non-Bt. cotton seed. The ELISA method which was used for detection of Bt. protein in milk samples had sensitivity to detect 0.002 ppm Bt. protein in milk.

Bt. protein in blood

Bt. Protein in the blood plasma samples of buffaloes collected on 35th day of experimental feeding was not detected in both the groups of buffaloes fed ration containing either Bt. cotton seed or non Bt. cotton seed. The ELISA method which was used for detection of Bt. protein in blood plasma samples had a sensitivity to detect 0.004 ppm Bt protein in blood plasma samples.

From the information obtained in the present study, it is concluded that Bt. cotton seed is as nutritious, palatable and wholesome as non-Bt. cotton seed as a feed for lactating buffaloes. Further more, no Bt. protein was detected in milk or blood plasma samples of buffaloes fed ration containing either Bt. or non-Bt. cotton seed.

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Table 3. Chemical composition of non Bt. and Bt. cotton seed (% dry matter basis)*

Nutrients	Non Bt. cotton seed	Bt. cotton seed
Organic matter	95.9	95.9
Crude protein	25.2	27.1
Ether extract	19.7	24.2
Total carbohydrates	51.0	44.6
Gross energy (Kcal/g)	4.82	5.05
Total gossypol (%)	1.52	1.23
Free gossypol (%)	1.39	1.14
Bt. protein (µg/g)	-	20

* **Source:** V. Subramaniam, Mahyco Kalaco Kalakal, Hyderabad (A.P.) (Personal communication)

Table 4. Performance of Murrah buffaloes fed ration containing non Bt. and Bt. cotton seed during experimental feeding period.

Particulars	Non-Bt. group	Bt. group
Av. Initial body weight (Kg)	442.1	468.9
Av. Final body weight (Kg)	459.2	483.4
Av. Body weight gain during 35 days (Kg)	17.1	14.5
Av. Weight gain per day (g)	488.6	414.3
Av. Dry matter intake/100Kg body weight	3.98	3.75
Av. Milk yield/day (Kg)	4.88	4.87
Av. 4% FCM yield/day (Kg)	8.47	8.04
Av. Fat % in milk	8.99	8.61

Evaluation of raw cottonseed meal derived from Bt cottonseed as a feed ingredient for Indian catfish, Magur (Clarias batrachus)

Chapter V

Annexure 4

PROJECT REPORT

CONSULTANCY PROJECT

Central Institute of Fisheries Education

(Deemed University)

Mumbai – 400061

FINAL TECHNICAL REPORT

Title of the Project

Evaluation of raw cottonseed meal derived from Bt-cotton seed as a feed ingredient for Indian catfish, Magur (Clarias batrachus)

8th Sptember, 2000 to 8th January, 2001

Name of the Investigaor(s)/ : **Dr. K.K. Jain**
Author(s) : **Dr. S. Raizada**
Dr. P.P. Srivastava

Performing Laboratory
Fish Nutrition and Physiology Division
Central Institute of Fisheries Education
(Deemed University)
Versova, Mumbai-400 061 (MS),India

Laboratory Project ID
Study : DPN-CR-2000-01
Experiment : BGCOT/CIFE/MAHY/200/1

Abbreviation

Bt.	:	Bacillus thuringiensis
CIFE	:	Central Institute of Fisheries Education
MAHYCO/MAHY	:	Maharashtra Hybrid Seeds Company
VM	:	Vitamin Mixture
MM	:	Mineral Misture
CMC	:	Carboxy Methyl Cellulose
FM	:	Fish Meal
GOC	:	Groundnut Oil Cake
FCR	:	Food conversion Ratio
FER	:	Feed Efficiency Ratio
g	:	Gram
ND	:	Not detected
CR	:	Contract Research
DPN	:	Digestive Physiology and Nutrition
BGCOT	:	Bt-gene incorporated cotton
ID	:	Identification

1. Title of the Project	:	**Evaluation of cottonseed me derived from Bt-cotton seed as feed ingredient for Indian caftis magur**
2. Name of the Investigator(s)	:	**Dr. K. ·K.Jain**
Author(s)	:	**Dr. S. Raizada**
		Dr. P. P. Srivastava
3. Implementing Institution	:	Central institute of: Fisheries Education' (Deemed University), Versova, Mumbai-400 061 (MS), India.
4. Laboratory/Division involved in experimentation	:	Fish Nutrition and Physiology Division
5. Funding Agency	:	Maharashitra Hybrid Seeds Compar Limited, Resham BhaMn, 4" near, 71 Veer Nariman Road, Mumbai - 400 02(India)
6. Date of Contract Research undertaken	:	8t" September, 2000
7. Tenure of Contract Research	:	4 months (from September 8, 2000 to January 8, 2001)
8. Date of completion of Contract Research	:	31st December, 2000.

NOTE: *Record retention : All study specific raw data; protocols, and fina technical reports will be retained at the implementing institute and i. will be confidential document until the sponsorer approves release.

9.	Objectives and purpose of Study as stated in the Contact Research	:	Annexure-I
10.	Experimental work giving full details of experimental setup, methods adopted, etc.	:	Annexure-II
11.	Results obtained and detailed analyses of results of present Contract research project	:	Annexure-III
12.	Condusions summarizing	:	Annexure-IV

13. Names and signature of investigator(s) :

Dr. K. K. Jain

Dr. S. Raizada

Dr. P. P. Srivastava

निदेशक / DIRECTOR
केन्द्रीय मत्स्य शिक्षा संस्थान (भा. कृ. अनु. प.)
Central Institute of Fisheries Education, ICAR
वर्सोवा, मुंबई / Versova, Mumbai – 400 061.

Annesure – I

Objectives

Objectives as stated in the Contract Research Project proposal from MAHYCO Mumbai.

1. To assess the growth and survival of Indian catfish, Clarias batrachus fed o a diet containing raw cotton seed meal derived from Bt. Gene incorporate *(Bacillus thuringiensis)* cotton.

2. To study the comparative growth and survival on feeding of given samples of cotton seed as

A	:	Bt. Cotton variety MRC-162
B	:	Non Bt. Cotton variety MRC-162
C	:	Commercial cotton variety NHH-44
D	:	Non-Cottonseed groundnut (control)

Purpose

This contract research was designed to assess whether raw cotton seed meal derived from Bt. gene incorporated cotton plant is as safe and nutritious for growth of the Indian catfish, Magur *(Clarias batrachus)* as the meal derived from the non-Bt. gene raw cotton-seed meal and commercial cotton variety.

A. *Test material:* The test material was defined as a cotton meal derived from Bt-gene cotton, ID # as Bt. cotton variety (MRC-162).

B. Control Material : The control material was defined as the cottonseed meal derived from the control, same germplasm cotton line and ID # a non-Bt. Cotton variety (MRC-162)

C. Commercial Material : The commercial material was defined as the cottonseed meal derived from the commercial lot of different germplam cotton line and ID # as commercial cotton variety NHH-44.

D. Laboratory Control Material : The laboratory control material was identified as the goundnut oil cake and ID # as GOC.

Samples of cottonseed meal of the test and control materials were evaluated for anti-nutrient contents to determine gossypol level. The were kept frozen and were powered to 0.5 mm (500 micron) size particle using Cyclotec (1093 sample mill, TECATOR Foss, Sweden) and used to feed preparation.

Annexure – II

Materials and Methods

Experimental diets (Table-1) were formulated to contain (crude protein 36-39 %). Experimental diets were formulated by substituting cotton seed meals @ 20 % (óAô contained gossypol, 1.23 %; óBô contained gossypol, 1.52 % and óCô contained gossypol, 1.59 %) with that of groundnut cake. Out of these three A contained free 1.14 % gossypol; óBô contained 1.39 % free gossypol, and óCô contained 1.35 % free gossypol (Table-2). These values are preheated values of raw ingredients. Twenty percent groundnut was fully replaced by cotton seeds óAô or óBô or óCô. Before mixing the ingredients óAô or óBô and óCô in rest of the others ingredients (Table-1) the ingredients óAô, óBô and óCô were autoclaved (at 15 Atmospheric pressure, and 121 ^{o}C for 20 minutes to ensure complete degradation / destruction and/or inactivation of gossypol from cotton seed and also denaturing of Aflatoxin, if at all present. The values of free and total gossypol in the feeds (autoclaved and non-autoclaved) are presented in Table-3.

Table 1 : Percentage composition (dry weight basis) of experimental diets 'A' to 'D' fed to catfish, *Clarias batrachus.*

Feed	FM (%)	Acetes (%)	Wheat Flour(%)	Wheat Bran(%)	(%)	GOC (%)	Cod (%)	VM* (%)	CMC (%)	Yeast (%)	Beefext (%)	Eggyolk (%)
A	50	10	8	4	20	-	2	2	2	0.5	0.5	1
B	50	10	8	4	20	-	2	2	2	0.5	0.5	1
C	50	10	8	4	20	-	2	2	2	0.5	0.5	1
D Control	50	10	8	4	-	20	2	2	2	0.5	0.5	1

FM = Fishmeal; GOC = Groundnut oil cake; CMC = Carboxymethyl cellulose; VM=Vitamin mix = MM = Mineral mix.

*VM+MM (2.5kg contains); Vitamin A, 5.000,000 IU; Vitamin D^3, 1,025,000 IU; Vitamin $B^{2\ 1.3}$ g; Vitamin E, 875 Units; Vitamin K, 1.0g; Calcium pantothenate, 2.5 g; Vitamin B^{12}, 7.5 mg; Choloride, 6% w/w; Calcium, 850 g, Manganese, 27.5 g; Iodine, 1.0 g; Zinc, 15.0 g; Iron, 7.5 g; Copper, 2.0g; Cobalt, 0.45 g (Alembic Chemicals, Vadodara, India, Lot No. V-9944)

Table 2 : Chemical Composition of raw Cottonseed Samples (on dry weight basis)

Sample	Oil (%)	Protein (%)	Ash (%)	Car bohy drate (%)	K.Cal/ 100g	Total Gossy-pol (%)	Free Gossy-pol # (%)	Aflato-xin[$#] (µg/g)
A	24.2	27.1	4.1	44.6	504.6	1.23	1.14	ND[*]
B	19.7	25.2	4.1	51.0	482.1	1.52	1.39	ND[*]
C	19.8	25.4	4.1	50.7	482.6	1.59	1.35	ND[*]

ND[*] = Not Detected.

$ = Aflatoxin content were analzed at Vimta Labs Limited, Hyderabad and data provided to the Nutrition Division of CIFE, Mumbai.

= Analyzed by Dr. V. Subramaniam of MAHYCO, Medak, Kollakal-502334 (Andhra Pradesh) and data provided to the Nutrition Laboratory, CIEF, Mumbai

The feed (A, B, C and D) were weighed (dry matter basis), as per the percentage of the ingredients (Table-1) and water was added to make moist feed. No known contaminants were expected in the diet that would interfere with the present study.

Table 3 : Total and free gossypol contents (g/100g) in samples of cottonseed incorporated feeds

Lab No.	Identificaiton Details	Free Gossypol#	Total Gossypol#
2533	Label A: Bt-cottonï Not autoclaved	0.04	0.09
2534	Label B: Non-*Bt*-Not autoclaved	0.03	0.09
2535	Label C: Control ï Not autoclaved	0.03	0.07
2536	Label A: Bt-cotton-autoclaved	0.02	0.09
2537	Label B: Non-Bt-autoclaved	0.02	0.07
2538	Lable C: Control ï autoclaved	0.02	0.07

All values are expressed on moisture-free basis.

Values are mean of at least two determinations.

= Anylyzed by Dr. V. Subramaniam of MAHYCO, Medak, Kollakal-502334 (Andhra Pradesh) and data provided to the Nutrition Laboratory, CIEF, Mumbai

Testing System and Procedure

The Experiment was conducted in 300 round plastic pools (Fig. 1 and 2) with continuous aeration. The water temperature was maintained at 28 ^{o}C using a thermostatic heater (Rena 300 Watt, France, Fig.3). Artificial shelters were provided to the fingerling (Fig.3). Each tub was filled with 100 L of water and fishes were stocked @ 10 fish/tank (Fig.4). There were five replicates of each treatment. Prior to

beginning of the experiment, catfish weighing 0.165 g ± 0.030 g (10 fish weighing 1.65 g were fed feed ôDôas conditioning feed for 15 days. Initially fish were fed @ 50 % of the body weight in two equal installments. The feed quantity was adjusted weekly on the basis of growth (Fig.5). After two weeks the quantity of feed was reduced to 20% of the body weight until the end of the experiment. The experiment was conducted for 28 days. After each weekly weighing, the fish were given a bath of acrifliavin @ 3 mg/L to prevent bacterial infection. Water temperature 28 ^{o}C, dissolved oxygen 7.2.7.8 mg/L, and Ph 8.2-8.4 were monitored during the experimental period and were found within normal limits required for the fish culture.

Data Collection and Proximate analysis of fish and feed

At the end ï of- 28 days feeding experiment, minimum of three fish from each plastic pool were taken for the purpose of pooled tissues analysis and final length and weight of individual using Soxtec system (Model : HT-2, 1045, Sweden) for lipid analysis; Kjeltec system (model : 2200 Kjeltec Autosystem, Sweden) for fibre analysis; Muffle furnace (Expo, Mumbai) for ash contents and oven (Newtronic, Mumbai) for moisture content. Growth, survival, feed conversion and fish body proximate composition were subjected to one-way analysis of variance and Duncanôs multiple range test to determine treatment differences ($P<0.05$). A studentôs ôtô ï test was used to statistically evaluate any differences in measured parameters between fish fed diets containing Bt. Cotton seed and non-Bt. Cotton seed (Steel and Torrie, 1960), and to other treatment groups.

Annexure – III

RESULTS

A. Survial

The data collected from the 28 days feeding study showed no statistical significant differences in survival of Magur *(Clarias batrachus)* fed diet containi Bt-Cottonseed (treatment óAô) compared to fish fed diet containing non-I Cottonseed (treatment óBô). Fish survival was depressed in treatment- (commercial ctton control) as compared to other feed treatments in t experiment. The highest fish survival rate was recorded in the control havi groundnut cake in place of cotton seed meal. This results were as expected sin groundnut is a standard ingredient in the feed for Magus, whereas, cottonseed not a normal feed ingredient. Results are tabulated in Table-4 and Graph-I.

Table 4: Survival percentage* on feeding feed 'A' to 'D' of all five replicates. Values within a column sharing a letter are significantly indifferent (Ducan's multiple range test, P<0.05).

Feed	Survival (%) 7th day	Suvival (%) 14th day	Survival (%) 21st day	Survival (%) 28th day
A	$90^{a} \pm 3.5$	$70^{a} \pm 5.2$	$60^{a} \pm 4.2$	$58^{a} \pm 6.4$
B	$100^{a} \pm 0.0$	$82^{a} \pm 5.5$	$76^{a} \pm 4.3$	$72^{a} \pm 7.1$
C	$90^{a} \pm 2.6$	$60^{a} \pm 4.5$	$52^{a} \pm 2.4$	$48^{b} \pm 0.2$
D Control	$100^{a} \pm 0.0$	$92^{b} \pm 1.3$	$90^{b} \pm 1.7$	$88^{b} \pm 3.4$

* = Data presented here are mean value of five replicates.

Values are mens (X ± SE) of 5 replicates.

B Growth and feed conversion

Fish growth and feed conversion data from the 28th day feeding study showed no statistically significant differences in growth or feed conversion of magur *(Clarias batrachus)* fed diet containing Bt. ï Gene cotton seed (treatment A compared to fish fed diet containing non-Bt. Gene cottonseed (treatment B) (Tables-5 and 6 and Graph-II). Growth rate and feed conversion of fish fed die containing Feed conversion ratio and efficiency were significantly affected in cotton meal based feeds in comparison to laboratory control feed (Table-6 and Graph-III).

Table 5 : Final weight (g) of C. *batrachus* fed for 28 days and growth over initial and laboratory control weights. Values within a column sharing a letter are significantly indifferent (Dancans' multiple range test, P<0.05)

Feed	Initial weight (g)	Final Weight (g)	(%) increase From initial	% decrease in respect of laboratory control
A	0.165 ± 0.02	0.4723^{a}±0.059	186^{a}	(-) 48^{a}
B	0.165 ± 0.02	0.4566^{a}±0.053	177^{a}	(-) 50^{a}
C	0.165 ± 0.02	00.2007^{b}±0.010	22^{b}	(-) 78^{a}
D Control	0.165 ± 0.02	0.9075^{c}±0.025	450^{c}	-

Values are means (X ± SE) of 5 replicates.

Table 6 : Growth and feed conversion of Magus, Clarias batrachus fed feed A to D, Values within a column sharing a letter are significantly indifferent (Ducan's siltiple range test(P<0.05)

Feed	Cotton meal (%)	Groundnut cake (%)	Weight gain (%) over initial	Feed conversion ratio	Feed efficiency ratio
A	20	-	187	3.10^a	0.32^a
B	20	-	177	3.18^a	0.31^a
C	20	-	22	3.48^a	0.28^a
D Control	-	20	450	1.98^a	0.51^b

FCR = Feed given (dry weight basis)/ weight gain (live weight) in 28 days

C. Proximate Composition

Proximate composition of feed and fish bodies are summarized in Table-7 & 8 and graphs ï IV,V,VI, VII and VIII respectively.

Table 7 : Proximate composition of feeds (A and D)

Feed	**Lipid * (%)**	**Protein * (%)**	**Ash* (%)**	**Carbohydrate* (%) NFE**	**Moisture (%)**
A	12.8±1.0	38.0±1.6	9.5±0.07	36.9±2.12	39.6±1.52
B	11.7±0.7	36.0±2.1	8.9±0.09	39.2±1.85	38.5±2.17
C	11.9±0.4	37.5±1.1	8.7±0.20	40.7±3.72	37.7±3.01
D	12.3±0.2	39.0±1.8	7.8±0.36	39.6±1.56	37.9±2.33

* On dry weight basis.
Values are means (X ± SE) of 3 replicates

Table 8 : Proximate composition of the bodies of *Clarias batrachus* fed feed A to D for 28 days. Values within a column sharing a letter are significantly indifferent (Ducan's multiple range test, P<0,05)

Feed	Moisture (%)	Protein$ (%)	Carbohydrate* (%)	Lipid$ (%)	Ash$ (%)
- (Initial)	84.36[a] ± 4.91	11.57[a] ± 0.72	0.39[a] ± 0.02	1,42[a] ± 0.06	2.93[a] ± 0.07
	79.56[b] ± 45.26	12.59[b] ± 40.64	0.56[b] ± 0.22	2.16[b] ± 0.11	4.83[b] ± 0.26
	78.54[b] ± 43.78	13.46[b] ± 0.77	0.52[b] ± 0.04	2.29[b] ± 0.09	4.67[b] ± 0.18
	83.41[a] ± 4.6.11	10.32[b] ± 0.39	0.41[b] ± 0.03	1.28[b] ± 0.05	3.91[b] ± 0.09
	74.72[b] ± 4.39	14.66[b] ± 0.51	0.68[c] ± 0.05	2.78[c] ± 0.31	5.79[c] ± 0.32

* Carbohydrate (%) = 100 ï (Moisture % + Protein % + Lipid % + Ash %)
$ On wet weight basis.
Values are meal (X + SE) of 3 estimation.

There were no statistically significant differences in proximate compostion of bodies of magur *(Clarias batrachus)* fed diet containing Bt Cottonseed compared to fish fed diet containing non-Bt Cottonseed in this experiment. (Table 8). Bodies of fish fed diets containing commercial control cottonseed were significantly lower in protein, corbohydrate and lipids compare to fish fed the other treatments in the experiment. Bodies of the fish fec groundnut cake had the highest content of Protein, carbohydrate, lipids and ash among the treatments in this experiment (Table 8). This result was as expected since groundnut is anormal ingredient in feed for magur, whereas cottonseed is not commonly used in feed for magur.

ANNEXURE – IV

Conclusions summarizing the results

1. Cotton seed meal contained total and free gossypol content in sample A 1.2 and 1.14, sample B 1.52 and 1.39, and sample C 1.59 and 1.35 percent respectively, However, on sterilization using Autoclave, the gossipol content also reduced below the threshold limit of toxicity as recommended by Dorsa *et al.* (1992).

2. There was no aflatoxin content in any of the cotton seed meal sample.

3. Weight gain, survival, feed conversion ratio, feed efficiency and body proximate composition were not significantly different between fish fed Bt Cottonseed as sompared to fish fed non-Bt. Cottonseed during the study.

4. The overall survival range between 40% to 88% is considered normal for fatfish, Magur *(Clarias batrachus)* fed with moist feed containing autoclaved raw cottonmeal or with groundnut cake. Also this range is consistent with the database for magur fed with various feed.

5. Survival, growth rate, feed conversion and composition of bodies of *Clarias batrachus* are statistically insignificant for the size used in the study fed with either autoclaved Bt. Cotton seed or non-Bt. Cotton seed meal. Lowest survival, growth, feed conversion and body proximate compostion were recorded in fish fed with diet containing the commercial control cottonseed whereas highest values were recorded for fish fed with the diet containing

groundnut and no cottonseed. The differences in survival, growth rate, feed conversion and body proximate composition between fish fed cottonseed in their diet compared to fish fed diet containing groundnut cake may be due to low levels of free gossypol, even after autoclaving, in the cottonseed diets. It has been shown that gossipol has toxic effects on catfish when included in the fish diet (Dorsa *et al*., 1992). Also, differences in the types of protein and lipids in cottonseed compared to groundnut cake may account for the differences in performance of fish fed cottonseed compared to fish fed groundnut cake.

6. Based on the data from the present study, Bt. Cotton variety MRC ï 162 (Sample-A) and Non-Bt. Cotton variety MRC-162 (Sample-B) provided the same nutrition growth performance results for magur. This result was an expected since Bt. Cottonseed is similar in composition as Non-Bt. Cottonseed (Beerberich *et al.*, 1996) since they share a common germplasm. The only difference between the Bt. and Non-Bt cotton lines is the presence of the B Protein, and this protein has been shown to be highly specific to the target insect pests, and has no deleterious effects to non-target organisms such as birds, fish and mammals (EPA, 1988).

7. In the case of sample óCô the commercial control cottonseed, growth performance and survival were comparatively poor. Since the commercial control may be of different germplasm from the Bt. and non-Bt cottons, its different genetic constituency may cause differences in growth performance in comparison to the Bt and non-Bt cottonseed.

statistically similar growth performance, and survival, and can be recommended for use in catfish nutrition for making wet feed following strict procedures of detoxificaiton for gossypol.

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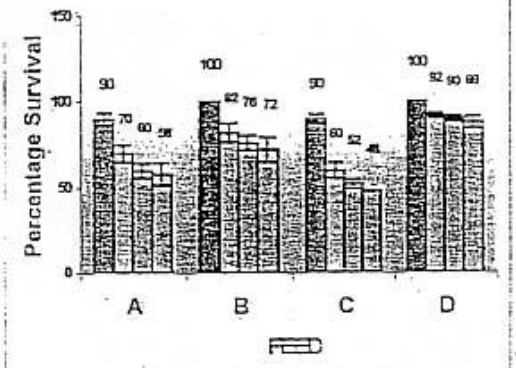
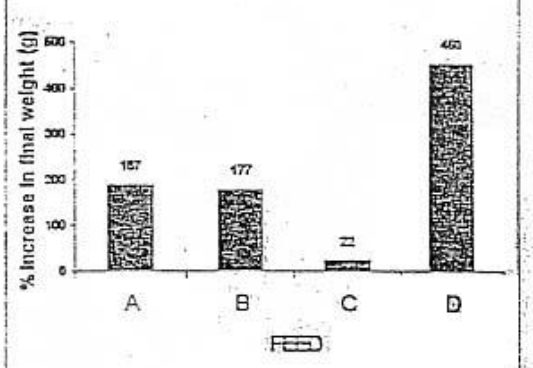
Dorsa, W.J., Robinette, R.H. Robinson, E.H., and W.E. Poe. 1982. Effects of dietary cottonseed meal and gossypol on growth of young channel catfish. Transactions of the American Fisheries Society 111:651-655.

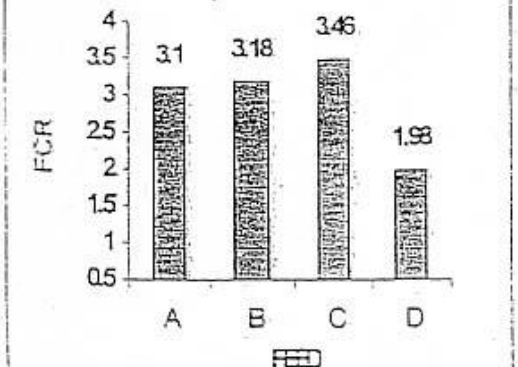
Graph I : Weekly survival percentage of *C. batrachus* fed feeds A to D.



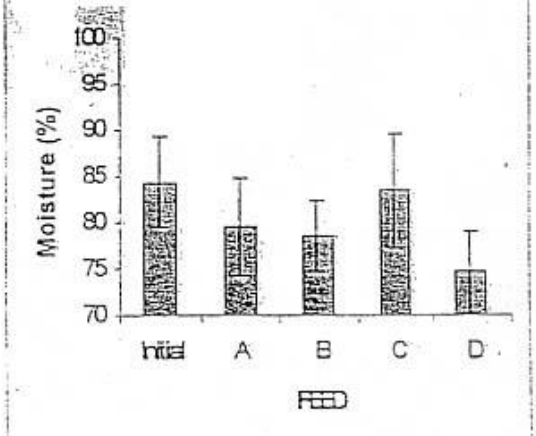
Graph II : Overall percent increase in final weight gain from initial weight of fish fed diets A to D.



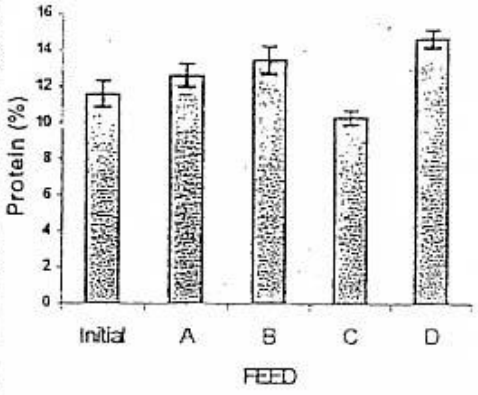
Graph III : Feed Conversion Ratio of feed (A to D) fed to *C. batrachus*



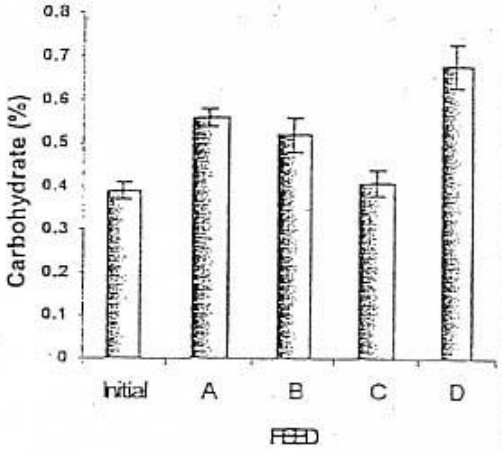
Graph IV : Moisture contents of fish bodies of *C. batrachus* fed feed A to D for 28 days



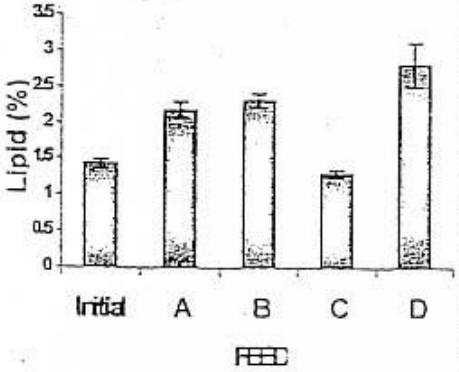
Graph V : Protein content of fish bodies of *C. batrachus* fed feed A to D for 28 days



Graph VI : Carbohydrate content of fish bodies of *C. batrachus* fed feed A to D for 28 days



Graph VII : Lipid content of fish bodies of *C. batrachus* fed feed A to D for 28 days



Graph VIII : Ash content of fish bodies of *C. batrachus* fed feed A to D for 28 days