

DRAFT REPORT

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

STUDY No.: 3585/02

SPONSORED BY

MAHARASHTRA HYBRID SEEDS COMPANY LIMITED

RESHAM BHAVAN

4th FLOOR 78, VEER NARIMAN ROAD

MUMBAI 400 020

INDIA

TEST FACILITY

TOXICOLOGY DEPARTMENT

RALLIS RESEARCH CENTRE

RALLIS INDIA LIMITED

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INDIA

TOXI-3585/02

088/4-CS to 088/7-CS-90-OG

PAGE No. 1/136

TABLE OF CONTENTS**PAGE**

QUALITY ASSURANCE STATEMENT	7
STATEMENT OF CONFIDENTIALITY	8
STATEMENT OF GLP COMPLIANCE	8
DECLARATION	8
STUDY DETAILS	9
STUDY PERSONNEL	10
LIST OF COMMONLY USED SYMBOLS AND ABBREVIATIONS	11
SUMMARY	13
INTRODUCTION	18
MATERIAL AND METHOD	20
TEST SPECIES	20
ACCLIMATIZATION	21
GROUPING	21
EXPERIMENTAL LAYOUT	21
HUSBANDRY	22
SAFETY PRECAUTIONS	23
TEST ITEM INFORMATION	24
TEST ITEMS AND CONTROLS INFORMATION	29
CHARACTERIZATION OF TEST AND CONTROL	
COTTON SEEDS	29
PREPARATION OF FEED CONCENTRATE AND ANALYSIS	
HAY	31
ROUTE OF TEST ITEM ADMINISTRATION	31
FEEDING OF GOAT FEED CONCENTRATE	31
FEEDIN OF HAY	31
OBSERVATIONS	
1. OPHTHALMOLOSCOPY	32
2. VETERINARY EXAMINATION, CLINICAL SIGNS	
AND PRE-TERMINAL DEATHS	32
3. BODY WEIGHT	32
4. CONSUMPTION OF HAY AND FEED CONCENTRATE ..	32
5. CALCULATION OF FEED CONVERSION/ENERGY	
EFFICIENCY PARAMETERS	33
6. CLINICAL LABORATORY INVESTIGATIONS	35
7. NECROPSY	38
8. HISTOPATHOLGY	39
STATISTICAL ANALYSES	40

contd.

TABLE OF CONTENTS

LIST OF APPENDICES

	APPENDIX	PAGE
INDIVIDUAL WEEKLY BODY WEIGHTS (kg) - MALES	1	89
INDIVIDUAL CUMULATIVE WEEKLY NET BODY WEIGHT GAINS (kg)- MALES	2	90
INDIVIDUAL WEEKLY BODY WEIGHTS (kg) - FEMALES ..	3	91
INDIVIDUAL CUMULATIVE WEEKLY NET BODY WEIGHT GAINS (kg)- FEMALES	4	92
INDIVIDUAL AVERAGE WEEKLY FEED CONSUMPTION (g/goat/day) - MALES	5	93
- FEMALES	6	94
INDIVIDUAL AVERAGE WEEKLY HAY CONSUMPTION (g/goat/day) - MALES	7	95
- FEMALES	8	96
INDIVIDUAL FEED CONVERSION AND ENERGY CONVERSION PARAMETERS - MALES	9	97
- FEMALES	10	98
INDIVIDUAL HAEMATOLOGICAL VALUES		
Sampling: Day (Pre-treatment) - MALES	11	99
- FEMALES	12	100
Sampling: Day 45 - MALES	13	101
- FEMALES	14	102
Sampling: Day 91 - MALES	15	103
- FEMALES	16	104
INDIVIDUAL CLINICAL CHEMISTRY VALUES		
Sampling: Day (Pre-treatment) - MALES	17	105
- FEMALES	18	106
Sampling: Day 45 - MALES	19	107
- FEMALES	20	108
Sampling: Day 91 - MALES	21	109
- FEMALES	22	110
INDIVIDUAL TERMINAL FASTING BODY WEIGHTS, ORGAN WEIGHTS AND ORGAN WEIGHT RATIOS - MALES.....	23	111
- FEMALES	24	112

TABLE OF CONTENTS

LIST OF APPENDICES

	APPENDIX	PAGE
INDIVIDUAL GROSS PATHOLOGICAL AND HISTOPATHOLOGICAL FINDINGS - MALES	25	113
- FEMALES	26	117

DEVIATIONS FROM APPROVED STUDY PLAN/AMENDMENT	27	121
--	----	-----

LIST OF ANNEXURES	ANNEXURE	PAGE
-------------------	----------	------

SUMMARY OF ANALYSIS: COMPOSITIONAL ANALYSIS, PESTICIDES RESIDUES AND AFLATOXINS OF COTTON SEEDS	1	123
---	---	-----

CERTIFICATE OF ANALYSIS	2	125
-------------------------------	---	-----

SUMMARY OF ANALYSIS : FEED CONCENTRATE (ANALYSIS ON "AS IS BASIS")	3	126
---	---	-----

ANALYSES OF MAJOR RAW MATERIALS (ENERGY AND PROTEIN SOURCE) USED FOR FORMULATION OF FEED CONCENTRATE (ANALYSIS ON "AS IS BASIS")	4	130
--	---	-----

SUMMARY OF ANALYSIS : HARIYALI HAY (ANALYSIS ON "AS IS BASIS")	5	131
---	---	-----

COMPOSITION AND ENERGY (TDN) OF GOAT FEED CONCENTRATE	6	132
--	---	-----

ANALYSIS REPORT – WATER SAMPLE	7	133
--------------------------------------	---	-----

CONTAMINANT ANALYSIS REPORT FOR WATER SAMPLE	8	134
--	---	-----

GLP CERTIFICATES	9	135
------------------------	---	-----

QUALITY ASSURANCE STATEMENT

The Study No.: 3585/02, entitled "Subchronic (90 day) Feeding Study with Bollgard II and Control Non-Bollgard II Cottonseed in Goats" has been inspected in accordance with the OECD Principles of Good Laboratory Practice for the testing of chemicals [C(97)186/Final].

This study was inspected and findings were reported to Management and to the Study Director on the dates shown below:

INSPECTION DATES	PHASE	REPORTING DATES
	INITIATION PHASE	
	IN LIFE PHASE	
	REPORTING PHASE	

Inspections were performed according to the Standard Operating Procedures of the test facility's Quality Assurance Unit. The report was inspected against the approved study plan and pertinent raw data.

Date:

(Mr.SATISH MURTHY V.)

Head, Quality Assurance Unit
Rallis Research Centre, Bangalore

STATEMENT OF CONFIDENTIALITY

This report contains **confidential** and **proprietary** information of Maharashtra Hybrid Seeds Company Limited, Resham Bhavan, 4th Floor 78, Veer Nariman Road, Mumbai 400 020, INDIA which will not be disclosed to anyone except the employees of this company or to persons authorised by law or judicial judgement without the expressed or written approval of Maharashtra Hybrid Seeds Company Limited, Resham Bhavan, 4th Floor 78, Veer Nariman Road, Mumbai 400 020, INDIA.

STATEMENT OF GLP COMPLIANCE

The Study No.: 3585/02 was performed in accordance with the OECD Principles of Good Laboratory Practice [C (97) 186/ Final].

This study was conducted based on the general principles of OECD Guideline for Testing of Chemicals, Section 4, No. 409, "Repeated Dose 90-day Oral Toxicity Study in Non-Rodents," adopted on 21 September, 1998 and as per 'The Revised Guidelines for Research in Transgenic plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant Parts, "Subchronic Oral Toxicity-Goats-90 Days Study for Genetically Engineered Seeds", Department of Biotechnology, Ministry of Science and Technology, Government of India, August 1998, and as per the Standard Operating Procedures.

This study was performed as per the mutually agreed study plan signed by the Study Director and Monitoring Scientists on 28.02.2005 and 07.03.2005, respectively. Amendment No 1 and 2 to the study plan signed by Study Director on 14.06.2005 and 22.06.2005 and Monitoring Scientists on 14.06.2005 and 24.06.2005, respectively.

DECLARATION

The Study Director hereby declares that the work was performed under his supervision and in accordance with the described procedures. It is assured that the reported results faithfully represent the raw data obtained during the experimental work. No circumstances have been left unreported which may have affected the quality or integrity of the data or which might have a potential bearing on the validity and reproducibility of this study.

The Study Director accepts overall responsibility for the technical conduct of the study as well as the interpretation, analysis, documentation and reporting of the results.

Date:

(Dr.S.M.SULAIMAN)
Study Director

STUDY DETAILS

Study Title : Subchronic (90 day) Feeding Study with Bollgard II and Control Non-Bollgard II Cottonseed in Goats

Test item : Two types of Cotton Hybrid F-2 Seeds with Bollgard II trait

Study Number : 3585/02

Study Director : Dr.S.M.Sulaiman

Sponsor : Maharashtra Hybrid Seeds Company Limited
Resham Bhavan 4th Floor
78, Veer Nariman Road
MUMBAI 400 020, INDIA

Monitoring Scientists : 1.Dr.M.K.Sharma
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INDIA

Test Facility : Toxicology Department
Rallis Research Centre
Rallis India Limited
Post Box No. 5813, Plot Nos. 21 & 22
Peenya II Phase, Bangalore 560 058, INDIA

Study Schedule:

Acclimatization : Start: 01.03.2005 End: 15.03.2005

Test item feeding : Start: 16.03.2005 End: 14.06.2005

Observations : Start: 16.03.2005 End: 17.06.2005

Termination (Euthanesia) : 14.06.2005 to 17.06.2005

STUDY PERSONNEL

The following personnel participated in the conduct of the study.

Name	Signature	Date
Dr.S.M.SULAIMAN M.V.Sc., Study Director Haematology, Clinical Biochemistry and Metabolism Section	_____	_____
Mr.P.J.PRAKASH M.Sc., Technical Co-ordinator Acute and Non-Rodent Section	_____	_____
Dr.D. SATHYANARAYAN SINGH B.V.Sc., Study Veterinarian Non-Rodent Breeding and Health Care section	_____	_____
Dr.C.SALEEM B.V.Sc., Technical Assistance Non-Rodent Breeding and Health Care section	_____	_____
Mr.M. RAMAKRISHNA BHAT M.Sc., Laboratory Investigations, Haematology, clinical Biochemistry and Metabolism Section	_____	_____

STUDY PERSONNEL contd.

Dr. GEETA NIRODY M.V.Sc
Pathologist,
Histopathology Section

Mr. S. RAMESH BABU M.Sc
Necropsy and Histotechniques,
Histopathology Section

Mr. M.VENKATESULU B.Sc.,
Data entry, Documentation, Data Analysis
and Report compilation
EDP Section

LIST OF COMMONLY USED SYMBOLS AND ABBREVIATIONS

ADF	Acid detergent fibre	mEq/l	milli Equivalent per litre
Alp	Alkaline phosphatase	mg	milligram
ALT	Alanine transaminase	min	minute
App	Appendix/Appendices	mm	millimeter
AST	Aspartate transaminase	mmol/l	millimole per litre
		mn	micron
BG II	Bollgard II	Mono	Monocyte
BUN	Blood Urea Nitrogen	NA	Not Applicable
Bwt	Body weight	Na	Sodium
		NAD	No Abnormality Detected
DM	Dry matter	Neut	Neutrophil
Eosi	Eosinophil	No.	Number
ELISA	Enzyme Linked Immunosorbent Assay		
F	Female	pg	picogram
fl	Femto litre	P.T	Prothrombin time
g	gram	RBC	Red Blood Corpuscle
G/l	Giga per litre	rpm	revolutions per minute
G.	Group		
Glu	Glucose	s	seconds
g/l	gram per litre		
		TDN	Total digestible nutrients
Hb	Haemoglobin		
Hct	Haematocrit	T/l	Tera per litre
		Tot.Pro	Total Proteins
K	Potassium		
kg	kilogram	U/l	Units per litre
l/l	litre per litre	WBC	White Blood Corpuscle
Lymp	Lymphocyte		
		%	per cent
M	Male	µmol/l	micromole per litre
m	meter		
mcg	microgram	°	Degree celcius

- Identification : By goat accession number and corresponding neck collar chain with coded metal tags and cage cards.
- Quarantine : Animals were quarantined for 93 days. The data on health, feed intake and body weight were recorded.

2. ACCLIMATIZATION

Fifteen days under experimental conditions after veterinary examination.

3. GROUPING

The animals procured for the study were weighed and placed in to four virtual groups (sex-wise) two days before initiation of treatment. Assignment to groups was done by using computer generated random numbers. The mean body weight of the study groups were compared for inter group difference and observed that there were no significant difference in the body weights between groups.

4. EXPERIMENTAL LAYOUT

Group No.	Study groups	Deciphered code name of the cotton seed	Sex	Goat numbers		No. of goats
				From	To	
G1	Feed concentrate fortified with AB12 crushed cotton seeds	MRC 7201 BG II cotton seeds	M	Gt 41	Gt 46	6
			F	Gt 47	Gt 52	6
G2	Feed concentrate fortified with AB34 crushed cotton seeds	MRC 7201 Non-BG II cotton seeds	M	Gt 53	Gt 58	6
			F	Gt 59	Gt 64	6
G3	Feed concentrate fortified with AB56 crushed cotton seeds	MRC 7301 BG II cotton seeds	M	Gt 65	Gt 70	6
			F	Gt 71	Gt 76	6
G4	Feed concentrate fortified with AB78 crushed cotton seeds	MRC 7301 Non-BG II cotton seeds	M	Gt 77	Gt 82	6
			F	Gt 83	Gt 88	6

M: Male F: Female

Note: the event number for Bollgard II cotton seeds is 15985

HUSBANDRY

Room: Goat experimental Facility

a. Conditions

Goats were housed in a small ruminant experimental facility with ambient temperature and adequate ventilation with 16 hours fluorescent light and 8 hours dark photoperiod. Fluorescent light was provided during 5.00 A.M. to 9.00 P.M. Experimental room conditions were temperature: 24-33°Celsius; relative humidity: 69-70%.

b. Accommodation

Goats were housed individually in concrete floor pens (Approx. size: Length 6 x Width 3 feet). All the animals will be let loose for socializing (sex-wise and group-wise) in runs (Approx. size: Length 10.5 x Width 8.8 feet each) for at 1 hour / day for seven days a week except on day 15 of acclimatization wherein the animals were not let-out for runs.

c. Feed and hay

The goats were maintained on *ad libitum* haryali (*Cynodon dactylon*) hay and concentrate feed prepared for the specific group which were offered in stainless steel hoppers for two hours. Based on the pre-determined feed concentrate consumption range, quantity of 500 grams of the feed concentrate per goat was offered.

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

SUMMARY

Two hybrids of Bollgard II cotton seeds MRC 7201 BG II cotton seeds and MRC 7301 BG II cotton seeds (event number 15985 containing Cry1Ac and Cry2Ab genes), supplied by Maharashtra Hybrid Seeds Company Limited, Dawalwadi, Tq.Badnapur, Dist. Jalna- 431 203 through Monsanto Research Centre, Bangalore was assessed for the wholesomeness and feed safety in relation with cottonseeds of control or conventional varieties that do not contain the Bollgard II trait (Non-Bollgard II counterparts) MRC 7201 Non-BG II cotton seeds and MRC 7301 Non-BG II cotton seeds. The cotton seed varieties were coded by the sponsor and the codes were deciphered at the end of in-life phase of the experiment. Powdered Cottonseeds of Bollgard II and control cotton varieties of Non-Bollgard II were administered to goats through the diet for 90 days. The feed concentrate was fortified with 12.5% of the respective varieties of cotton seed powder.

The experiment consisted of 4 groups: G1 group received feed concentrate fortified with MRC 7201 BG II cotton seeds, G2 group (concurrent control group to G1 group) received feed concentrate fortified with MRC 7201 Non-BG II cotton seeds, G3 group received feed concentrate fortified with MRC 7301 BG II cotton seeds and G4 group (concurrent control group to G1 group) received feed concentrate fortified with MRC 7301 Non-BG II cotton seeds.

Each group consisted of 12 (6 male + 6 female) young adult healthy Osmanabadi breed of goats, aged 8-9 months, adapted to stall feeding and the concentrate feed for a minimum period of 13 weeks. All the goats were vaccinated against Foot and Mouth disease, Haemorrhagic septecemia, Enterotoxemia and Peste de petits ruminants and treated with anthelmintic and ectoparasitocidal agent.

The body weight range at the start of treatment was; Males: 12.1-23.8 kg and Females: 13.8-22.0 kg. Quantity of 500 grams of the feed concentrate prepared for each group was offered to individual goats for 2 hours daily after removing the hay. After 2 hours, the left over feed concentrate was removed and haryali hay (*Cynodon dactylon*) was offered *ad libitum*.

All the major ingredients used for preparation of feed concentrate were analysed for the composition and based on the analysed data the feed concentrate was formulated for each group to attain the defined level of crude protein content. The hay and the prepared feed concentrate for each group were analysed for moisture, crude protein, crude fat, crude fibre, total carbohydrates, total ash, acid insoluble ash, nitrogen free extract, calcium, phosphorus, magnesium, iron, manganese, copper and zinc. In addition to these parameters acid detergent fibre was analysed for hay. The crude protein content (dry matter basis) in the concentrate feed of each group as determined by repeated periodic analysis was in the range of; G1: 18.9-19.9%; G2: 19.0-19.9%, G3: 19.2-19.9% and G4: 19.1-20.4%. All the major raw materials and the formulated feed concentrate for each group were analysed for aflatoxin.

The animals were housed individually in floor pens (Approx. size: L 6 x B 3 feet) with filtered air, adequate ventilation and illumination. The goats were let loose in groups (sex-wise and group-wise) in paved and covered runs daily for 1 hour for 7 days a week except on day 15 of acclimatization wherein the animals were not let-out for runs. The daily room temperature and relative humidity were recorded.

All the goats were observed twice daily for clinical signs and pre-terminal deaths, weekly for changes in body weight and fortnightly for physical examination. Daily consumption of feed concentrate and hay of individual goats was measured. Rectal temperatures were recorded daily for first 15 days of treatment period and weekly thereafter. Laboratory investigations for haematology and clinical chemistry were performed prior to the start of the treatment (day: -1: pre-treatment), interim (day 45) and at termination (day 91). At termination all the goats were subjected to a detailed necropsy. Organs were collected, weighed and preserved.

Under the testing conditions described briefly above, the following results were obtained:

1. PHYSICAL AND OPHTHALMIC EXAMINATION, CLINICAL SIGNS AND PRE-TERMINAL DEATHS

The Physical and ophthalmic examination did not reveal any abnormality. There were no clinical signs or pre-terminal death in any of the goats.

2. BODY WEIGHTS AND NET BODY WEIGHT GAINS

The body weights and net body weight gains of the two hybrids of Bollgard II cotton seed fed groups were comparable to their concurrent control groups.

3. FEED AND HAY CONSUMPTION

The feed consumption of two hybrids of Bollgard II cotton seed fortified feed concentrate was not statistically different from their concurrent control groups (Non- Bollgard II cotton seed fortified feed concentrate).

The hay consumption in two hybrids of Bollgard II cotton seed fed groups was not statistically different from their concurrent control groups (Non- Bollgard II cotton seed fed groups). However, in G1 group males, the hay consumption was higher during week 3 and lower during week 10. In G1 group females, the hay consumption was lower during weeks 1, 2 3 and 7. In G3 group females, the hay consumption was higher during weeks 8 and 11.

These changes are incidental and were considered to be of no physiological significance.

4. FEED CONVERSION AND ENERGY EFFICIENCY PARAMETERS

There were no significant difference in the feed conversion efficiency and energy corrected feed conversion efficiency parameters between the two hybrids of Bollgard II cotton seeds in comparison to their respective concurrent control groups.

5. LABORATORY EXAMINATIONS

Haematology:

There were no significant difference in the haematological parameters between the two varieties of Bollgard II cotton seeds and their respective concurrent control groups except for incidental changes in the values of erythrocyte count in G1 and G3 group males, haematocrit in G1 and G3 group males and females, leucocytes in G3 group males and Prothrombin time in G1 group males at different periods of analysis.

These changes in the haematological parameters were marginal changes and although significant and they are not considered to be of physiological significance.

Clinical Chemistry:

There were no significant difference in the clinical chemistry parameters between the two hybrids of Bollgard II cotton seeds and their respective concurrent control groups, except for incidental changes in the values of blood urea nitrogen in G1 group females and G3 group males, AST in G3 group males, sodium and potassium in G3 group females at different period of analysis. These changes in the clinical chemistry parameters were marginal changes and although significant and they are not considered to be of physiological significance.

Fasting body weights, organ weights and organ weight ratios:

There were no treatment related changes in terminal fasting body weights, organ weights and their ratios to body weight.

Gross Necropsy and Histopathaology:

There were no treatment related gross and histopathological findings.

Conclusion:

It is concluded that based on the health, growth and physio-pathological parameters analysed during the experiment that there is no differences between the two hybrids of Bollgard II cotton seed fed groups (MRC 7201 BG II cotton seeds and MRC 7301 BG II cotton seeds) in comparison to their respective concurrent control Non- Bollgard II cotton seed fed groups (MRC 7201 Non-BG II cotton seeds group and MRC 7301 Non-BG II cotton seeds).

Date:

(Dr. S.M.SULAIMAN)
Study Director

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

INTRODUCTION

Cotton is the leading plant fiber crop produced in the world and the most important in India. In addition, cotton-seed provides an important source of oil for human consumption and cottonseed and the processed cottonseed meal for animal feed. India ranks number one in the world for total area planted to cotton, but the country is ranked third in total cotton produced. One major limitation to cotton production in India is damage caused by insect pests. The cotton crop is damaged by about 130 species of insects of which the lepidopteran insects are the most important. Conventional chemical pesticides have been used to control these pests. However, use of these pesticides is costly to the grower, often pose environmental hazards, and have limited efficacy due to development of resistance in target pest populations.

As an effective and environmentally superior approach to control insect pests, Bollgard®¹ cotton was developed by insertion of a gene from a naturally occurring bacterium, *Bacillus thuringiensis* subsp. *Kurstaki*, into the chromosome of cotton. This enabled production of the Cry1Ac protein in the cotton plant, and this protein is active against lepidopteran insect pests. Bollgard cotton has been adopted broadly by growers worldwide, including India, since commercial introduction in 1996. The Cry1Ac protein in Bollgard cotton provides effective protection from feeding damage by lepidopteran insect pests, and growers using Bollgard cotton typically apply significantly less insecticide to control these pests, realizing higher yields, and achieving greater profitability using these improved Bollgard cotton varieties as compared to conventional cotton varieties.

¹ Bollgard® is a registered trademark of Monsanto Technology LLC

During acclimatization period, the goats were fed with concentrate feed fortified with 12.5% of control cotton seeds (AB91: Deciphered code name – NHH 44 hybrid Non- Bt cotton seeds). All the major raw materials used for preparation of feed concentrate, the formulated feed concentrate for each group presented in Annexure 4. The analysis report of the feed concentrate and hay are presented in Annexures 3 and 5 respectively. Composition and Energy (TDN) of goat feed concentrate presented as Annexure 6.

d. Water: *ad libitum*

Protected water: Deep borewell water passed through activated charcoal filter and exposed to UV rays in 'Aquaguard' water filter manufactured by Eureka Forbes Ltd., Mumbai- 400 001 was offered in stainless steel bowls. Water analysis report and contaminant analysis report are enclosed as Annexure 6 and Annexure 7 respectively. Interval of contaminant analysis for water was once a year. Results presented in this report are the closest to the study period.

SAFETY PRECAUTIONS

Gloves, cap and face mask in addition to protective clothing and shoes were used.

TEST ITEM INFORMATION (as furnished by the Sponsor)
(For use during acclimatization period)

Name : AB 91

Name to be used in the report : NHH hybrid Non- Bt cotton seed

Code by test facility : 088/1-CS

Batch No. : 134

Lot No. : 53

Sample No. : 4

Batch produced by : Monsanto Research Centre
Bangalore

Batch produced on (date) : 09.10.2004

Date of expiry : 08.10.2005

Supplied by : 1. Maharashtra Hybrid Seeds Company Limited,
Dawalwadi, Tq.Badnapur, Dist. Jalna- 431 203
2. Monsanto Research Centre
Bangalore

Date of receipt at test facility : 25.02.2005

Purity to be stated in the report : Not applicable

Physical appearance : Crushed seeds

Storage conditions : Cold storage (Below -15°C)

TEST ITEM INFORMATION (as furnished by the Sponsor)
(For use during treatment period)

Name : AB 12

Name to be used in the report : MRC 7201 BG II cotton seeds

Code by test facility : 088/4-CS

Batch No. : 176

Lot No. : 23

Sample No. : 2

Batch produced by : Monsanto Research Centre
Bangalore

Batch produced on (date) : 09.10.2004

Date of expiry : 08.10.2005

Supplied by : 1. Maharashtra Hybrid Seeds Company Limited,
Dawalwadi, Tq.Badnapur, Dist. Jalna- 431 203
2. Monsanto Research Centre
Bangalore

Date of receipt at test facility : 25.02.2005

Purity to be stated in the report : Not applicable

Physical appearance : Crushed seeds

Storage conditions : Cold storage (Below -15°C)

TEST ITEM INFORMATION (as furnished by the Sponsor)
(For use during treatment period)

Name : AB 34

Name to be used in the report : MRC 7201 Non-BG II cotton seeds

Code by test facility : 088/5-CS

Batch No. : 174

Lot No. : 218

Sample No. : 3

Batch produced by : Monsanto Research Centre
Bangalore

Batch produced on (date) : 09.10.2004

Date of expiry : 08.10.2005

Supplied by : 1. Maharashtra Hybrid Seeds Company Limited,
Dawalwadi, Tq.Badnapur, Dist. Jalna- 431 203
2. Monsanto Research Centre
Bangalore

Date of receipt at test facility : 25.02.2005

Purity to be stated in the report : Not applicable

Physical appearance : Crushed seeds

Storage conditions : Cold storage (Below -15°C)

A new genetically modified cotton plant, Bollgard II cotton, has been developed using particle acceleration plant transformation procedures to insert *Cry2Ab* insect control gene from *Bacillus thuringiensis* var. *Kurstaki* into the Bollgard cotton genome. Therefore, Bollgard II cotton contains two proteins, Cry1Ac and Cry2Ab, that have insecticidal activity against lepidopteran insect pests of cotton.

Bollgard II cotton provides equivalent or increased control of major insect pests of cotton with additional control of sporadic pests, such as beet and fall armyworm. Furthermore, combining the Cry2Ab protein with Cry1Ac protein provides an additional tool to delay the development of pest resistance to the Cry1Ac protein in Bollgard cotton, as Cry2Ab is a different Bt protein class than Cry1Ac. The Bollgard II trait has been introduced into Indian cotton hybrids using traditional plant breeding methods to provide Indian cotton growers an additional tool to protect the cotton crop from lepidopteran insect damage.

OBJECTIVE

The objective of this study was to compare the wholesomeness and feed safety of Bollgard II cottonseeds with cottonseeds of control or conventional varieties that do not contain the Bollgard II trait (Non-Bollgard II counterparts). Cottonseeds of Bollgard II and control cotton varieties of Non-Bollgard II counterparts were administered to goats through the diet for 90 days.

MATERIAL AND METHOD

1. TEST SPECIES

Animals	: Goats (<i>Capra hircus</i>)															
Breed	: Osmanabadi															
Source	: Osmanabadi Goat Marketing Services MIDC, Osmanabad, Maharashtra															
Justification for Selection of test species	: Goat is a model of species envisaged to assess the toxicity of transgenic seeds as per 'The Revised Guidelines for Research in Transgenic plants and Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant Parts, " Subchronic Oral Toxicity-Goats-90 Days Study for Genetically Engineered Seeds ", Department of Biotechnology, Ministry of Science and Technology, Government of India, August 1998. Hence goat is used in the study.															
Vaccination/Health status	: Vaccinated against Foot and Mouth disease, Peste des petits ruminants, Haemorrhagic septecemia, and Enterotoxaemia. The animals were treated with anthelmintic and ectoparasitocidal agent.															
No. of animals/group	: Six males + Six females															
Mean Body Weight (kgs) \pm SD at start of treatment:	<table><tr><td></td><td>Males</td><td>Females</td></tr><tr><td>G1:</td><td>17.8 \pm 3.21</td><td>16.4 \pm 1.42</td></tr><tr><td>G2:</td><td>16.2 \pm 3.25</td><td>16.3 \pm 1.07</td></tr><tr><td>G3:</td><td>19.8 \pm 2.42</td><td>17.4 \pm 1.31</td></tr><tr><td>G4:</td><td>18.6 \pm 1.75</td><td>18.3 \pm 2.31</td></tr></table>		Males	Females	G1:	17.8 \pm 3.21	16.4 \pm 1.42	G2:	16.2 \pm 3.25	16.3 \pm 1.07	G3:	19.8 \pm 2.42	17.4 \pm 1.31	G4:	18.6 \pm 1.75	18.3 \pm 2.31
	Males	Females														
G1:	17.8 \pm 3.21	16.4 \pm 1.42														
G2:	16.2 \pm 3.25	16.3 \pm 1.07														
G3:	19.8 \pm 2.42	17.4 \pm 1.31														
G4:	18.6 \pm 1.75	18.3 \pm 2.31														
No. of groups	: 4															
Age (at the start of treatment)	: 8-9 months															

TEST ITEM INFORMATION (as furnished by the Sponsor)
(For use during treatment period)

Name : AB 56

Name to be used in the report : MRC 7301 BG II cotton seeds

Code by test facility : 088/6-CS

Batch No. : 314

Lot No. : 09

Sample No. : 6

Batch produced by : Monsanto Research Centre
Bangalore

Batch produced on (date) : 09.10.2004

Date of expiry : 08.10.2005

Supplied by : 1. Maharashtra Hybrid Seeds Company Limited,
Dawalwadi, Tq.Badnapur, Dist. Jalna- 431 203
2. Monsanto Research Centre
Bangalore

Date of receipt at test facility : 25.02.2005

Purity to be stated in the report : Not applicable

Physical appearance : Crushed seeds

Storage conditions : Cold storage (Below -15°C)

TEST ITEM INFORMATION (as furnished by the Sponsor)
(For use during treatment period)

Name : AB 78

Name to be used in the report : MRC 7301 Non-BG II cotton seeds

Code by test facility : 088/7-CS

Batch No. : 231

Lot No. : 45

Sample No. : 7

Batch produced by : Monsanto Research Centre
Bangalore

Batch produced on (date) : 09.10.2004

Date of expiry : 08.10.2005

Supplied by : 1. Maharashtra Hybrid Seeds Company Limited,
Dawalwadi, Tq.Badnapur, Dist. Jalna- 431 203
2. Monsanto Research Centre
Bangalore

Date of receipt at test facility : 25.02.2005

Purity to be stated in the report : Not applicable

Physical appearance : Crushed seeds

Storage conditions : Cold storage (Below -15°C)

TEST ITEMS AND CONTROLS INFORMATION

The test and the control items were supplied by the sponsor as crushed cotton seeds and it was used as such for blending with the feed concentrate. The test item and the controls were blinded and provided as codes by the sponsor. Two sealed envelopes were prepared by the sponsor, one of the envelope was sent to the Management (Chief Technology Officer), Rallis Research Centre, Bangalore and the other envelope was retained by the sponsor. The codes were provided to the Study director by the sponsor at the end of the in-life phase for the preparation of the draft report. The crushed cotton seeds were stored at -15.1 to -25 degrees Celsius.

CHARACTERIZATION OF TEST AND CONTROL COTTONSEEDS

The test cottonseeds used in this study were cotton hybrids A and B (Filial -Two or F-2) that contain the Bollgard II trait (Cry1Ac and Cry2Ab genes). The corresponding control cottonseeds were cotton hybrids A and B (Filial -Two or F-2) that do not contain the Bollgard II trait (Non-BG II). The test and the control cottonseeds were characterised by the sponsor prior to their use in this study, using ELISA (Annexure 2).

The sponsor has provided the testing facility with results of analyses of all the samples of the test and control cottonseed for the presence of mycotoxins and pesticides. The sponsor has also provided the test facility with results of compositional analyses of the test and control cottonseeds. The compositional analyses includes at least proximates (protein, fat, carbohydrates, ash) and gossypol. The sponsor has used certified and/or Non-GLP facility for these analyses. Summary data from these analyses is presented as Annexure 1.

The authenticity of the test and control items and analytical data was provided by the sponsor.

PREPARATION OF FEED CONCENTRATE AND ANALYSIS

The individual feed ingredients were selected and procured in sufficient quantities for the experimental period, stored at 6 to 8° C and were subjected to proximate analysis. Those ingredients which required powdering (grounding) were put in the milling machine and powdered individually making sure the milling equipment is properly cleaned between ingredients and stored at 6-8°C.

The major raw material which includes maize, groundnut cake, wheat bran and cane molasses were analysed for the parameters as presented in Annexure 4.

The feed concentrate for each of the test group was prepared by inclusion of respective type of crushed cotton seed for each group i.e. MRC 7201 BG II cotton seeds for G1 group, MRC 7201 Non-BG II cotton seeds for G2 group, MRC 7301 BG II cotton seeds for G3 group and MRC 7301 Non-BG II cotton seeds for G4 group.

Based on the crude protein content of the selected feed ingredients, feed concentrate was formulated for the specific group. The required quantity of the feed ingredients was weighed to compute the nutritional requirement range.

The amount of 12.5% of respective crushed cotton seeds was included for the formulation of the each diet. The blending of the diets was performed weekly.

A premix was prepared using mineral mixture, salt and Vitamin premix in approximately 5% of wheat bran and mixed manually for 5 minutes. Then the premix and all the other ingredients were blended in the ribbon blender for 15 minutes and then stored in labelled feed bags for further use. The prepared feed concentrate was aliquoted group-wise for the quantities required for daily use and stored in cold storage (-18 to -29°C). The required quantity of feed, one aliquot of each group was taken from cold storage on a daily basis and a quantity of 500 grams per goat was provided in stainless steel hoppers for two hours. The left over quantity of feed concentrate was weighed and discarded daily. The feed concentrate prepared during weeks 1, 5 and 9 and composite samples from each batch of the feed concentrate prepared during weeks 2, 3 and 4; 6, 7 and 8; 10, 11, 12 and 13 were subjected for analysis of the parameters as presented in Annexure 3.

HAY

Good quality hay (*Cynodon dactylon*) was procured and cleaned to remove extraneous matter. The hay was analysed once a month for parameters as presented in Annexure 5.

ROUTE OF TEST ITEM ADMINISTRATION

The test and control items were administered through the diet. This route of administration was selected because it represents the most likely route of exposure to goat species in their natural habitat.

FEEDING OF GOAT FEED CONCENTRATE

The study groups animals received feed concentrate specifically prepared for that group in stainless steel hoppers with a feeding time of two hours per day for 90 days.

FEEDING OF HAY

The hay was provided to goats of all groups *ad libitum* in the stalls except during the time of concentrate feeding and fasting period as required for blood collection.

OBSERVATIONS

1. OPHTHALMOSCOPY

Ophthalmic examination was performed in all animals prior to start of test item feeding and on day 90.

2. VETERINARY EXAMINATION, CLINICAL SIGNS AND PRE-TERMINAL DEATHS

Goats were observed twice daily for clinical signs and pre-terminal deaths. Physical examination which includes auscultation of lungs and heart, recording of heart rate, respiration rate, rectal temperature and observation for discharges if any was performed before grouping, at initiation of treatment on day (-1) and once every two weeks of the experimental schedule.

The goats were observed for clinical signs and pre-terminal deaths twice daily. Rectal temperature were recorded daily for 15 days (day 1 to day 15 of treatment period) and weekly thereafter.

3. BODY WEIGHT

Individual body weights were recorded at start of treatment (pre-treatment) and at weekly intervals.

4. CONSUMPTION OF HAY AND FEED CONCENTRATE

At the start of the study, all the dedicated containers for hay and feed were labelled with Study No., group number and goat number and these were used throughout the study. The hay was provided *ad libitum* to all the goats in the stalls except during the time of concentrate feeding and during fasting period as required for blood collection.

The feed concentrate - 500 grams specifically prepared for the group was offered to individual goat for two hours per day. Initial weight of the hay and feed was recorded on a daily basis. The left over quantity of the hay and feed concentrate was weighed, recorded and discarded.

Each day spillage (wastage) of the hay and feed concentrate was collected, weighed, recorded and considered for calculation of feed and hay consumption.

Feed and hay consumption was measured daily. The daily feed and hay consumption was calculated as:

$$\text{Daily feed/hay cons. (g)} = \text{Feed/hay offered(g)} - \text{left over feed/hay} - \text{spillage of feed/hay}$$

Average daily feed/hay consumption data is presented for each week of the experimental period by taking the mean of 7 days of daily feed/hay consumption. The individual goat feed/hay consumption data is expressed as g/goat/day.

Note: During week 13, the average daily feed/hay consumption data is presented by taking the mean of 6 days of daily feed/hay consumption.

5. CALCULATION OF FEED CONVERSION/ENERGY EFFICIENCY PARAMETERS:

The feed/ energy conversion parameters were calculated as follows based on the feed concentrate and hay consumption of individual goats for 90 days and the body weight gain.

FORMULAE FOR CALCULATION OF FEED/ENERGY EFFICIENCY

$$\text{TDN Kg of individual Feed ingredient} = \text{TDN}^{\#} \% \text{ of the ingredient used for the formulation of feed concentrate} \times \text{Quantity of the ingredient}$$

#: reported book values

$$\text{ENERGY (TDN) ON FED BASIS OF EACH GROUP FEED} = \frac{\text{Total TDN Kg of all the ingredients}}{\text{Total quantity of the feed prepared}} \times 100$$

$$\text{TDN \% OF HAY} = 97.6 - 0.974 \times X \text{ (\% ADF on a 100\% DM basis)}$$

$$\text{TDN CONTENT OF HAY ON FED BASIS} = \text{TDN \% of Hay} \times \text{\% Dry matter in the Hay}$$

*: Constant values

$$\text{TDN INTAKE (g/day individual goat)} = \text{Feed consumed (g/day as fed)} \times \text{TDN content of feed fed} + \text{Hay consumed (g/day as fed)} \times \text{TDN content of hay as fed}$$

ACTUAL FEED CONVERSION:

$$\text{a. FEED: GAIN} = \frac{\text{Total amount of hay + feed concentrate consumed for 90 days (g)}}{\text{Weight gain (g)}}$$

$$\text{b. GAIN: FEED} = \frac{\text{Weight gain (g)}}{\text{Total amount of hay + feed concentrate consumed for 90 days (g)}}$$

ENERGY CORRECTED FEED CONVERSION:

$$\text{c. ENERGY (TDN): GAIN} = \frac{\text{Total TDN consumed for 90 days (g)}}{\text{Weight gain (g)}}$$

$$\text{d. GAIN: ENERGY (TDN)} = \frac{\text{Weight gain (g)}}{\text{Total TDN consumed for 90 days (g)}}$$

Note: Body weight gain = Weight on day 90 – Initial weight on day 1.

7. Sodium (mEq/l): Easylyte electrolyte analyser (Medica Corporation, USA)

8. Potassium (mEq/l): Easylyte electrolyte analyser (Medica Corporation, USA)

d. Analyses of blood samples for the Cry 1 Ac and Cry2Ab protein:

Plasma samples collected for clinical chemistry at termination, were aliquoted and stored at $\leq -20^{\circ}\text{C}$ until further analysis for the presence of Cry 1 Ac and Cry2Ab protein using ELISA specific for the detection of the Cry 1 Ac and Cry2Ab protein.

The ELISA was performed as follows:

Procedure of ELISA for the detection of Cry1Ac protein in goat plasma samples

1. To a pre-coated plate (as supplied by the manufacturer : DesiGen, A division of Mahyco Seeds Ltd, Dawalwadi, Jalna- 431 203, Maharashtra), 100 μl / well of Cry1Ac conjugate was added.
2. 100 μl of the plasma sample to be analysed was added to the appropriately positioned wells.
3. 100 μl of sample extract buffer was added in blank well.
4. The plate was washed four times with 10 X wash buffer manually.
5. The plate was dried by patting (well down) on blotting paper to remove excess buffer
6. 100 μl of freshly prepared 1X substrate was added to each well.
7. The plate was incubated at room temperature in dark for at least 15 minutes.
8. 100 μl of the stop solution was added after incubation.
9. The absorbance was measured at 450 nm using Diasorin ETI-LAB ELISA micro plate reader.

Interpretation:

1. The absorbance value ≤ 0.150 was considered as "Negative" for the presence of Cry1Ac protein.
2. The absorbance value > 0.150 was considered as "Positive" for the presence of Cry1Ac protein.

Procedure of ELISA for the detection of Cry2Ab protein in goat plasma samples

1. To a pre-coated plate (as supplied by the manufacturer: DesiGen, A division of Mahyco Seeds Ltd, Dawalwadi, Jalna- 431 203, Maharashtra), 100 µl / well of Cry2Ab conjugate was added.
2. 100 µl of the plasma sample to be analysed was added to the appropriately positioned wells.
3. 100 µl of sample extract buffer was added in blank well.
4. The plate was washed four times with 10 X wash buffer manually.
5. The plate was dried by patting (well down) on blotting paper to remove excess buffer
6. 100 µl of freshly prepared 1X substrate was added to each well.
7. The plate was incubated at room temperature in dark for at least 15 minutes.
8. 100 µl of the stop solution was added after incubation.
9. The absorbance was measured at 450 nm using Diasorin ETI-LAB ELISA micro plate reader.

Interpretation:

1. The absorbance value ≤ 0.100 will be considered as "Negative" for the presence of Cry2Ab protein.
2. The absorbance value > 0.100 will be considered as "Positive" for the presence of Cry2Ab protein.

The processing and reading of the plasma samples was performed separately for the detection of Cry1Ac and Cry2Ab proteins. Positive control, Negative control and a blank sample well was maintained for each plate for validation and quality control check for ELISA.

7. NECROPSY

All the surviving animals at termination were fasted overnight (water allowed) and euthanised by exsanguination under thiopentone sodium (intravenous) anaesthesia and necropsied. The details of necropsy findings were recorded from all animals.

The following organs were collected from all animals and preserved in 10% buffered neutral formalin:

- | | |
|-------------|--|
| 1. Adrenals | 8. Spleen |
| 2. Kidneys | 9. Ovaries |
| 3. Testes | 10. Stomach (Rumen, Reticulum, Omasum, Abomasum) |
| 4. Liver | 11. Heart |
| 5. Thymus | 12. Small intestine (Duodenum, Jejunum, Ileum) |
| 6. Lungs | |
| 7. Colon | |

The following tissues were weighed. The organ weights and organ weights as percentage of body weights will be determined.

- | | |
|--------------------------------|------------|
| 1. Adrenals | 5. Brain |
| 2. Heart | 6. Kidneys |
| 3. Liver | 7. Spleen |
| 4. Gonads (testes and ovaries) | |

8. HISTOPATHOLOGY

Histopathological evaluation was performed on the preserved organs showing gross lesions. On gross pathology the testis of one animal (Gt 42) of G1 group exhibited small size testes (unilateral) and one animal (Gt 53) of G2 group showed kidney (unilateral) adhesion with capsule. These two organs were processed for histopathology. The tissues were processed for routine paraffin embedding and 5 micron sections were stained with Harris/Mayer's Haematoxylin Eosin. Unused tissues were archived.

STATISTICAL ANALYSES

Analysis of body weight, net body weight gain, feed and hay consumption, clinical laboratory results and organ weight data was statistically analysed. The data were compared by Bartlett's test for homogeneity of intra-group Variances. Where ever the variances proved to be heterogeneous, the data was transformed using appropriate transformation. The data with homogeneous intra group variances was subjected to one-way analysis of variance (ANOVA - Snedecor and Cochran, 1987). Following ANOVA, when 'F' was found to be significant, Dunnett's pairwise comparison (Scheffe 1953) of means of two types of cotton seed with Bollgard trait was compared, respectively, to its non Bollgard control mean. Males and females were analysed separately.

All analyses and comparisons are evaluated at 5% ($P \leq 0.05$) level. Throughout this report statistically significant differences ($P \leq 0.05$) indicated by the aforementioned tests are designated by the superscripts as stated below:

+/-: Significantly higher (+)/lower (-) than its concurrent control group

RESULTS AND DISCUSSION

GENERAL

The experimental design, test item feeding schedule and schedule of laboratory investigations are presented in Table 1.

IN-LIFE DATA

a. OPHTHALMOSCOPY, VETERINARY EXAMINATION, CLINICAL SIGNS AND PRE-TERMINAL DEATHS: Tables 2 & 3

Ophthalmoscopy: Ophthalmic examinations did not reveal any abnormality.

Veterinary examination: The veterinary examination did not reveal any visible abnormality at any period of the experimental schedule.

Clinical signs and pre-terminal deaths: There were no clinical signs or pre-terminal death in any of the goats.

Rectal temperature recorded daily for 15 days (day 1 to day 15 of treatment period) and weekly thereafter was normal.

Note: All comparisons are made between the two hybrids of Bollgard II cotton seed fed groups (MRC 7201 BG II cotton seeds[G1 group] and MRC 7301 BG II cotton seeds[G3 group]) and their respective concurrent control groups(MRC 7201 Non-BG II cotton seeds[G2 group] and MRC 7301 Non-BG II cotton seeds[G4 group]). i.e G1 group is compared with G2 group(concurrent control group for G1) and G3 group is compared with G4 group(concurrent control group for G3).

b. BODY WEIGHTS: Tables 4 & 6; App. 1 & 3; Figure 1A – 1D

There was no statistical significant difference in the body weights of two hybrids of Bollgard II cotton seed fed animals and their concurrent control groups.

c. CUMULATIVE WEEKLY NET BODY WEIGHT GAINS:

Tables 5 & 7; App. 2 & 4; Figure 2A – 2D

There was no statistical significant difference in the cumulative net body weight gains of two hybrids of Bollgard II cotton seed fed animals and their concurrent control groups.

d. FEED CONSUMPTION: Tables 8 & 9; App. 5 & 6; Figure 3A – 3D

The feed consumption of two hybrids of Bollgard II cotton seed fortified feed concentrate was not statistically different from their concurrent control groups (Non- Bollgard II cotton seed fortified feed concentrate).

e. HAY CONSUMPTION: Tables 10 & 11; App. 7 & 8; Figure 4A – 4D

The hay consumption in two hybrids of Bollgard II cotton seed fed groups was not statistically different from their concurrent control groups (Non- Bollgard II cotton seed fed groups). However, in G1 group males, the hay consumption was higher during week 3 and lower during week 10. In G1 group females, the hay consumption was lower during weeks 1, 2 3 and 7. In G3 group females, the hay consumption was higher during weeks 8 and 11. These changes were considered to be of no physiological significance.

f. FEED CONVERSION AND ENERGY EFFICIENCY PARAMETERS:

Tables 12 & 13; App. 9 & 10

There were no significant difference in the feed conversion efficiency and energy corrected feed conversion efficiency parameters between the two hybrids of Bollgard II cotton seeds in comparison to their respective concurrent control groups.

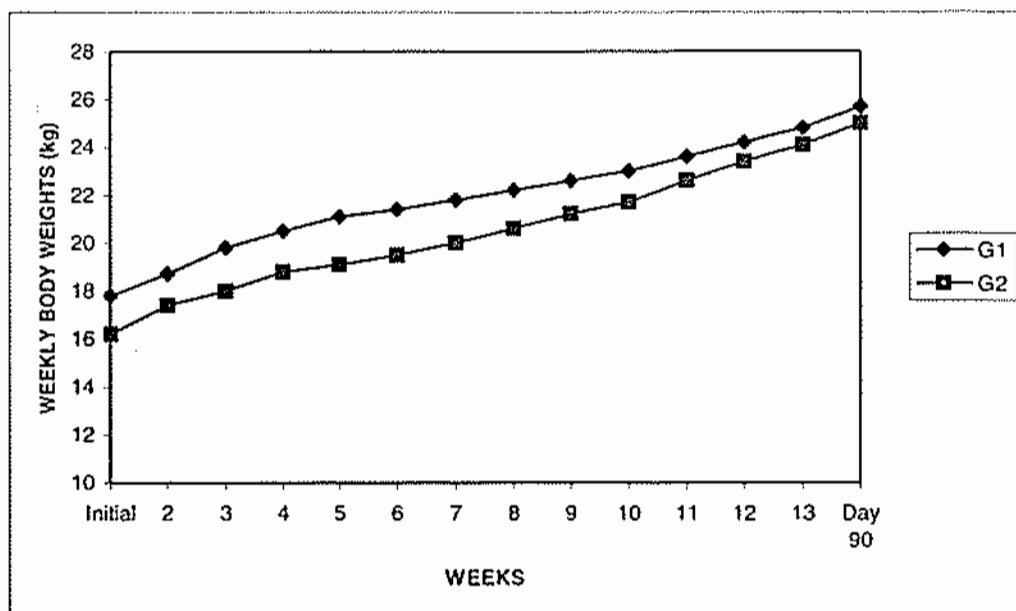


FIG 1A. Weekly Mean Body Weights of male goats (n=6) fed with feed concentrate fortified with MRC 7201 BG II Cotton seeds (G1) and MRC 7201 Non-BG II Cotton seeds (G2), for 90 days.

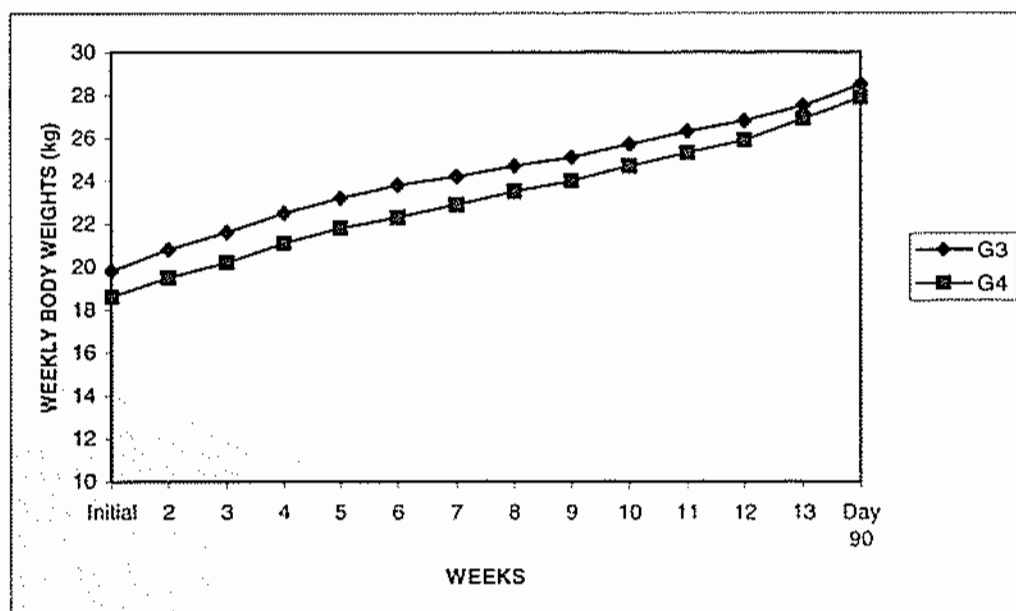


FIG 1B. Weekly Mean Body Weights of male goats (n=6) fed with feed concentrate fortified with MRC 7301 BG II Cotton seeds (G3) and MRC 7301 Non-BG II Cotton seeds (G4), for 90 days.

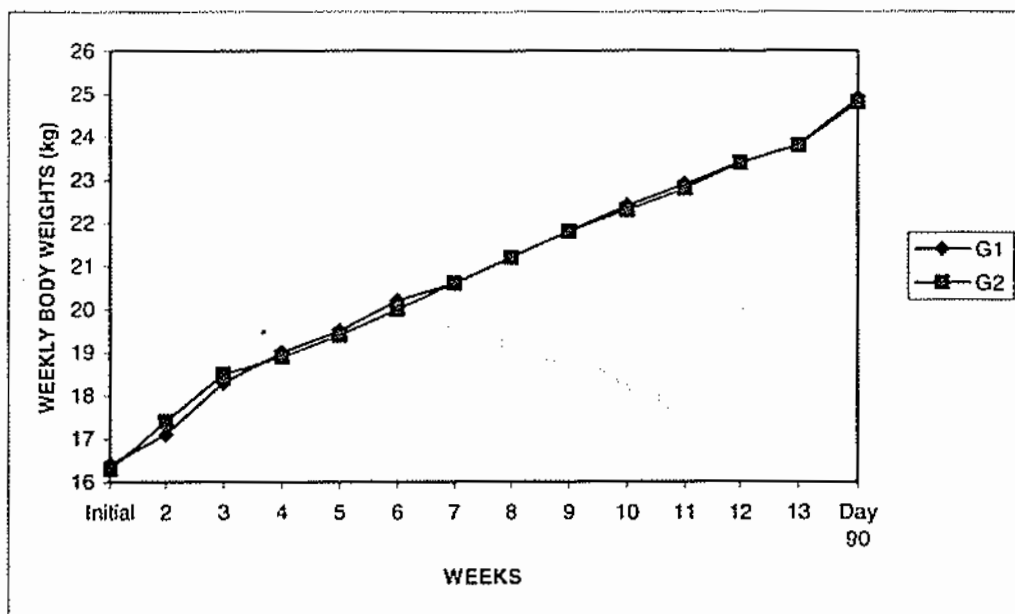


FIG 1C. Weekly Mean Body Weights of female goats (n=6) fed with feed concentrate fortified with MRC 7201 BG II Cotton seeds (G1) and MRC 7201 Non-BG II Cotton seeds (G2), for 90 days.

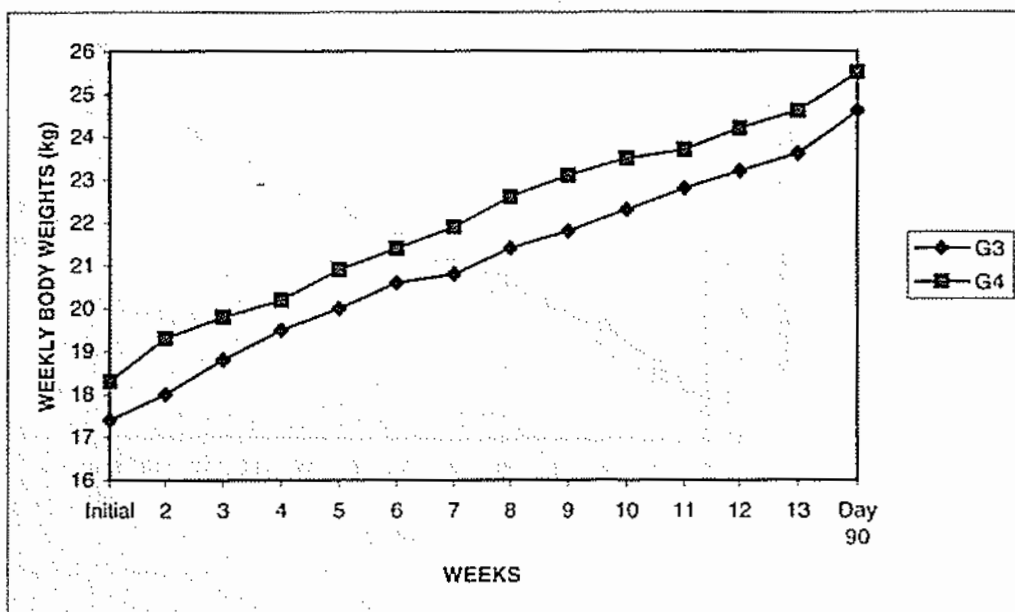


FIG 1D. Weekly Mean Body Weights of female goats (n=6) fed with feed concentrate fortified with MRC 7301 BG II Cotton seeds (G3) and MRC 7301 Non-BG II Cotton seeds (G4), for 90 days.

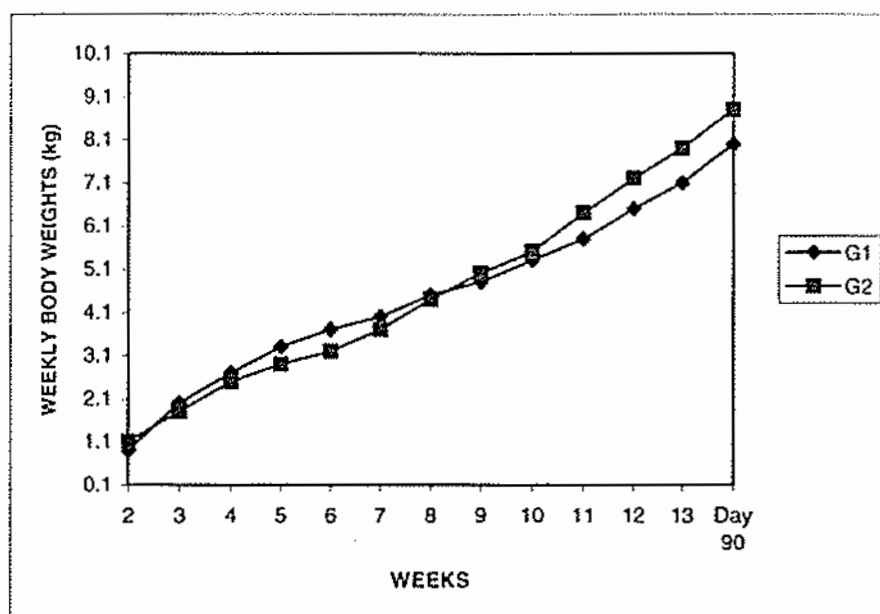


FIG 2A. Cumulative Weekly Mean Net Body Weight Gains of male goats (n=6) fed with feed concentrate fortified with MRC 7301 BG II Cotton seeds (G3) and MRC 7301 Non-BG II Cotton seeds (G4), for 90 days.

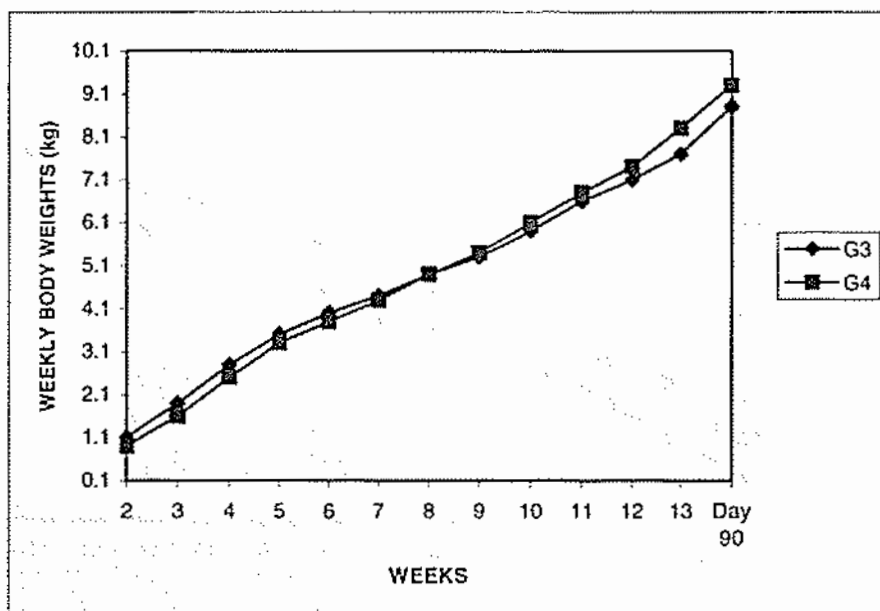


FIG 2B. Cumulative Weekly Mean Net Body Weight Gains of male goats (n=6) fed with feed concentrate fortified with MRC 7301 BG II Cotton seeds (G3) and MRC 7301 Non-BG II Cotton seeds (G4), for 90 days.

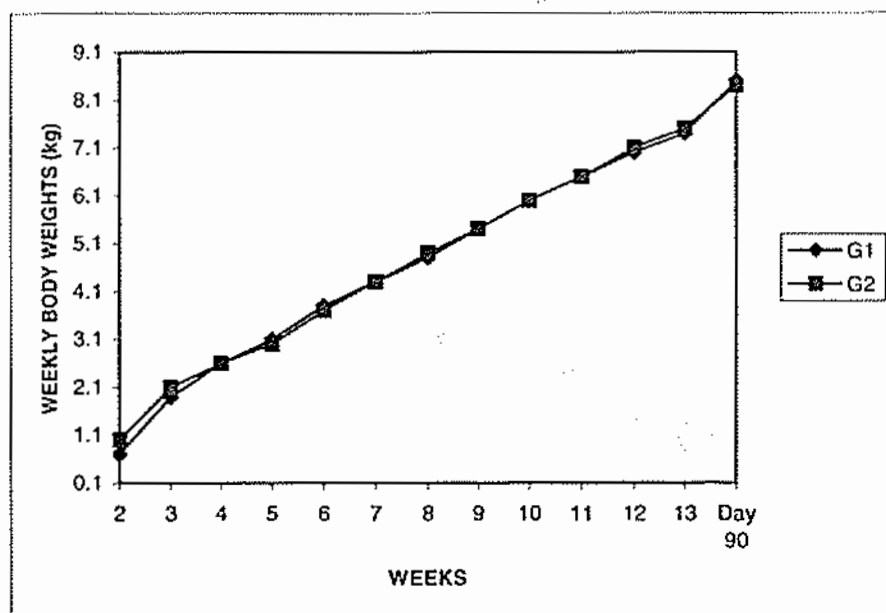


FIG 2C. Cumulative Weekly Mean Net Body Weight Gains of female goats (n=6) fed with feed concentrate fortified with MRC 7201 BG II Cotton seeds (G1) and MRC 7201 Non-BG II Cotton seeds (G2), for 90 days.

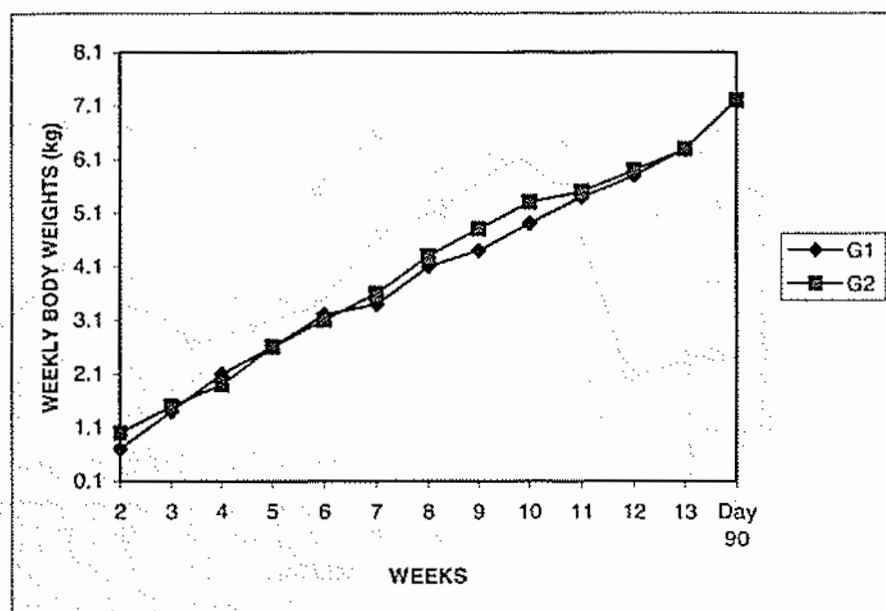


FIG 2D. Cumulative Weekly Mean Net Body Weight Gains of female goats (n=6) fed with feed concentrate fortified with MRC 7301 BG II Cotton seeds (G3) and MRC 7301 Non-BG II Cotton seeds (G4), for 90 days.

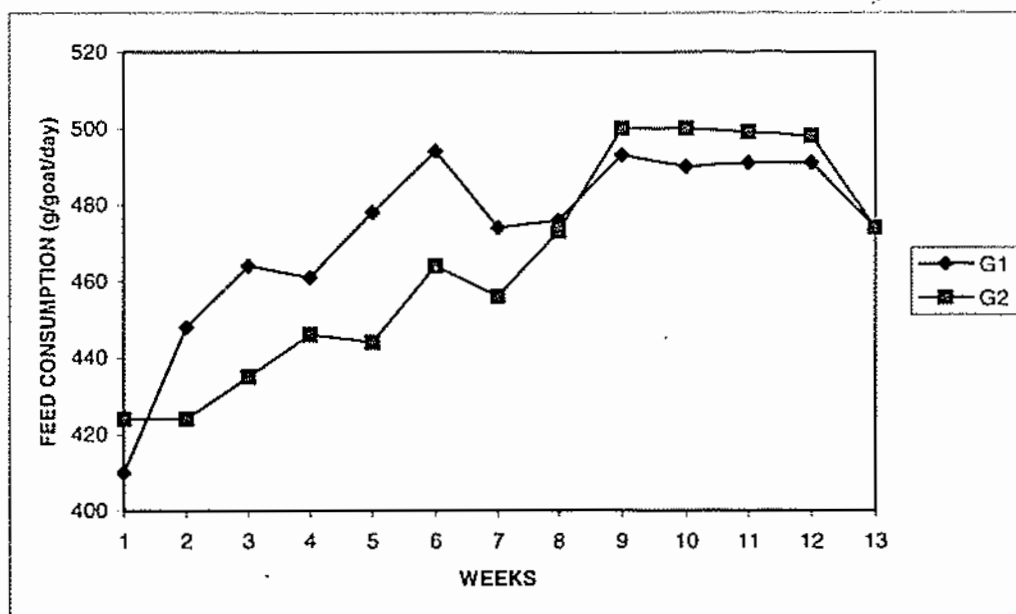


FIG 3A. Average Weekly Feed Consumption of male goats (n=6) fed with feed concentrate fortified with MRC 7201 BG II Cotton seeds (G1) and MRC 7201 Non-BG II Cotton seeds (G2), for 90 days.

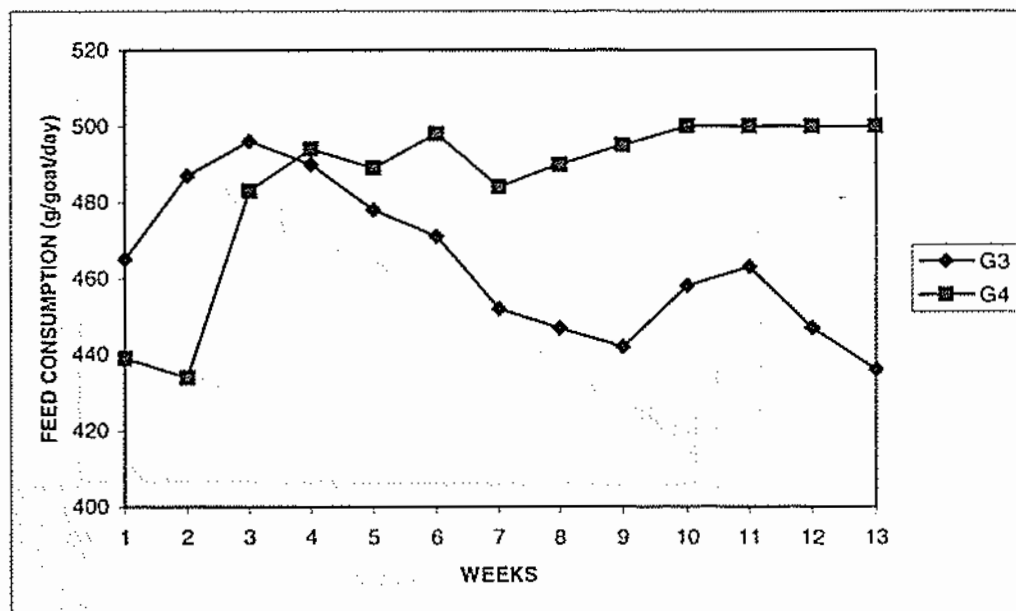


FIG 3B. Average Weekly Feed Consumption of male goats (n=6) fed with feed concentrate fortified with MRC 7301 BG II Cotton seeds (G3) and MRC 7301 Non-BG II Cotton seeds (G4), for 90 days.

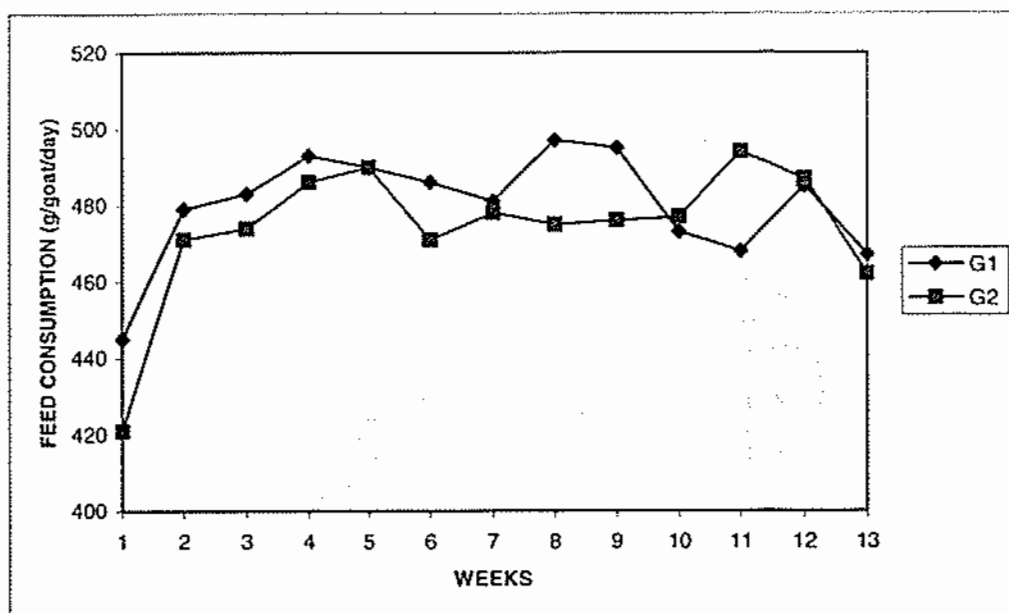


FIG 3C. Average Weekly Feed Consumption of female goats (n=6) fed with feed concentrate fortified with MRC 7201 BG II Cotton seeds (G1) and MRC 7201 Non-BG II Cotton seeds (G2), for 90 days.

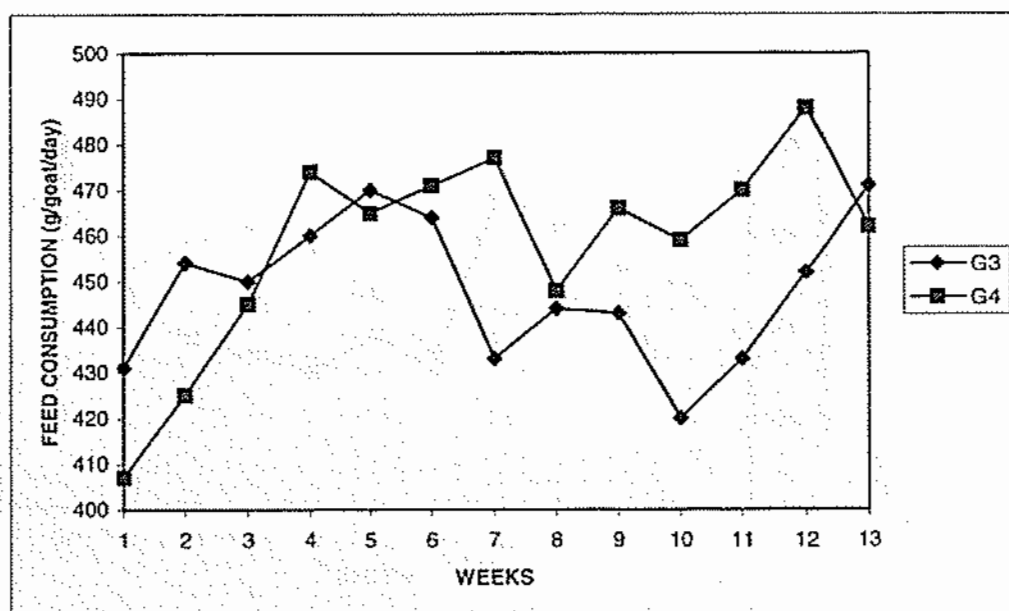


FIG 3D. Average Weekly Feed Consumption of female goats (n=6) fed with feed concentrate fortified for 90 day with MRC 7301 BG II Cotton seeds (G3) and MRC 7301 Non-BG II Cotton seeds (G4), for 90 days.

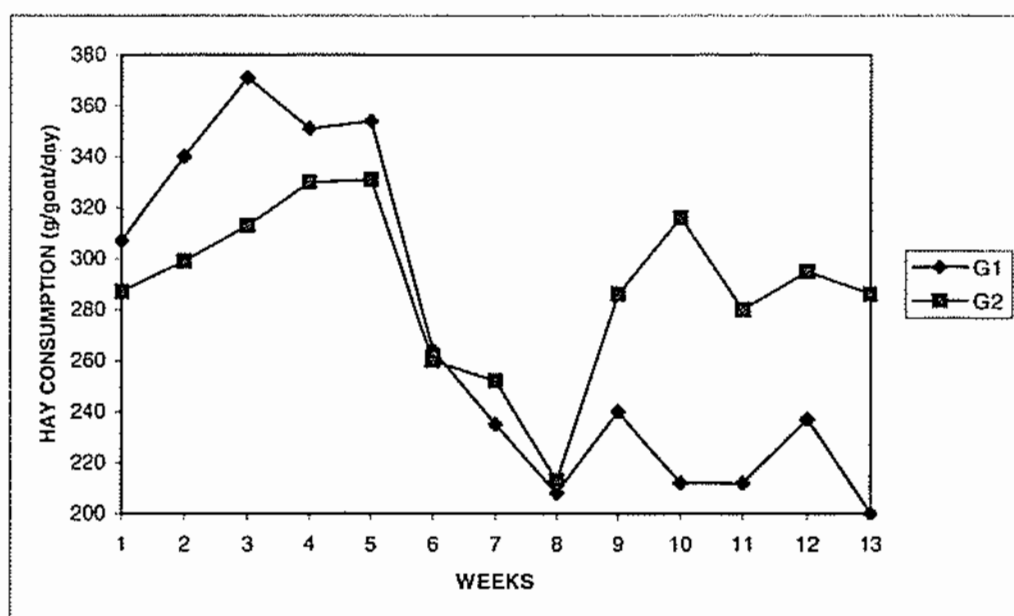


FIG 4A. Average Weekly Hay Consumption of male goats (n=6) fed with feed concentrate fortified with MRC 7201 BG II Cotton seeds (G1) and MRC 7201 Non-BG II Cotton seeds (G2), for 90 days.

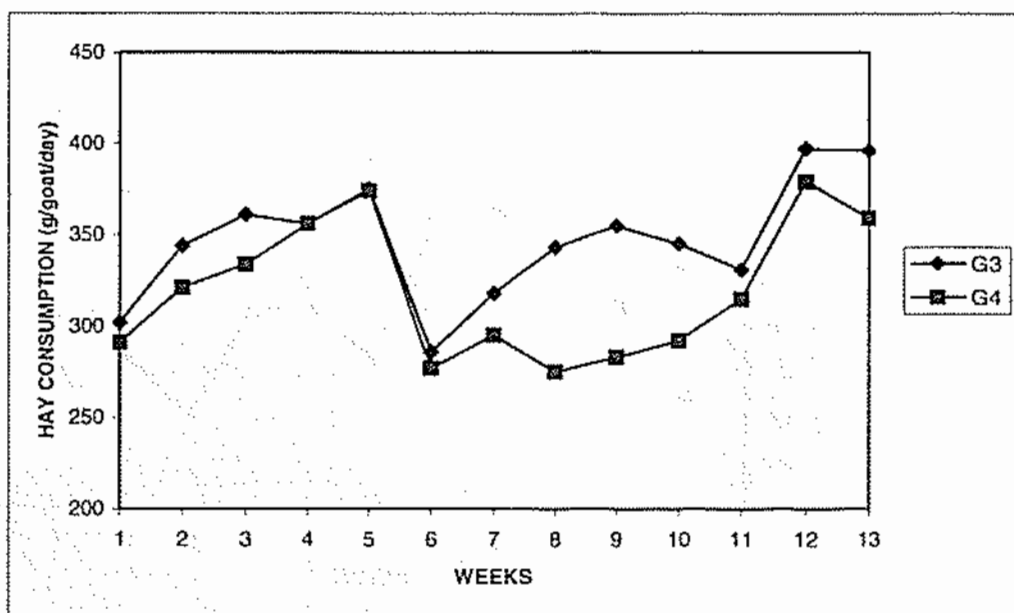


FIG 4B. Average Weekly Hay Consumption of male goats (n=6) fed with feed concentrate fortified with MRC 7301 BG II Cotton seeds (G3) and MRC 7301 Non-BG II Cotton seeds (G4), for 90 days.

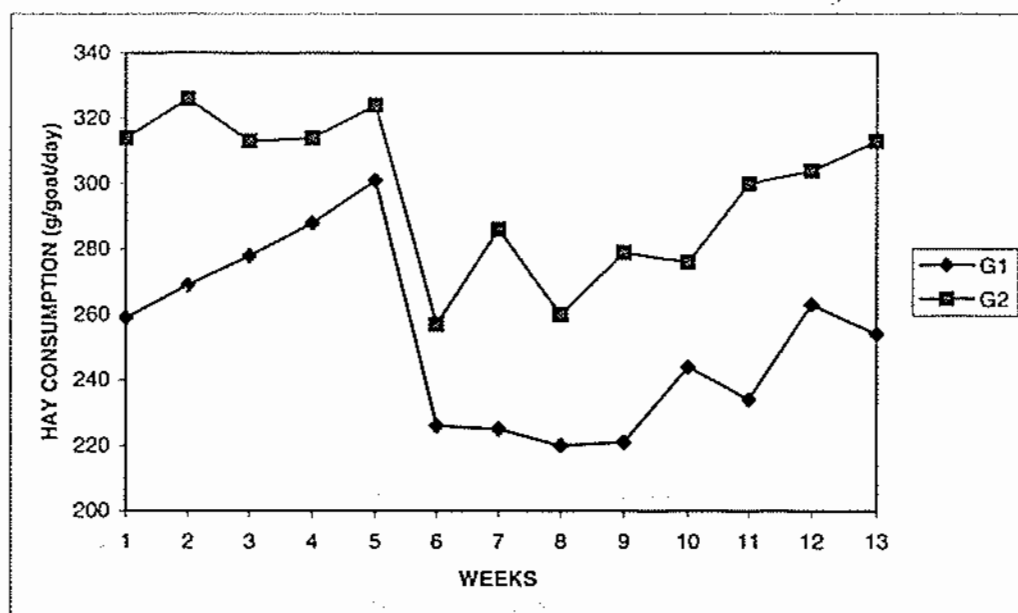


FIG 4C. Average Weekly Hay Consumption of female goats (n=6) fed with feed concentrate fortified with MRC 7201 BG II Cotton seeds (G1) and MRC 7201 Non-BG II Cotton seeds (G2), for 90 days.

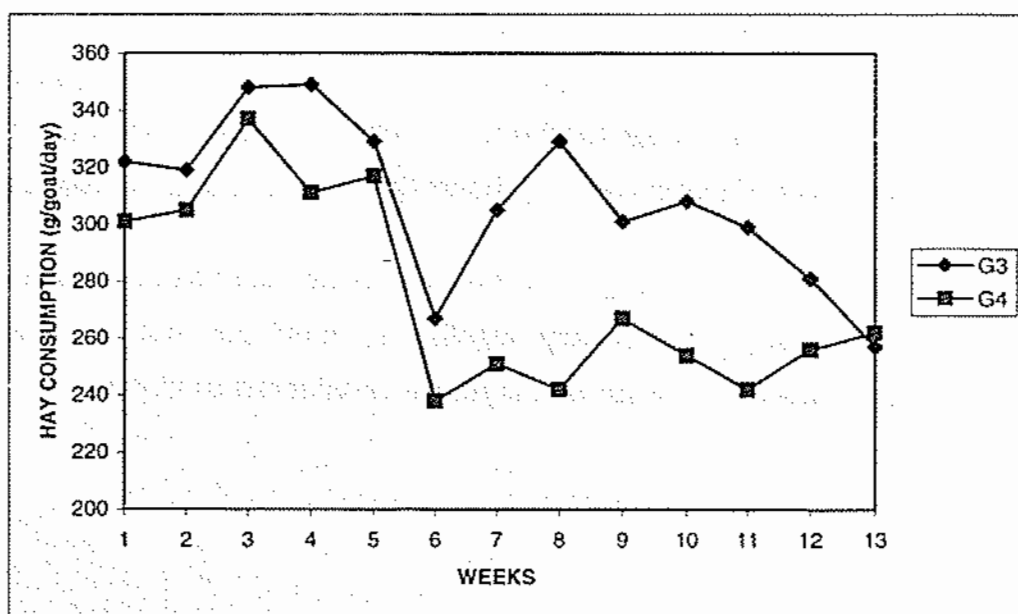


FIG 1D. Average Weekly Hay Consumption of female goats (n=6) fed with feed concentrate fortified with MRC 7301 BG II Cotton seeds (G3) and MRC 7301 Non-BG II Cotton seeds (G4), for 90 days.

g. LABORATORY INVESTIGATIONS:

i. Haematology: Tables 14 – 19; App. 11 - 16

All the parameters at pre-treatment were normal.

Males:

There were no significant difference in the haematological parameters between the two hybrids of Bollgard II cotton seeds and their respective concurrent control groups except for the lower values of erythrocyte count and Prothrombin time in G1 group and higher value of erythrocyte in G3 group on day 45 sampling. At the day 91 sampling, haematocrit value in G1 group and leucocyte count in G3 group were lower.

Females:

There were no significant difference in the haematological parameters between the two hybrids of Bollgard II cotton seeds and their respective concurrent control groups except for the lower value of haematocrit in G1 group on day 45 sampling and higher value of haematocrit in G3 group on day 91 sampling.

However these changes in the haematological values are marginal changes and although statistically significant, they are not considered to be of physiological significance and hence considered incidental.

ii. Clinical Chemistry: Tables 20 – 25; App. 17 - 22

All the parameters at pre-treatment were normal.

Males:

There were no significant difference in the clinical chemistry parameters between the two hybrids of Bollgard II cotton seeds and their respective concurrent control groups, except for changes in G3 group wherein blood urea nitrogen was lower (on day 45 sampling) and AST was higher (on day 91 sampling).

Females:

There were no significant difference in the clinical chemistry parameters between the two hybrids of Bollgard II cotton seeds and their respective concurrent control groups, except for marginally lower value of blood urea nitrogen in G1 group and marginally higher value of sodium in G3 on day 45 sampling. The potassium was marginally higher in G3 group on day 91 sampling.

However, these changes in the clinical chemistry values lower value of blood urea nitrogen, higher values of AST, sodium and potassium and are marginal changes and although statistically significant, they are not considered to be of physiological significance and hence considered incidental.

iii. Analyses of blood samples for the Cry 1 Ac and Cry2Ab protein: Tables 26 & 27

Plasma samples analysed using ELISA were negative for the presence of Cry 1 Ac and Cry2Ab protein.

h. ORGAN WEIGHTS: Tables 28 & 29; App. 23 and 24

MRC 7201 BG II Cotton seeds:

There was no difference in the terminal fasting body weight in both males and females. There was a significant increase in the absolute weight of the adrenals. In males, as compared to the concurrent control, which was considered incidental as there was no change in the relative weight (ratio to body weight).

MRC 7301 BG II Cotton seeds:

There was no difference in the terminal fasting body weight and absolute weights of organs in both males and females. There was a significant decrease in the relative weight of the liver in males as compared to the concurrent control. This was considered to be of no biological significance as the decrease was low (11%) and there were no gross changes.

i. GROSS AND HISTOPATHOLOGICAL FINDINGS:

Tables 30 & 31; App. 25 and 26

There were no treatment related gross findings. Only two gross lesions were seen, testes(unilateral)-small sized in a G1 male and kidney(unilateral)-adhesion with capsule in a G2 male.

Microscopic No.	group	No.	
G3	MRC 7301 BG II	Gt65	NAD
	Tissues not examined		
	Cotton seeds		
		Gt66	NAD
	Tissues not examined		
		Gt67	NAD
	Tissues not examined		
		Gt68	NAD
	Tissues not examined		
		Gt69	NAD
	Tissues not examined		
		Gt70	NAD
	Tissues not examined		

1: Minimal, 2: Mild, 3: Moderate, 4: Severe, a: Focal/Multifocal, d: Diffuse
contd.

TOXI-3585/02
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PAGE No. 115/136

contd. APPENDIX 25

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II
AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

INDIVIDUAL GROSS PATHOLOGICAL AND
HISTOPATHOLOGICAL FINDINGS-MALES

Group	Study	Goat	Gross
Microscopic No.	group	No.	
G4	MRC 7301 NON-BG II	Gt77	NAD
	Tissues not examined		
	Cotton seeds		

Tissues not examined,	Gt78	NAD
Tissues not examined	Gt79	NAD
Tissues not examined	Gt80	NAD
Tissues not examined	Gt81	NAD
Tissues not examined	Gt82	NAD

 1: Minimal, 2: Mild, 3: Moderate, 4: Severe, a: Focal/Multifocal, d: Diffuse

TOXI-3585/02
 088/4-CS TO 088/7-CS/90-OG
 PAGE No. 116/136

APPENDIX

26

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

INDIVIDUAL GROSS PATHOLOGICAL AND HISTOPATHOLOGICAL FINDINGS-FEMALES

Group	Study	Goat	Gross
No.	groups	No.	
G1	MRC 7201 BG II	Gt47	NAD
Tissues not examined			
Cotton seeds			
		Gt48	NAD
Tissues not examined			
		Gt49	NAD
Tissues not examined			

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II
AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

INDIVIDUAL GROSS PATHOLOGICAL AND
HISTOPATHOLOGICAL FINDINGS-MALES

Group Microscopic No.	Study group	Goat No.	Gross
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G1	MRC 7201 BG II	Gt41	NAD
Tissues not examined Cotton seeds			

		Gt42	TESTES(Unilateral):Small sized
TESTES: Atrophy-seminiferous tubules 4			-3.8 cm

		Gt43	NAD
Tissues not examined			

		Gt44	NAD
Tissues not examined			

		Gt45	NAD
Tissues not examined			

		Gt46	NAD
Tissues not examined			

1: Minimal, 2: Mild, 3: Moderate, 4: Severe, a: Focal/Multifocal, d:
Diffuse contd.

TOXI-3585/02
088/4-CS TO 088/7-CS/90-OG
PAGE No. 113/136

APPENDIX 25

contd.

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II
AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

INDIVIDUAL GROSS PATHOLOGICAL AND
HISTOPATHOLOGICAL FINDINGS-MALES

Group	Study	Goat	Gross
Microscopic			
No.	group	No.	
G2	MRC 7201 NON-BG II	Gt53	KIDNEY(Unilateral):Adhesion
	KIDNEYS: Inflammation chronic-capsule 1a		
	Cotton seeds		with capsule
	Basophilic tubules 1a		

Tissues not examined Gt54 NAD

Tissues not examined Gt55 NAD

Tissues not examined Gt56 NAD

Tissues not examined Gt57 NAD

Tissues not examined Gt58 NAD

1: Minimal, 2: Mild, 3: Moderate, 4: Severe, a: Focal/Multifocal, d: Diffuse contd.

TOXI-3585/02
088/4-CS TO 088/7-CS/90-OG
PAGE No. 114/136

APPENDIX 25

contd.

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II
AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

INDIVIDUAL GROSS PATHOLOGICAL AND
HISTOPATHOLOGICAL FINDINGS-MALES

Group	Study	Goat	Gross
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Tissues not examined Gt50 NAD

Tissues not examined Gt51 NAD

Tissues not examined Gt52 NAD

1: Minimal, 2: Mild, 3: Moderate, 4: Severe, a: Focal/Multifocal, d: Diffuse contd.

TOXI-3585/02
088/4-CS TO 088/7-CS/90-OG
PAGE No. 117/136

APPENDIX 26

contd.

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II
AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

INDIVIDUAL GROSS PATHOLOGICAL AND
HISTOPATHOLOGICAL FINDINGS-FEMALES

Group	Study	Goat	Gross
Microscopic			
No.	groups	No.	
G2	MRC 7201 NON-BG II	Gt59	NAD
Tissues not examined			
Cotton seeds			
		Gt60	NAD
Tissues not examined			
		Gt61	NAD
Tissues not examined			
		Gt62	NAD
Tissues not examined			
		Gt63	NAD

Tissues not examined

Tissues not examined

1: Minimal, 2: Mild, 3: Moderate, 4: Severe, a: Focal/Multifocal, d: Diffuse
contd.

TOXI-3585/02
088/4-CS TO 088/7-CS/90-OG
PAGE No. 118/136

contd.

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II
AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

INDIVIDUAL GROSS PATHOLOGICAL AND HISTOPATHOLOGICAL FINDINGS-FEMALES

Group	Study	Goat	Gross
No.	groups	No.	
G3	MRC 7301 BG II	Gt71	NAD
	Tissues not examined		
	Cotton seeds		
		Gt72	NAD
	Tissues not examined		
		Gt73	NAD
	Tissues not examined		
		Gt74	NAD
	Tissues not examined		
		Gt75	NAD
	Tissues not examined		
		Gt76	NAD
	Tissues not examined		

 1: Minimal, 2: Mild, 3: Moderate, 4: Severe, a: Focal/Multifocal, d:
 Diffuse contd.

TOXI-3585/02
 088/4-CS TO 088/7-CS/90-OG
 PAGE No. 119/136

APPENDIX 26

contd.

SUBCHRONIC (90 DAY) FEEDING STUDY WITH BOLLGARD II
 AND CONTROL NON-BOLLGARD II COTTONSEED IN GOATS

INDIVIDUAL GROSS PATHOLOGICAL AND
 HISTOPATHOLOGICAL FINDINGS-FEMALES

Group	Study	Goat	Gross
Microscopic			
No.	groups	No.	
G4	MRC 7301 NON-BG II	Gt83	NAD
Tissues not examined			
Cotton seeds			
		Gt84	NAD
Tissues not examined			
		Gt85	NAD
Tissues not examined			
		Gt86	NAD
Tissues not examined			
		Gt87	NAD
Tissues not examined			
		Gt88	NAD
Tissues not examined			

 1: Minimal, 2: Mild, 3: Moderate, 4: Severe, a: Focal/Multifocal, d:
 Diffuse

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040 1

Gossypol Check Anx3-6 Rev

Lab No.	Identification No.	Moisture (%)	Oil (%)	Protein (%)	Ash (%)	Carbohydrate (%)	K calories per 100 g	* Total Gossypol (%)
13425	MRC-7201 BG II	8.0	19.5	24.3	3.2	45.0	452.7	0.87
13426	MRC-7202 Non-Bt	7.8	19.8	26.2	3.6	42.6	453.4	0.91
13427	MRC-7301 BG II	7.4	20.5	23.6	3.4	45.1	459.3	0.76
13428	MRC-7301 Non-Bt	8.2	19.3	23.5	4.0	45.0	447.7	0.94
13429	NHH-44	7.9	20.2	22.6	3.8	45.5	454.2	0.99