FEEDING STUDIES IN LAYING HENS

WITH

Bt COTTONSEEDS

Report for:

METAHELIX LIFE SCIENCES PRIVATE LIMITED
PLOT NO.3, KIADB 4th PHASE, BOMMASANDRA,
BANGALORE-560 099, INDIA

Prepared by:

DEPARTMENT OF TOXICOLOGY
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PROJECT No.	**: TOX-346H**
STUDY	**: FEEDING STUDIES IN LAYING HENS**
COMPOUND	**: Bt COTTONSEEDS**
REPORT No.	**: 000046376**
Date	**: 14.05.2007**

QUALITY ASSURANCE STATEMENT

This is to certify that the work described in the study report entitled 'Feeding Studies in Laying Hens' with 'Bt Cottonseeds' has been conducted with

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SCIENTIFIC PERSONNEL INVOLVED IN THE STUDY

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(Scientist Pathology)

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STUDY DIRECTOR SCIENTIST PATHOLOGY HEAD, DEPT. OF TOXICOLOGY

Approved for issue

DEPUTY DIRECTOR
(MANAGEMENT)

INDEX

SUMMARY

In the assessment and evaluation of the toxic characteristics of a substance, determination of feeding studies is an important observation.
This study was hence, designed to conduct "Feeding studies in Laying Hens" provided by M/s Metahelix Life Sciences Private Limited, in laying hens.

Three groups consisting of 10 hens, individually, were designated for the study. The first group was kept as control and were fed on the standard layer feed only, the second group of laying hens was administered with the 'Non-Bt Cottonseeds (Sample-I)' in powdered form at the rate of 10% of the total diet along with the standard layer feed while the third group of laying hens was similarly administered with 'Bt Cottonseeds (Sample-II)' in powdered form at the rate of 10 % of the total diet along with the standard layer feed.

No toxic signs and symptoms / mortality was observed in any test group as well as the control group of animals.

Under the conditions of this study, the laying hens of 'Bt Cottonseeds (sample II)' group did not induce any treatment related observable toxic effects, when compared to the corresponding group of 'Non-Bt Cottonseeds (Sample-I)' laying hens and the control group of laying hens.

INTRODUCTION

Cotton is the main fibre crop that India produces. India ranks number one in the world for total area planted with cotton, but the country is ranked third in total cotton production. One of the major drawbacks for cotton production is the damage caused by the insect pests. The use of pesticide to control these pests is a very costly affair, moreover the pesticide often pose a threat to the environment.

As an effective and environmentally superior approach to provide tolerance against these insects and pests, Bt Cotton was produced by modifying the conventional cotton by insertion of a gene from a naturally occurring bacterium.

OBJECTIVE

To assess the effects of feeding Bt Cottonseeds on the production of the eggs, composition of the egg shell, locomotor and general behaviour of the laying hens as compared to feeding non-transgenic cotton seeds grown under identical conditions.

TEST SUBSTANCE

The sponsor is responsible for necessary characterization and evaluation of the test substance. The details of the test substance provided by the sponsor are as follows:

PRODUCT NAME	:	NON-Bt COTTONSEEDS (SAMPLE -I) & Bt COTTONSEEDS (SAMPLE-II)
SPONSOR	:	METAHELIX LIFE SCIENCES PRIVATE LIMITED
MATERIAL DESCRIPTION	:	YELLOWISH BROWN COLOURED POWDER
PACKED IN	:	BROWN COLOURED PAPER CARTONS
DATE OF COMMENCEMENT OF STUDY	:	19.03.2007
DATE OF COMPLETION	:	08.04.2007

Note: For characterization details of test samples, see Annexure-I provided by the sponsor.

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EXPERIMENTAL DESIGN

Total No. of birds used : 30 egg laying hens

Duration : 21 days

Acclimatization : 7 days

No. of birds / Group : 10 birds

Age of the birds : 8-14 months

Route of administration : Dietary

HUSBANDRY

All the birds of treatment and control groups were housed inside a controlled environment conditions. The laying hens were taken outside the cages atleast twice a week for exercise. Each laying hen held an identifiable number.

EXPERIMENTAL PROCEDURE

Initially all laying hens of Group II and III were fed a diet containing commercial 'Non-Bt Cottonseeds (Sample-I)' for one week, prior to the beginning of the study for acclimatization purposes while the Group I were fed on standard layer diet. After acclimatization Group II was administered with the 'Non-Bt Cottonseeds (Sample-I)' in powdered form at the rate of 10% of the total diet along with the standard layer feed whereas, the Group III was similarly administered with 'Bt Cottonseeds (Sample-II)' in powdered form at the rate of 10 % of the total diet along with the standard layer feed.

OBSERVATIONS

Laying hens of all the groups were observed once daily for all health related observations. The body weights were recorded weekly and the feed consumption was recorded daily. The birds were also observed for behavioural abnormality, locomotor ataxia and paralysis. The numbers of eggs were recorded daily and eggs were analyzed for their composition on day 0, day 14 and day 21.

Observation Period: 21 days

RESULTS

Clinical observations

Clinical observation of the hens revealed no remarkable changes in the health status of laying hens of any experimental group like behavioral abnormality, locomotor ataxia and paralysis. All the birds had normal gait and posture. There was no significant difference in the body weight of laying hens of any of the groups (Table 1-3).

The egg production of all the groups (Group I control, Group-II fed on Non-Bt Cottonseeds and Group-III fed on Bt Cottonseeds) was comparable to its control counterpart (Table 4-6).

The eggs were analyzed for their protein, fat, phosphorous, calcium and shell thickness and all the parameters were found in the normal range and were comparable in all the groups (Group-III fed on Bt Cotton seeds, Group-

II fed on Non-Bt Cotton seeds and Group-I control group) of the laying hens (Table 7-9).

Calculation and Evaluation of Data

The student 't' test was used for the statistical analysis of data to find out the level of significance i.e. 'P' value.

Conclusion

The feeding trial of transgenic and non- transgenic cottonseeds in the egg laying hens did not reveal any appreciable change.

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TABLE- 1: BODY WEIGHT IN GRAMS OF THE HENS OF GROUP I (CONTROL) AT WEEKLY INTERVALS

HEN No.	Day 0	Day 7	Day 14	Day 21
1	1243	1285	1306	1332
2	1217	1252	1273	1305
3	1166	1190	1212	1248
4	1204	1240	1263	1298
5	1210	1239	1270	1297
6	1140	1168	1191	1220
7	1210	1244	1267	1295
8	1130	1158	1181	1217
9	1160	1187	1222	1251
10	1221	1259	1284	1312
Mean	**1190.1**	**1222.2**	**1246.9**	**1277.5**
S.D.	**38.11**	**42.94**	**42.23**	**40.23**

TABLE - 2: BODY WEIGHT IN GRAMS OF THE HENS OF GROUP II (FED ON NON-Bt COTTONSEEDS) AT WEEKLY INTERVALS

HEN No.	Day 0	Day 7	Day 14	Day 21
1	1168	1193	1222	1251
2	1240	1280	1304	1332
3	1163	1199	1222	1255
4	1243	1278	1304	1336
5	1211	1235	1255	1280
6	1192	1236	1265	1291
7	1176	1218	1246	1270
8	1114	1138	1169	1197
9	1156	1185	1221	1250
10	1222	1246	1278	1304
Mean	**1188.5**	**1220.8**	**1248.6**	**1276.2**
S.D.	**40.96**	**43.76**	**41.94**	**42.12**

TABLE- 3: BODY WEIGHT IN GRAMS OF THE HENS OF GROUP III (FED ON Bt COTTONSEEDS) AT WEEKLY INTERVALS

HEN No.	Day 0	Day 7	Day 14	Day 21
1	1110	1135	1157	1183
2	1131	1167	1194	1223
3	1203	1213	1238	1249
4	1145	1186	1221	1248
5	1143	1169	1192	1220
6	1122	1134	1323	1350
7	1255	1278	1319	1352
8	1205	1245	1288	1318
9	1167	1189	1220	1249
10	1196	1238	1264	1293
Mean	**1167.7**	**1195.4**	**1241.6**	**1268.5**
S.D.	**23.0**	**47.68**	**5.58**	**57.33**

TABLE- 4: TOTAL EGG PRODUCTION OF THE HENS OF GROUP I (CONTROL) IN 21 DAYS

Hen No.	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
1	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
2	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1
3	0	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
4	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1
5	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0	1
6	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1
7	0	1	1	1	0	1	1	1	0	1	1	1	0	1	1	1	0	1	1	1	0	1
8	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
9	0	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
10	1	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1

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TABLE - 5: TOTAL EGG PRODUCTION OF THE HENS OF GROUP II (FED ON NON-Bt COTTONSEEDS) IN 21 DAYS

Hen No.	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
1	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1
2	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1
3	1	1	0	1	1	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1
4	0	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1
5	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
6	0	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1
7	1	0	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	0	1	0	1	1
8	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1
9	0	1	1	1	0	1	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1
10	1	1	0	1	1	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0	1

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TABLE- 6: TOTAL EGG PRODUCTION OF THE HENS OF GROUP III (FED ON Bt COTTONSEEDS) IN 21 DAYS

Hen No.	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21
1	1	0	1	1	1	0	1	1	1	1	0	1	1	1	1	0	1	1	0	1	0	1
2	1	1	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1
3	1	1	1	0	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1
4	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
5	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	0	1
6	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
7	1	1	1	1	0	1	1	1	1	0	1	1	0	1	1	1	1	1	1	0	1	1
8	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1
9	0	1	1	1	1	1	0	1	1	1	1	0	1	1	1	0	1	1	1	1	0	1
10	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1

TABLE- 7: MEAN COMPOSITION OF EGGS ON DAY 0

PARAMETERS	GROUP-I (Control)	GROUP-II (Non-Bt Cottonseeds)	GROUP-III (Bt Cottonseeds)
PROTEIN (% / MASS)	14.75	14.03	13.20
FAT (% / MASS)	5.80	5.77	6.60
PHOSPHOROUS (mg / 100 mg)	126.13	137.38	158.6
CALCIUM (mg / 100 mg)	39.98	49.30	39.50
SHELL THICKNESS (mm)	0.45	0.43	0.30

TABLE- 8: MEAN COMPOSITION OF EGGS ON DAY 14

PARAMETERS	GROUP-I (Control)	GROUP-II (Non Bt Cottonseeds)	GROUP-III (Bt Cottonseeds)
PROTEIN (% / MASS)	14.88	14.61	14.29
FAT (% / MASS)	6.67	6.61	6.97
PHOSPHOROUS (mg / 100 mg)	137.01	154.68	140.38
CALCIUM (mg / 100 mg)	52.76	49.13	48.60
SHELL THICKNESS (mm)	0.34	0.33	0.31

TABLE- 9: MEAN COMPOSITION OF EGGS ON DAY 21

PARAMETERS	GROUP-I (Control)	GROUP-II (Non Bt Cottonseeds)	GROUP-III (Bt Cottonseeds)
PROTEIN (% / MASS)	14.11	14.54	14.12
FAT (% / MASS)	6.67	8.22	6.02
PHOSPHOROUS (mg / 100 mg)	139.90	146.71	157.13
CALCIUM (mg / 100 mg)	53.05	61.22	52.59
SHELL THICKNESS (mm)	0.42	0.39	0.40

Table 10: FEED CONVERSION RATIO (FCR) OF DIFFERENT GROUPS AT THE END OF EXPERIMENTATION

S.No.	GROUP	FCR
1.	**Control**	**227.24**
2.	**Non-Bt Cottonseeds (Group I)**	**228.02**
3.	**Bt Cottonseeds (Group II)**	**262.08**

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TEST CERTIFICATE

000046376

Issued to :
METAHELIX LIFE SCIENCES PVT. LTD.
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Kind Attn: DR. M.J. VASUDEVA RAO , PRESIDENT

J.O.No. TOX-346H
Reg.No. 4612570
Date 15-05-2007
GC-01 (REV-04)
Your Ref.No. --
Date

Sample Particulars:
One sample of "Bt Cottonseeds" was received for Feeding study in laying hens.

TEST RESULTS

Material Description : Non-Bt Cottonseeds (Sample-I)- Yellowish brown coloured powder
Bt Cottonseeds (Sample-II)- Yellowish brown coloured powder.

Sponsor : Metahelix Life Sciences Private Limited
Plot no.3, KIADB 4th Phase, Bommasandra,
Bangalore-560 099, India.

Result

Feeding study in laying hens

Under the conditions of this study, the 21 days feeding studies on laying hens with 'Bt Cottonseeds (Sample-II)' and Non-Bt Cottonseeds (Sample-I)', as a feed supplement, did not induce any observable toxic effects in the egg laying hens as compared to its control counterpart.

(Annexure enclosed)
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DOR : 06-11-2006
DOC : 14-05-2007

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METAHELIX
Germinating Hope

PCR & ELISA CONFIRMATION OF BIOSAFETY COTTONSEED MATERIAL

Objective: Quality Control of the cottonseed material from cry1C-9124 based intrahirsutum hybrids to be used for the biosafety studies; despatched on 11th September 2006.

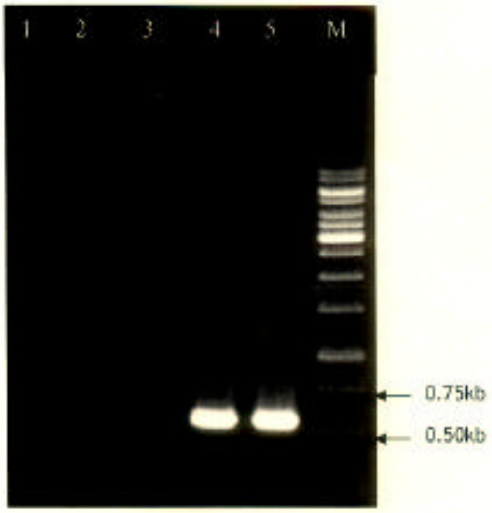
1. Confirmation of transgenic nature by PCR specific to the transgene
2. Confirmation of presence of Cry1C protein and its quantitation by ELISA

PCR confirmation

PCR was performed on Eppendorf Mastercycler Gradient machine with the following primers:

Upper:5'-CCT CGC CAT TCT TCG TGA TTC C
Lower:5'-GGT TGG CCT CCC TTC CGT AGA TA

1. H_2O CONTROL
2. -VE CONTROL (LEAF)
3. NON TRANSGENIC SEED DNA
4. TRANSGENIC SEED DNA
5. +VE CONTROL



EXPECTATION- 0.58 KB

Results and conclusion

As expected amplification from *cry1C* was observed in case of transgenic and positive control proving the presence of the gene. Water and negative controls were clear indicating the absence of gene.

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ELISA confirmation

Quantitative ELISA for Cry1C protein was performed using the Quantiplate kit for Cry1C (Envirologix, USA; Catalog No. AP 007) according to the manufacturer's protocol

Sl no	Entry ID	A_{450}	Cry1C concentration (µg/g on fresh wt)
1	Blank	0.09	NA
2	1 ppb standard	0.3	0.92
3	5 ppb standard	1.44	5.2
4	10 ppb standard	2.21	9.93
5	Nontransgenic	0.092	NA
6	Transgenic	2.9	13.08

Results

The absorbance value observed at 450nm for nontransgenic sample was nearly the same as blank and no colour development was observed in case of nontransgenic. Blue colour development was observed in case of transgenic samples indicating the presence of Cry1C protein.

Declaration

I hereby declare that the certificate of quality presented above is true to the best of my knowledge and is made on the basis of experiments carried out in our premises.

Vai. Ramanathan

Head - Genomics