Comparative Studies on the Chemical Composition of Cottonseed of BGII cotton hybrids (containing *cry1Ac & cry2Ab2 genes*) and their non-BGII counterparts

Report

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Material And Methods :

Cottonseed production :

Cotton hybrids comprising of seven BGII hybrids, MRC-6301 BGII, MRC-6322 BGII, MRC-6703 BGII, MRC-6201 BGII, MRC-6160 BGII, MRC-6326 BGII and MRC-6221 BGII and their respective controls (i.e. Non-BGII counterpart hybrids MRC-6301, MRC-6322, MRC-6703, MRC-6201, MRC-6160, MRC-66326, and MRC-6221) were chosen for the study. One popular transgenic cotton hybrid containing *cryIA(c)* gene, MECH-162 Bt and one popular commercial non-Bt hybrid NHH-44 were used for comparison. Cotton crop was raised in two field locations, Jalna in Maharashtra state and Shamshabad in Andhra Pradesh state. The crop was sown on 30 June 2002 and 31 July 2002 respectively in three field replications. After boll maturity, three pickings of kapas were done at both locations and at each picking, kapas from the three field replications were pooled. After completion of the third picking, the samples from three pickings were pooled for uniformity of sampling.

Cottonseed processing :

The pooled kapa was ginned and acid delinted. Ginned, delinted and dehusked seeds, called meat, were used for total gossypol and fatty acid analyses. The husk was manually removed and the endosperm (meat) was separated. The whole seed or meat was ground in a coffee grinder to obtain uniform flour particles and used for analysis.

Preparation of oil cake in laboratory :

Ginned, delinted and dehusked kernels were ground in a coffee grinder. A quantity of 15g of the ground flour was extracted with 25 ml of hexane in a 50 ml culture tube by shaking on a tube-rotator for 30 min. The hexane extract was collected in a beaker and the meal was re-extracted twice with 25 ml hexane and extracts were pooled. Hexane was evaporated to obtain unrefined cottonseed oil. The partially defatted meal thus obtained (oil cake) was ground in a mortar and pestle to uniform particle size and stored in a refrigerator. The oil cake had a mean oil content of 7.3% in Jalna location and 7.7% in Shamshabad location.

Proximate analysis :

Proximate analysis (protein, oil, ash, carbohydrate and moisture contents) of cottonseed (whole seed) was performed using approved methods. Oil content was determined by Soxhlet extraction method (AOAC 1990a). Protein content was estimated by determining the total nitrogen according to mocro-Kjeldahl method and the values were multiplied by the factor 6.25 to calculate the total protein (AOAC 1990b). Ash content was measured according to AOAC method (1990c). Moisture content was determined by loss on

Study title:

Comparative Studies On The Chemical Composition Of Cottonseed Of BGII cotton Hybrids (containing *cry1A(c) and cry2A(b) genes*) And Their non-BGII counterparts.

Introduction :

Cotton is an important commercial fiber crop of global importance. Its other commodity products are seed cotton, cottonseed oil, and cottonseed meal. Cottonseed is a rich source of oil for human consumption and processed cottonseed represents a potentially rich source of low cost protein. Cottonseed and cottonseed meal are also used animal feed. Cotton crop is frequently infested with insect pests such as American bollworm, spotted bollworm etc. Crop protection to control insect pests thru transgenic approach is effectively used around the world. Development of transgenic cotton that expresses insecticidal crystal protein *genes cry1A(c)* and *cry1A(b)* from *Bacillus thuringiensis (Bt)* var. *kurstaki* has resulted in lines with improved resistance to lepidopteran insect pests (Pearlak *et al.*, 1990). Transgenic crops such as cotton and corn expressing insecticidal proteins using genes from Bt are under large scale cultivation since 1996 in USA (Tabashnik *et al.*, 1997). It has been reported that these insecticidal proteins cause no deleterious effect on organisms such as beneficial insects, birds, fish and mammals including humans. Berberich *et al*, 1996 compared nutrients including protein, fat, carbohydrates, ash, moisture, fatty acids, amino acids, and anti-nutrients including gossypol, cyclopropionic fatty acids, aflatoxins of insect-protected cotton lines with their parent controls. This group concluded that the insect-protected cotton lines produced by the insertion of *cry1A(c)* gene is compositionally equivalent and are as nutritious as the seed produced by any other cotton varieties in the market. Our previous studies using four cotton hybrids containing *cry1A(c)* gene and their non-*Bt* counterparts also confirmed the above observations.

It is reported that crop species containing two Bt genes are more effective in the insect pest management and retards the development of resistance of insect pests towards the crop. Accordingly our scientists have developed cotton hybrids expressing two insecticidal crystal protein *genes cry1A(c)* and *cry 2A(b)* (BGII hybrids). The objective of this study is to compare the chemical composition, fatty acid profile, and total gossypol content of seven Bollgard II (BGII) cotton hybrids with their non-BGII counterparts.

Results and Discussion :

The plant variety produced through genetic manipulation is required to be safe and equivalent or superior in various constituents to its traditional counterpart. To elucidate this, a comparative study using seven cotton hybrids that were genetically engineered with *Cry1(c) and Cry2A(b) genes*- MRC-6301 BGII, MRC-6322 BGII, MRC-6703 BGII, MRC-6201 BGII, MRC-6160 BGII, MRC-6326 BGII, MRC-6221 BGII and their respective non-BGII counterpart were undertaken. In order to have comparison, one commercial Bt cotton hybrid containing one Bt gene MECH-162 Bt and a non-Bt commercial cotton hybrid NHH-44 were also used in this study.

There are three commodities in cotton— seed cotton, cottonseed oil and cottonseed meal, that have commercial and nutritional value in terms of their use for human food and animal feed. The composition of these commodities indirectly give their food and feed value. The primary use of seed cotton (non-delinted) is for cattle feed. The cottonseed meal is used almost exclusively as animal feed, primarily for cattle. Cottonseed is not a major food source for human consumption due to the presence of anti nutrients like gossypol etc. Refined cottonseed oil is used for human consumption. The chemical composition of cottonseed, fatty acid profile of cottonseed oil and total gossypol content in cottonseed meat as well as oil cake were reported.

Proximate composition of cottonseed :

The levels of major chemical constituents such as oil, protein, ash and carbohydrates in cottonseed were reported in Table 1 for both the locations. The calorie values were derived by calculation.

The oil content varied from 22.1 to 26.0% with a mean of 23.6% for the seven BGII cotton hybrids while it ranged from 22.4 to 23.9% with a mean of 23.1% for their corresponding non-BGII cotton hybrids at Jalna location. At Shamshabad location, it ranged from 21.5 to 25.0% with a mean value of 23.6% for BGII hybrids while it ranged from 22.1 to 25.2% with a mean of 23.1% for non-BGII hybrids. The oil content in the two local checks is within the above mentioned ranges. This data clearly suggests that there are no appreciable differences in oil content between BGII and non-BGII cotton hybrids at both locations.

The protein content varied from 25.4 to 27.3% with a mean value of 26.5% for the seven BGII cotton hybrids while it ranged from 21.9 to 25.0% with a mean value of 24.0% for their corresponding non-BGII cotton hybrids at Jalna location. At Shamshabad location

the protein content ranged from 24.3 to 27.2% with a mean of 26.1% for BGII hybrids while it ranged from 22.9 to 24.6% with a mean of 23.8% for non-BGII hybrids. The protein content in the two local checks is within the above mentioned ranges. This data

drying at 100° C to constant weight as described in AOAC method (1990d). Carbohydrate was estimated by difference using the fresh weight-derived data and the following equation (USDA 1975a):

% Carbohydrate = 100% - (% protein + % oil + % ash + % moisture)

Calories were calculated using the factors with the fresh weight-derived data and following equation (USDA 1975b):

Calories (kcal/100g) = (4 * % protein) + (9 * % oil) + (4 * carbohydrate)

Measurement of Total Gossypol :

Cotton meat or oil cake samples were extracted with acidified aminopropanol in dimethylformamide solution. Total gossypol content was determined using aniline reaction procedure (AOCS 1989). The gossypol levels were corrected to moisture free basis. The reference standard, gossypol obtained from Sigma Chemical Company, U.S.A. was used.

Fatty acid composition :

Cottonseed (meat) flour was extracted with petroleum ether (60-80° C) and the solvent was evaporated under nitrogen. Fatty acid methyl esters were prepared from the oil, thus obtained, according to the method of Metcalfe et al (1966). The oil (50 mg) was saponified with 1.3 ml of 0.5 N sodium hydroxide in methanol by heating in a boiling water bath for 5 min. The contents were cooled and interesterified with 14% boron trifluoride in methanol by heating in the boiling water bath for 5 min. The contents were cooled to room temperature and 2 ml of saturated sodium chloride solution was added. The solution was shaken by repeated inversions for 2 min. followed by addition of 2 ml of petroleum ether, and shaken on a tube rotator to extract fatty acid methyl esters into the petroleum ether extract. The contents were centrifuged and petroleum ether layer was transferred into a glass vial and flushed with nitrogen gas and stored in a refrigerator for further analysis.

Fatty acid methyl esters were analyzed in a Chemito 8510 Gas Chromatograph (GC) equipped with a flame ionization detector (FID), a temperature programmable oven and a Chemito 5000 data processor. The fatty acid methyl esters were separated on a glass column (6 feet long with 1/8th inch diameter) packed with 3% SP-2310 + 2% SP-2300. Nitrogen gas at 50 ml/min., was used as carrier gas. The temperature of injector port and detector port was maintained at 260° C. The column temperature was held at 190° C for 4 min. initially, followed by programming at an increase of 10° C/min. to a final temperature of 250° C and held at 250° C for 2 min. The individual peaks of the sample were identified by matching with the retention times of the peaks of the reference standards (Nucheck Inc., USA). Individual fatty acids were expressed as per cent of total fatty acids in the sample.

Total gossypol content :

Gossypol is a polyphenolic pigment present in cotton that can cause toxicity problems in food and feed products of cottonseed (Berardi and Goldblatt, 1980).

Gossypol has both desirable and undesirable effects. However, it is often noted as an anti-nutritional component. Total gossypol levels were determined in cottonseed meat and oil cake of BGII cotton hybrids and their respective controls and the data is shown in the Table 2. There were no appreciable differences in total gossypol content in cottonseed meat and oil cake between BGII and non-BGII cotton hybrids at both locations.

The gossypol content expressed as per cent in oil cake is higher to that of the per cent in cottonseed meat. This is expected because the content in oil cake is expressed after defatting the whole seed. Thus the difference in quantities are in order.

Summary And Conclusions :

Cotton crop is heavily infested with insect pests, resulting in reduced yield and poor quality produce. Crop protection using genetic engineering, particularly through the use of *Bacillus thuringiensis gene* technology is effectively used around the world. The oil from cottonseed is used for human consumption and the seed with linters is used as animal feed. There has been speculation about the safe use of genetically modified cotton, in terms of its chemical composition. Earlier reports from the literature indicated that cottonseed produced from such genetically modified (insect-protected) cotton cultivars are compositionally equivalent to that from parental variety as well as commercial cultivars.

We report here the results from the extensive studies made using seven BGII cotton hybrids containing *cry1A(c)* and *cry2A(b) genes* and their respective controls. These cotton hybrids were grown in two field locations, Jalna and Shamshabad. Various chemical constituents were determined in cottonseed samples. From the results of our analysis it is concluded that the composition of various constituents such as oil, protein, ash, carbohydrates, calories and fatty acid composition in cottonseed of the BGII cotton hybrids is nearly similar to that untreated controls in both the field locations. It was also observed that the levels of total gossypol which is an anti-nutritional component, in cottonseed meat and oil cake are also similar in BGII and non-BGII cotton hybrids. The results of the present study conform well with our earlier studies with four cotton hybrids containing the *cry1A(c)* gene. Our results clearly suggests that the cottonseed from cotton protected with two cry genes is nutritionally nearly similar to the untreated cotton hybrids and safe for its utilization.

suggests that there are appreciable differences in protein content between BGII and non-BGII cotton hybrids. BGII cotton hybrids had slightly higher protein content than non-BGII hybrids at both locations.

The ash content was small compared to other constituents which is around 3.8%. The contents were similar between BGII and non-BGII cotton hybrids at both locations.

The major constituent of cottonseed is carbohydrates. The content ranged from 44.8 to 47.7% with a mean value of 46.2% for the seven BGII cotton hybrids while it ranged from 47.5 to 51.1% with a mean of 49.0% for their corresponding non-BGII cotton hybrids at Jalna location. At shamshabad location it ranged from 44.0 to 49.7% with a mean value of 46.6% for BGII hybrids while it ranged from 46.7 to 50.3% with a mean of 49.2% for non-BGII hybrids. BGII cotton hybrids had slightly lower values than non-BGII hybrids at both locations. Since carbohydrate is a derived parameter and BGII cotton hybrids had slightly higher protein content, it is expected that BGII cotton hybrids would have slightly lower carbohydrate content than non-BGII cotton hybrids.

The values for most of the above constituents were similar to those reported for cottonseed in the literature (Berberich *et al.*, 1996). Published data are not available for carbohydrates and calories. But these are not used as important parameters for cottonseed. The calorie content did not show appreciable variation among the BGII cotton hybrids and controls in both the locations.

Fatty acid profile :

Fatty acid profiles were evaluated in the oils extracted from meat samples of the BGII and non-BGII cotton hybrids and also the local checks. It was observed that linoleic, palmitic, and oleic acids are the major fatty acids and account for nearly 96% of the total fatty acids. The fatty acid composition is shown in Table II.

The linoleic acid content varied from 54.1 to 55.8% with a mean value of 55.0% for the seven BGII cotton hybrids while it ranged from 53.8 to 56.5% with a mean of 54.8% for non-BGII cotton hybrids at Jalna location. At Shamshabad location it ranged from 53.2 to 56.9% with a mean of 54.5% for BGII hybrids while it varied from 52.4 to 57.7% with a mean of 54.9% for non-BGII hybrids. The linoleic acid content for the two local checks is within the above ranges. This data clearly suggests that there are no appreciable differences in linoleic acid content between BGII and non-BGII cotton hybrids.

Similar trends were observed for palmitic and oleic acids also and there were no appreciable differences between BGII and non-BGII cotton hybrids at both the locations in them.

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ANNEXURE 7.2.3

Effects of feeding cottonseed produced from cotton containing Bollgard®, Bollgard II or Roundup Ready® on feed intake, milk production and composition in lactating dairy cows in Argentina

INTA FINAL REPORT

Study Title: Effects of Feeding Cottonseed Produced From Cotton containing Bollgard®, Bollgard II or Roundup Ready® on Feed Intake, Milk Production and Composition in Lactating Dairy Cows in Argentina

Laboratory Project ID: Carta de Acuerdo entre la Compañía Monsanto y el Centro Regional Santa Fe del Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina

Monsanto Study No. 00-04-36-04

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Study number: 00 04 36 04

Experiment Number/s: one (1)

Title: Effects of Feeding Cottonseed Produced From Cotton containing Bollgard®, Bollgard II or Roundup Ready® on Feed Intake, Milk Production and Composition in Lactating Dairy Cows in Argentina

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Study Initiation Date: September/ 15th, 2000

Record Retention: All study specific raw data, protocols and final reports will be retained at Monsanto St. Louis MO.

Sample Retention: Feed and milk samples are going to be retained until at Experimental Station Rafaela INTA, Argentina until Sponsor directs otherwise.

Signatures of Final Report Approval:

Clinical Investigator — Date: 30 Sept. 2001

Sponsor Representative — Date: Sept. 24, 2001

Study Monitor — Date: 24 SEP 2001

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Attachments

ABBREVIATIONS

ADF	Acid detergent fiber
ADL	Acid detergent lignin
AMPA	Amino methyl phosphonic acid
Band+MF	tray + fresh material (DM determinations)
BCS	body condition score
BG	Bollgard
B.t.	*Bacillus thurigenesis*
BW	body weight
CP	crude protein
d	day
DM	dry matter
DMI	dry matter intake
EC or SC	body condition score (Spanish)
EE	ether extract
EEA	Agric.Experimental Station
g	grams
IA	artificial insemination (Spanish)
ID	identification
idem,	ditto
INTA	National Inst.Agric.Technology (Argentina)
Kg	kilogram
M	meters
M:A	sample "A" (chemical analysis)
M:B	sample "B" (chemical analysis)
MF	fresh material (Spanish, DM determinations)
MS	dry matter (Spanish)
NDF	neutral detergent fiber
NFC	nonfibrous carbohydrates
NFS	non-fat solids
per	period
ppm	parts per million
PS	dry weight (Spanish, DM determinations)
RP	cow identification number (Spanish)
RR	Roundup Ready
SD	standard deviations
SE	standard errors
THI	temperature humidity index
TMR	total mixed ration
T°C	temperature Celsius degrees
trat	treatment
V	varieties or treatment (chemical analysis)

Translations (frequently used words in this experiment)

ENGLISH TO	SPANISH	SPANISH TO	ENGLISH
1 alfalfa hay	heno de alfalfa	1 agua	water
2 AM & PM	M & T	2 algodón	cotton
3 ash	ceniza	3 alimento	feed
4 back	atrás	4 alta	high
5 bags	bolsas	5 año	year
6 body	cuerpo	6 atrás	back
7 body condition	condición corporal	7 bajo	low
8 body weight	peso vivo	8 bolsas	bags
9 box	corral	9 cargar	charge
10 burn	quemar	10 celo	heat
11 calving	parto	11 ceniza	ash
12 charge	cargar	12 con	with
13 corn silaje	silaje de maíz	13 condición corporal	body condition
14 cotton	algodón	14 consumo	intake
15 cottonseed	semilla de algodón	15 corral	box
16 cow	vaca	16 cuadrados	squares
17 crude protein	proteína bruta	17 cuerpo	body
18 daily	diaria	18 día	day
19 dairy cattle	ganado lechero	19 diaria	daily
20 dairy farm	tambo	20 dieta	diet
21 date	fecha	21 eliminación	disposal
22 day	día	22 ensayo, experimento	trial
23 diet	dieta	23 envío	shipment
24 disposal	eliminación	24 esperanza	hope
25 dry	seco	25 estrés por calor	heat stress
26 entry	ingreso	26 estudio	study
27 evening	tarde	27 fecha	date
28 fat	grasa	28 fibra	fibre
29 feed	alimento	29 firma	signature
30 feeding standard	tabla de alimentación	30 firmar	sign
31 fibre	fibra	31 ganado lechero	dairy cattle
32 fill	llenar	32 grasa	fat
33 fresh weight	peso fresco	33 harina de soja	soybean meal
34 health	salud	34 heno de alfalfa	alfalfa hay
35 heat	celo	35 hielo	ice
36 heat stress	estrés por calor	36 horario	timetable
37 high	alta	37 humedad	moisture
38 hope	esperanza	38 ingreso	entry
39 ice	hielo	39 inventario	inventory
40 insemination	servicio, inseminación	40 investigación	research
41 intake	consumo	41 leche	milk
42 inventory	inventario	42 llenar	fill
43 low	bajo	43 M & T	AM & PM
44 milk	leche	44 mañana	morning
45 milk (verb)	ordeñar	45 mes	month
46 milking routine	rutina de ordeñe	46 mezcla	mix

ENGLISH TO SPANISH SPANISH TO ENGLISH

47 mix	mezcla	47 mezclado	mixing
48 mixing	mezclado	48 muestra	sample
49 moisture	humedad	49 nombre	name
50 month	mes	50 número	number
51 morning	mañana	51 oferta	offer
52 name	nombre	52 ordeñar	milk (verb)
53 non-fat solids	solidos no grasos	53 papel	paper
54 number	número	54 parto	calving
55 offer	oferta	55 periodos	periods
56 paper	papel	56 peso	weight
57 periods	periodos	57 peso fresco	fresh weight
58 pregnancy	preñez	58 peso vivo	body weight
59 refusal	rechazo, remanente	59 pezón	teat
60 research	investigación	60 preñez	pregnancy
61 sample	muestra	61 proteína bruta	crude protein
62 seeds	semillas	62 quemar	burn
63 shipment	envío	63 rechazo, remanente	refusal
64 sign	firmar	64 rutina de ordeñe	milking routine
65 signature	firma	65 salud	health
66 soybean meal	harina de soja	66 seco	dry
67 squares	cuadrados	67 semana	week
68 study	estudio	68 semilla de algodón	cottonseed
69 teat	pezón	69 semillas	seeds
70 time	tiempo	70 servicio, inseminación	insemination
71 timetable	horario	71 silaje de maíz	corn silaje
72 treatment	tratamiento	72 sin	without
73 trial	ensayo, experimento	73 solidos no grasos	non-fat solids
74 water	agua	74 tabla de alimentación	feeding standard
75 week	semana	75 tambo	dairy farm
76 weight	peso	76 tarde	evening
77 with	con	77 tiempo	time
78 without	sin	78 tratamiento	treatment
79 year	año	79 vaca	cow

1.0 Summary

Cotton was modified by the introduction of a specific DNA sequence (Bollgard® BG, and Bollgard II BGII) to provide tolerance towards insect pests. Cotton has also been modified to impart tolerance to the herbicide Roundup® (Roundup Ready®; RR). Utilization of these crops offers producers an alternative strategy for managing weed pressure and insect pests. Insect tolerance was conferred to the plant by inclusion of a gene for Cry protein derived from *Bacillus thuringiensis* (B.t.). Herbicide tolerance is inferred to the plant by inclusion of the gene for CP4 EPSPS. Ginned cottonseed (linted) is utilized extensively in dairy cattle rations as an energy, fiber and protein source. This experiment evaluated the effect of feeding dairy cows with cottonseeds containing *cry1Ac* (Bollgard®), *cry1Ac* and *cry2Ab* (Bollgard II), *CP4 EPSPS* (Roundup Ready®) genes and control non-transgenic cottonseed on dry matter intake, milk yield and milk composition.

Twelve lactating multiparous Argentinean Holstein cows weighing about 570 kg (BW) were used in a 4x4 Latin square design, with 3 squares each containing 4 cows, four 4-week periods and four treatments. The cottonseeds treatments were from control non-genetically modified cotton (DP50), Bollgard cotton (DP50B) containing the Cry1Ac gene, Bollgard II cotton (DP50BII) containing both *cry1Ac* and *cry2Ab* genes and Roundup Ready cotton (DP50RR) containing the CP4 EPSPS gene. All cows received the same diet of corn silage, alfalfa hay, ground corn, soybean meal, minerals and vitamins. The individual cottonseed treatments (2.75 kg/cow/d) or about 10% of the total dry matter intake were hand mixed in with the diet.

There were no significant differences ($P<0.05$) in total dry matter intake (DMI) or cottonseed intakes, ranging from 23.4 to 23.9 and 2.25 to 2.29 kg DM/cow/d, respectively among treatment groups (Table 1). Milk yield, milk composition and body condition score (BCS) were comparable ($P>0.05$) among the treatments. Milk yield when averaged over all treatment groups was > 26.5 kg per cow per day containing a mean of 3.58, 3.14, 5.0, 8.9 and 0.02 % fat, protein, lactose, solids nonfat and N-urea, respectively. Body condition score averaged 2.3 for all cows over all treatment groups

A subsample of milk samples taken from cows fed Bollgard II or control DP50 cottonseed during the 4th week of each period were tested in duplicate by PCR followed by Southern blot analysis for the presence of a portion of the transgene *cry1Ac*. Milk

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samples from cows fed either Bollgard, Bollgard II, Roundup Ready or control cottonseed were also tested for the presence of an endogenous single copy of cotton gene *acp I*. Forty-eight milk samples were assayed for the *acp I* gene and twenty-four milk samples were assayed for *cry1Ac* gene. Neither gene was detectable in any of these test samples at an estimated limits of detection (LOD) of 5pg of genomic DNA, with the exception of one sample. Milk from one cow ID2442 fed control DP50 cottonseed tested positive for *acp I* but the result could not be reproduced. Forty-eight milk samples collected during the fourth week of the 28 day testing period from cows fed Bollgard, Bollgard II, Roundup Ready or nontransgenic control (DP50) cottonseed were analyzed by Western blot for Cry1Ac, Cry2Ab2 and CP4-EPSPS proteins. All samples tested negative for these proteins at LODs of 100 ppb (100 ng/g), 10 ppb (10 ng/g), and 10 ppb (10 ng/g) for Cry1Ac, Cry2Ab2 and CP4-EPSPS, respectively.

Table 1. Effect of different genetically modified cottonseeds on daily dry matter intake, milk yield, milk composition and body condition score of dairy cows (values reported as least square means).

Parameter	DP50	DP50B	DP50BII	DP50RR	SE**	P≤
DMI, kg/d*	23.4	23.8	23.9	23.7	1.12	0.648
Cottonseed, kg/d	2.25	2.29	2.28	2.27	0.086	0.854
Milk yield, kg/d	26.9	26.7	27.6	27.4	2.88	0.686
Milk composition						
Fat, %	3.59	3.60	3.52	3.59	0.186	0.777
Protein, %	3.16	3.14	3.14	3.14	0.072	0.947
Lactose, %	4.97	5.01	5.05	5.00	0.064	0.277
Non-fat solids, %	8.84	8.92	8.96	8.90	0.096	0.394
N-Urea mg/100ml	18.77	19.49	20.66	20.01	1.958	0.267
Body Condition Score (1 - 5 point scale)	2.30	2.30	2.30	2.34	0.076	0.515

* kg/d: kilogram per day
** Standard error
Where DP50, DP50B, DP50BII and DP50RR represent the control, Bollgard, Bollgard II and Roundup Ready cottonseeds, respectively.

Results of this study indicate that genetically modified cottonseed varieties (Bollgard, Bollgard II and Roundup Ready) supported similar performance as control (non-transgenic) cottonseed and did not affect dry matter intake, milk yield, milk composition and body condition score of dairy cows under controlled feeding conditions.

2.0 Introduction

2.1 Background

Cotton was modified (Bollgard; BG and Bollgard II; BGII) to provide tolerance towards insect pests. Cotton has also been modified to impart tolerance to the herbicide Roundup (Roundup Ready; RR). Utilization of these crops offers producers an alternative strategy for managing weed pressure and insect pests. Insect tolerance was conferred to the plant by inclusion of a gene for Cry protein derived from *Bacillus thuringiensis* (B.t.). Herbicide tolerance was conferred to the plant by incorporation of the gene for CP4 EPSPS. Ginned cottonseed (linted) is utilized extensively in dairy cattle rations as an energy, fiber and protein source. This study was conducted to compare lactating dairy cow performance when fed cottonseed from modified cotton: BG containing Cry1Ac protein, BGII containing Cry1Ac and Cry2Ab proteins), and RR (containing the CP4 EPSPS protein) with cottonseed from a conventional non transgenic variety.

2.2 Purpose

The primary purpose of this experiment was to assess the effects of feeding ginned Bollgard, Bollgard II and Roundup Ready fuzzy cottonseed on feed intake, milk production and milk composition in lactating dairy cattle compared with cottonseed from the parental non transgenic line grown under identical conditions and harvested at the same physiological maturity.

3. Materials and Methods

3.1. Test Substances. The test substances were cottonseed (DP50BII) from genetically modified Bollgard II cotton containing both Cry1Ac and Cry 2Ab (also referred to as Cry X) proteins, Bollgard cottonseed (DP50B) from cotton containing Cry1Ac protein and Roundup Ready cottonseed (DP50RR) from cotton containing CP4 EPSPS protein. The test substances were generated under production plan 99-04-36-01 (Attachment 15).

A sample (~400 g) of each cottonseed product was sent to Monsanto in a labeled (study number, date collected, treatment letter and color, initials of the person performing the sampling) plastic bag on dry ice for glyphosate residue analysis.

3.2. Control Substance. The control substance was non transgenic parental line DP50 generated under production plan 99-04-36-01 (Attachment 15). Test and control substances were generated under the same conditions.

A sample (~400 g) of each cottonseed product was sent to Monsanto in a labeled (study number, date collected, treatment letter and color, initials of the person performing the sampling) plastic bag on dry ice for glyphosate residue analysis.

3.3. Test and Control Substance Storage. During the experiment, test and control cottonseed were stored at ambient temperature in labeled paper bags on dry concrete floor in a completely enclosed shed.

3.4. Test and Control Substance Administration. Cottonseed from test or control lines was hand blended at feeding with total mixed rations in individual feeders and fed to each dairy cow.

3.5. Test and Control Substance Preparation and Accountability. Test and control substance accountability were maintained throughout the study. The amount of each substance harvested, ginned, used and in inventory was recorded. Samples of each substance were retained by the Sponsor. Excess test and control product were disposed of according to the Sponsor's directions.

Prior to the start of study, test and control substances were sampled (~800 g) and sent (ambient temperature) to Monsanto in a labeled plastic bag with the study number, date collected, treatment letter "E","F","G", or "H" (as follow, E = DP50; F = DP50B; G = DP50BII and H = DP50RR) and color of bag, and the initials of the person doing the sampling.

Cottonseed samples (~200 g) were characterized at Monsanto using event specific PCR to confirm the identity of the test substances and the control substance. Also, representative samples of each cottonseed product (~200 g) were sent by the Monitor to Romer Labs for mycotoxin analysis, to Covance Labs for gossypol analysis and sent to Dairy One, DHI Forage Analysis Laboratory for nutrient analysis.

4.0 Test System

4.1. Site. This study was conducted at Instituto Nacional de Tecnología Agropecuaria's (INTA) research farm located at Rafaela, Santa Fe, Argentina. The record-keeping system included individual milk weights at each milking (2 times per day), feed intake and refusals and all medications, therapies and other management information for each cow. Also, recording of weather information was gathered .

4.2. Animals. This study included 12 lactating multiparous dairy cattle (approximately 42 to 64 days in milk at the beginning of the trial). Animals selected from INTA's research herd were randomly assigned to specific treatments 7 days before initiation of the first treatment feeding.

4.3. Identification. All cows were identified by three different means (a tattoo and two ear tags).

4.4. Safety. Safety procedures were followed according to practices currently used at the site. Appropriate security measures were in place to prevent tampering, destruction or theft of the test and control substances.

4.5. Animal Care and Facilities. Established site practices were followed for management, health care, reproduction, milking and feeding. Diets were formulated to meet or exceed the CNCPS Model (Cornell University) and 1989 U.S. National Research Council nutrient requirements for dairy cattle. Animals were maintained in individuals pens (1.4 x 4.0 m each) with rubber floor. The facilities were totally open with a half shadow (85%) roof. Feed and water were provided *ad libitum* throughout the trial so that milk yield was not limited. The study file documentation described the following practices:

a) Feeding program, including ration specifications and ingredients, feed and ingredient sampling (Attachment 1a, 1b, 1c). Feed was offered such that a minimum of a 10% refusal was recorded and refusals were disposed of in a secure location and burned.

b) Milking program, animal cooling during hot days and daily cow management (Attachment 2)

c) Mastitis identification and treatment (Attachment 3). In addition, validation of the following systems was included in the study file:

1. Milk meter accuracy records and electronic capture of milk.
2. Accuracy of scales for measuring feed offered and refused, mixing, and weighing of cattle.
3. Facilities construction and description.

5.0 Experimental Design and Conduct

5.1. Design. This was a 4 x 4 Latin Square design with 4 lactating multiparous Holstein Friesian cows and four treatments per square. Each square was replicated three times so a total of 12 animals were used. Cows were randomly assigned into three groups. Each block of four cows was assigned to a square. Cows in each square/block were randomly assigned to treatments for the first period. Thereafter, cows in each square were assigned to the treatments for periods 2 through 4 in the sequence described in Table 2. Each of the four periods were 28 days in duration. The first 21 days of each period were used for adaptation to treatment, the next seven days were used to determine treatment effects on feed intake, milk yield, and milk composition.

Table 2
4 X 4 Latin Square Design

Square 1 ---------- Diet ----------

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
2670	E	F	H	G
2442	F	G	E	H
2671	G	H	F	E
2584	H	E	G	F

Square 2 ---------- Diet ----------

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
2574	E	G	F	H
2591	F	E	H	G
2663	G	H	E	F
2586	H	F	G	E

Square 3 ---------- Diet ----------

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
2579	E	F	G	H
2401	F	H	E	G
2416	G	E	H	F
2555	H	G	F	E

Where: Letters designate individual treatment codes, as follow: E = DP50; F = DP50B; G = DP50BII and H = DP50RR.

5.2. Treatments. Corn grain, protein supplement, alfalfa hay, corn silage, minerals and vitamins were blended in a mixer truck into a total mixed ration (TMR). The TMR was weighed into the feeders and the respective cottonseed treatment was added (1.375 kg as is basis in the morning and 1.375 as is basis in the evening) and hand blended with the TMR such that a total of 2.75 kg of cottonseed was offered per cow per day. Total mixed ration was fed for about a 10% refusal from week 1 through 3 and for about 5% refusal during week 4, based on a mean consumption during week 3.

5.3. Treatment Assignment. Animals were randomly assigned to study 7 days before the beginning of the treatment period.

5.4. Study Duration. The study included four 28-day periods for a total of 112 days. For Square 1 in period 4, the study included three 28-day periods (1 through 3) and one 35 day period for a total of 119 days. During this additional 5th week, the same measurements were collected as in the 4th week.

5.5. Animal and Milk Disposition. Animals remained on study until the end of the fourth period. The removals of animals either prematurely or at the end of the study were recorded on an Animal Release form. No milk from cows in this study entered the food chain until 30 days after the completion of the study. The location and means of milk disposal were documented and the data archived in the site study file.

6.0 Observations, Examinations and Tests

6.1. Daily Observations. Cows were observed daily by designated individuals. All health-related observations and/or medicines administered were recorded. The original data is archived at Monsanto and copies retained by the investigator . Electronic records were used when data collection was validated and documentation filed. All electronic data were printed out, signed and dated by the individual responsible for collection of those data.

6.2. Feed Intake. Quantity of feed offered and refused were recorded daily throughout the treatment period (Attachment 5).

6.3. Diets. Diets were formulated by the Investigator to meet or exceed the CNCPS Model (Cornell University) and 1989 U.S. National Research Council nutrient requirements for dairy cattle (Attachment 1a). The same proportion of corn grain, soybean meal, alfalfa hay and corn silage were used in the control and transgenic diets, and the same amount of cottonseed was offered to each cow.

6.4. Feed Composition. Prior to the start of study, all ingredients were sampled and analyzed for moisture, acid detergent fiber, neutral detergent fiber, crude protein, fat, calcium, phosphorus, magnesium, potassium, sodium and ash (Forage Analysis Laboratory, EEA Rafaela INTA, Argentina and Faculty of Chemistry, Santa Fe, Argentina). Total mixed rations were sampled on an experimental period basis. Samples of the commingled feed were analyzed for moisture, acid detergent fiber, neutral detergent fiber, crude protein, fat, calcium, phosphorus, magnesium, potassium, sodium and ash (same laboratories as above).

Corn silage samples were collected weekly for dry matter determination by the study site and rations adjusted to accommodate any variation in feeds (Attachment 4). This information was also compared to the dry matter content of mixed diets, which were obtained weekly to evaluate mixing times. In addition, samples of the dietary ingredients were taken from each lot and/or batch before they were incorporated into the ration. These samples were retained until the analytical results from the corresponding TMRs had been evaluated. If the nutrient values from the TMRs did not match what was expected from the formulations, the individual ingredient samples were analyzed for their nutrient content to determine if an ingredient's nutrient composition had significantly changed from what was used in the formulation. If so, the nutrient composition for the ingredient was updated and the TMR reformulated for the future production of TMR.

6.5. Body Weight and Body Condition Score. Body condition was recorded one week before and every two weeks after calving and on day two during the first period. On Day 28 of each period, body weights were measured and body condition scored using the 5 point system (quarter point basis) developed by Cornell University. The same two people did body condition scoring independently over the course of the study.

6.6. Milk Yield and Composition. Individual milk yields were recorded after each milking. Consecutive evening and morning milk samples were collected (Attachment 2a) daily during the last 7 days of each period and were analyzed for fat, protein (N*6.38), SNF (solids nonfat), MUN (milk urea nitrogen) and lactose. Milk analysis was carried out with a Milkoscan Model 4000, Foss Electric, DK DK-3400, Hillorod, Denmark (Sancor Cul Milk Cooperative, Sunchales, Argentina). Thirty milliliter samples of milk were taken aseptically from each cow at each milking during the seven day collection period and stored in labeled containers (cow ID, date, study number, AM or PM milking or their Spanish equivalent M and T respectively). Samples were stored at -5 to -20 degrees °C for future analysis as requested by the Sponsor. A subsample of the milk samples taken from the fourth week of each period were sent to Monsanto to determine the presence of transgenic DNA and protein.

6.7. Reproduction. Individual animal reproduction data were collected. The original data was sent to and archived by Monsanto. The information included breeding and medicines or reproductive aids used and results of reproduction examinations. The pregnancy status of each animal was documented within two weeks of when animals were removed from the study. These data were used to address unexpected variances in milk production.

6.8. Adverse Experiences. Provisions were made to record all adverse experiences or reactions to test or control substances but none had occurred.

6.9. Data Handling. For square 1 Period 4, the fifth week of data collection was added due to a mistake that occurred. The mistake involved putting cows on the wrong treatments for the first week. This was corrected so all cows were placed on the correct treatments for week 2. Thus, the cows had a two week adaptation period rather than the intended three week adaptation, as result, the fifth week was added. The results during this extra-week were affected by the high environmental temperatures and Thermal Humidity Index (THI). For this reason and the fact they the cows had adapted to the diets during the two weeks, the fourth week results were used and the fifth week's data were rejected. Monsanto Study Monitor and Co-Monitors reviewed the raw data periodically and were responsible for transfer of the originals or exact copies to Monsanto's Biotechnology Regulatory Science Archive. All raw data were transferred to Monsanto (via Study Monitor) within 2 months after the final report was completed. The data will be stored in Monsanto's Biotechnology Regulatory Science Archive in Chesterfield for a minimum of two years after study completion. An exact copy of the data will remain at the test facility (accessible to the Investigator) for five years after study completion.

7.0 Data Analysis

Data were analyzed using the MIXED procedure in SAS (1996). The statistical model accounted for variation due to cow, square and period along with comparisons among the treatments. Treatment means were reported as least squares means with the associated standard error. Observations that are at least two standard deviations outside the treatment means were investigated. Observations deemed to be outliers were eliminated from the analysis. Also, all data from the 7 days of data collection in each period were visually evaluated between cows and days (see Attachments 6 and 7) to assess possible errors.

8.0 Results and Discussion

8.1 Chemical Composition of Feeds

Chemical composition of the individual ingredients used in this trial are shown in Attachment 8. The mean composition (dry matter basis) of Mixed Ration was: 32% alfalfa hay, 28% corn silage, 22% corn grain, 17% soybean meal and 2% minerals and vitamins. Whole fuzzy cottonseeds contributed 2.75 kg/cow/day (as is basis) or about 10% of the total diet dry matter. The chemical composition of Mixed Ration (Table 3) was comparable to composition when calculated using the nutrient content and the proportion of its components in the diet.

Table 3 Chemical composition of mixed ration and the different cottonseeds ¥

Feeds Analyses	Units	Mixed Ration	Cottonseeds (Treatments) DP50 (E)	DP50B (F)	DP50BII (G)	DP50RR (H)
Dry Matter	%	53.8±2.70	91.4±1.51	91.6±1.22	91.2±1.36	91.2±0.87
Crude Protein	%	18.1±0.70	17.6±4.41	18.1±2.73	16.7±1.38	19.6±1.70
NDF	%	36.7±1.36	52.6±1.84	52.7±1.24	52.8±1.62	48.8±2.19
ADF	%	23.0±2.26	37.3±0.74	37.5±1.14	39.4±1.81	34.9±2.32
ADL	%	4.23±0.36	8.0±0.76	8.5±0.88	7.7±0.90	8.3±1.07
Ether Extract	%	4.1±0.98	15.9±1.04	15.4±0.45	14.2±1.02	17.3±0.62
Ash	%	9.4±0.38	4.3±0.23	4.2±0.31	3.9±0.39	4.3±0.37
NFC §	%	*31.7*	*9.9*	*9.6*	*12.4*	*10.0*
Calcium	%	0.96±0.246	0.14±0.005	0.15±0.010	0.15±0.008	0.14±0.008
Phosphorus	%	0.49±0.032	0.64±0.049	0.61±0.087	0.58±0.052	0.61±0.051
Magnesium	%	0.26±0.034	0.38±0.035	0.34±0.017	0.36±0.019	0.36±0.019
Potassium	%	2.5±0.41	1.4±0.15	1.6±0.11	1.4±0.13	1.4±0.12
Sodium	%	1.3±0.17	0.02±0.007	0.02±0.005	0.03±0.004	0.02±0.003
Iron	ppm	399±18.8	37.6±2.07	37.1±2.42	37.3±3.01	38.3±3.65
Zinc	ppm	56.0±14.34	34.0±7.09	37.5±5.58	36.1±7.79	35.0±7.56
Copper	ppm	10.4±1.02	4.6±0.30	4.5±0.45	4.5±0.58	4.7±0.35
Manganese	ppm	85.0±9.09	13.5±0.76	13.0±0.53	13.1±1.13	13.8±0.71

¥ Means and Standard Deviations, each value n=8
§ NFC: Nonfibrous carbohydrates = 100-(Crude Protein+NDF+ Ether Extract+Ash)

The nutrient composition of the different cottonseeds used in this experiment were similar (Table 3). When compared with nutrient composition of cottonseeds published by NRC (2001), the cottonseed in this study had a lower CP, Ether Extract (EE), ADL and minerals in general when using the analyses of Experimental Station Rafaela (Attachment 9). The range of CP content of genetically modified cottonseeds was from 16.7 (DP50BII) to 19.6% (DP50RR) compared to NRC information (average of 23.5% CP). The mean EE of cottonseeds was almost 16% compared to 19.3% for NRC. The ADL average of cottonseeds used in this experiment was 8.2% against 12.9% of NRC. The analytical values determined by DHI Forage Testing Lab (Attachment 9) fell within the range reported by NRC (2001).

Some important differences were observed comparing the same materials analyzed in DHI Forage Analysis Laboratory (Dairy One) and the Forage Analysis Laboratory at INTA Rafaela (Attachment 9). Crude protein, acid detergent lignin, neutral detergent fiber, acid detergent fiber were much higher when analyzed by Dairy One compared to analyses conducted at the Experimental Station Rafaela (Attachment 9) regardless of cottonseed type. The corresponding calculated energy levels were also different since the equations incorporate the various fiber fractions. According to Ensminger *et al.* (1990),

variations in composition of feed are unusual today, because of improved methods of analysis and computerized blending equipment. However, the authors indicate that nutrient variations of 10 to 15% are normal due to differences from farm to farm, year to year, sampling, varieties, soil fertility, weather, maturity of harvest and differences in Laboratory Testing Techniques. Regardless of these differences, all cottonseed products were analyzed using the same procedure whether it was at INTA or Dairy One. Therefore, no bias was introduced resulting in no impact on the outcome of this study.

The differences in chemical composition, in particular comparisons with NRC (2001), could be related to soil and climatic conditions and the way cotton is grown in Argentina, which is mainly without fertilizers and under dry conditions. Van Soest (1994 gives a special reference to the effects of the environment on feed chemical composition. He indicated that light and temperature are the most important variables, but nitrogen fertilizer and water supply are also considered as environmental factors which affect nutritive value of feeds. All cottonseed used in this study was grown in Argentina under similar conditions (Attachment 15); therefore, eliminating potential confounding of the interpretation of the results.

Mycotoxins, except marginal zearalenone toxin levels corrected for hull contents in two treatments (DP50 and DP50BII), and pesticide residues were not detected in any cottonseed samples (see Attachment 10). The laboratory could not analyze the samples for citrinin and diacetoxyscirpenol due to complex sample matrix. According to Coppock *et al.* (1987) and Arieli (1998), the contents of free gossypol were in a normal range for all the samples, DP50 (0.374%), DP50B (0.330%), DP50BII (0.262%), and DP50RR (0.384%) cottonseed. Aminomethylphosphonic acid (AMPA) residues were below 0.05 ppm for all cottonseed products and glyphosate residues ranged from 0.05 to 0.09, <0.05 to 0.07, <0.05 to 0.06, and 0.20 to 0.47 ppm for DP50, DP50B, DP50BII and DP50RR, respectively. Only DP50RR cotton was sprayed with glyphosate during growth in the field. Test substance characterization confirmed the test substances to contain the expected transgenic events with the exception of DP50BII (Bollgard II). PCR analysis of DP50BII indicated the sample was contaminated with Roundup Ready. The PCR product produced a faint band, lower in intensity than the control, likely due to insect pollination, as the DP50BII plot was grown adjacent to the DP50RR plot . The low level of Roundup Ready contamination in the Bollgard II seed does not impact the study as there were no differences observed between any of the treatments, including cows fed Roundup Ready cottonseed. In addition, the nontransgenic control was confirmed to be free of the test events, and so the appropriate comparison could still be made in order to assess any potential effects of feeding transgenic cotton to cows.

8.2 Dry Matter Intake, Body Weight and Body Condition Score

The effect of the treatments on intake, body weight and body condition score during the experiment are presented in Table 4 and Attachment 11. Cottonseed was offered at a

fixed amount of 2.75 kg (as is)/cow/d for all the treatments, achieving an average intake of cottonseeds of 2.3 kg DM/cow/d. Genetically modified cottonseeds Bollgard, Bollgard II and Roundup Ready did not affect total DMI of the animals. These results are consistent with Clark and Ipharraguirre (2001) who concluded that genetically modified crops did not affect feed intake and had similar feeding value for lactating cows when compared to their conventional counterparts.

Body weights and body condition scores were not affected by treatments (Table 4 and Attachment 11). However, significant differences ($P<0.01$) in body weights and body condition scores were observed among experimental periods unrelated to treatment. Animals lost body weight from period 1 (573 kg) to period 4 (559 kg) with a significant variation in body condition score (Table 4). These observations and the fact that the animals have had high consumption levels of dry matter are discussed together considering the hot weather that occurred during the trial (see Weather Conditions 8.4).

8.3 Milk Yield and Milk Composition

Milk production and milk composition were not affected by the different genetically modified cottonseeds when compared with control non-transgenic cottonseed (Table 5). These results are in accordance with Clark and Ipharraguirre (2001). Milk yield when averaged over all treatment groups was more than 26.5 kg of milk/cow/d, containing on average 3.58, 3.14, 5.0 and 8.9% fat, protein, lactose and non-fat solids, respectively. Concentration of N-urea in milk, ranged from almost 19 (DP50) to 20.7 mg N-urea/100 ml of milk in treatment DP50BII. These values indicate that the animals were fed with similar and relatively well balanced diets (Gustaffson, 1993; Linn and Olson 1995; Broderick and Clayton, 1997).

No transgenic DNA (specifically Cry1Ac) or plant protein (specifically *acp I*) was detected in any of the milk samples tested from cows fed Bollgard II cottonseed or nontransgenic control (DP50)cottonseed. No Cry1AC, Cry2Ab2 or CP4 EPSPS protein was detected by Western blot analysis in any of the milk samples tested from cows fed Bollgard, Bollgard II, or Roundup Ready cottonseed, respectively as reported in Study 01-01-36-29 (in progress).

8.4 Weather Conditions and Animal Behavior

Two variables, Daily Minimum Temperature (T°C min) and Temperature Humidity Index (THI), were used to describe the weather condition during the lapse of time when the experiment was carried out (Figure 1).

The thermal neutral zone of Holstein dairy cattle is about 5 to 20°C, but can vary among animals and adaptation periods. Temperatures below or above the thermal neutral range alter intake and metabolic activity and thus the maintenance requirements of the animal

(NRC, 2001). Also, the THI obtained by equation from the relative humidity and the air temperature offers the possibility to evaluate the body comfort of the animals. A THI value above 72 may affect dry matter intake and as a consequence lactational performance.

In our study, the minimum temperatures were between 10 to 20°C (Figure 1) providing a relatively good environment for thermal neutral zone during the nights. But, an average THI of 70 was registered during the days with values above 72 during almost all the experiment (Figure 1).

The eating behavior of animals during the nights, achieving high DMI, probably indicated an adaptation mechanism of the animals to these conditions. Others have shown that cows consume more during the night when environmental temperatures are lower and heat stress is reduced (Beede and Shearer, 1991). However, based on the DMI and the energy content of the diets, there was an excess of energy available beyond the energy needed for milk production and normal maintenance. This energy probably was used by the cows to maintain body temperature as a result of increased maintenance requirements since body weights were not increased during this time. Moreover, as was previously indicated there was a trend for animals to lose body weight. The effects of environmental temperature on the cows in this study should not affect the quality of the data since no bias would be introduced based on the experimental design employed in this study.

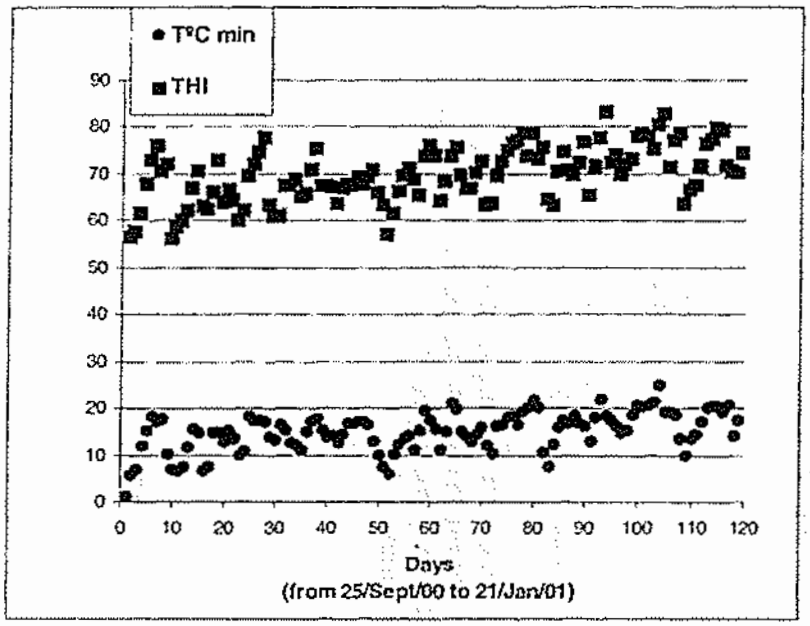

Figure 1 Daily Minimum Temperatures (T°C min) and Temperature Humidity Index (THI) from 25/Sep/00 to 15/Jan/01 at the Experimental Station Rafaela INTA, Argentina

Table 4 Total dry matter and cottonseed intakes, body weight and body condition scores

	Treatments*						Periods ¥					
Cottonseeds	DP50	DP50B	DP50BII	DP50RR	SE	P<	1	2	3	4	SE	P<
Intakes, kg/cow/d												
Total DMI	23.4	23.8	23.9	23.7	1.12	0.648	24.9^{a}	25.2^{a}	23.7^{b}	21.1^{c}	0.63	0.01
Cottonseed	2.25	2.29	2.28	2.27	0.086	0.854	2.20^{b}	2.39^{a}	2.14^{b}	2.36^{a}	0.063	0.01
Body weight, kg	567.4	567.3	566.8	568.7	24.95	0.967	573.2^{a}	572.5^{a}	565.5^{b}	558.9^{b}	24.76	0.01
Body condition score §	2.30	2.30	2.30	2.34	0.076	0.515	2.27^{bc}	2.41^{a}	2.25^{c}	2.33^{b}	0.070	0.01

* DP50: Control; DP50B: Bollgard; DP50BII: Bollgard II; DP50RR: Roundup Ready
¥ and ^{a,b,c} Means within row with different letter are significantly different P<0.01
§ Scale 1 (thin cow) to 5 (fat cow)
SE Standard error

Table 5 Effect of different cottonseeds on milk yield and milk composition (Least Square Means and Standard Errors)

	Treatments*					
Cottonseeds	DP50	DP50B	DP50BII	DP50RR	SE	P<
Milk yield, kg/d	26.9	26.7	27.6	27.4	2.88	0.686
Milk composition, %						
Fat	3.59	3.60	3.52	3.59	0.186	0.777
Protein	3.15	3.14	3.14	3.13	0.072	0.947
Lactose	4.97	5.01	5.04	5.00	0.064	0.277
Non-fat solids	8.84	8.91	8.96	8.90	0.096	0.394
N-urea, mg/100ml	18.77	19.49	20.66	20.01	1.958	0.267
Components yield, kg/d						
Fat	0.95	0.95	0.95	0.97	0.071	0.858
Protein	0.84	0.84	0.86	0.85	0.084	0.808
Lactose	1.34	1.34	1.39	1.37	0.145	0.461
Non-fat solids	2.37	2.38	2.47	2.44	0.250	0.486
N-urea, g/d	5.17	5.19	5.75	5.54	0.780	0.180

* DP50: Control; DP50B: Bollgard; DP50BII: Bollgard II; DP50RR: Roundup Ready

An extra week of data collection was decided due to a mistake that occurred in Square 1 during the first week in Period 4. The results on this extra-week were affected by the high temperatures and THI. For this reason they were rejected. Data collection from Square 1 in Period 4 was obtained with 2 weeks of animal adaptation to the diet.

9.0 Conclusions

Results of this study indicate that genetically modified cottonseed (Bollgard, Bollgard II and Roundup Ready) supported similar performance as non transgenic cottonseed (DP50) and did not affect dry matter intake, milk yield, milk composition and body condition score of dairy cows under controlled feeding conditions.

10.0 References

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Attachment 1a

Feeding Program and Ration Formulation Procedures

pp. 29-31

ATTACHMENT 1a

Feeding Program and Ration Formulation Procedures

The ration will be formulated for the first time the week before beginning the adaptation period (August 14-18, 2000). Afterwards, necessary adjustments will be made weekly according to the amounts ingested and the dry matter in the high moisture components (corn silage).

Each new ingredient lot will be analyzed and the ration will be reformulated according to the chemical composition of the new lot when it is used.

These are the steps to be followed:

1) The "CPM Dairy. Beta, version 1.5, Cornell-Penn-Miner, 1998" dairy cattle ration calculation software will be used.
2) Nutritional requirements will be established based on:

Animals

a) Live weight: taking the average value plus 1.5 of the standard deviation from
first weighing made, before the beginning of the adaptation period.
b) Body condition, taking the average value plus 1.5 the standard deviation for the first determination, before the beginning the adaptation period.
c) Live weight and body condition will be adjusted progressively during the trial, according to subsequent determinations.
d) Milk production. The first determination will correspond to the week prior to the trial and the later ones will serve for subsequent ration adjustments. The average value of the cows plus 1.5 the standard deviation will be taken.
e) Lactation status: 70 days of lactation will be taken as the basis at the beginning of the trial.
f) Lactation number: 2 lactation periods will be taken as the average.
g) The chemical composition of the milk will be set at 3.6% G [fat] and 3.3% PB
[raw protein].
h) Environment: the mean daily temperature of the week in question will be taken. If there are changes, they will be adjusted progressively.
i) After adaptation, the week prior to the measurement period, the TMR will be adjusted according to the consumption achieved.

Food

a) The chemical composition of each ingredient will be filed in the software database, taking the information from the following sources:

- *Ad hoc* analysis of each food in the INTA Rafaela EEA laboratory, according to the protocol for: dry matter; raw protein; ash; neutral detergent fiber; acid detergent fiber; acid detergent lignin, ethereal extract, calcium, phosphorus, potassium, magnesium, chlorine and sodium.
- Amino acid composition and degradability of the nitrogenized fractions and the carbohydrates from the "CPMDairy" software database, from the INTA Rafaela EEA laboratory database and from the dairy cattle NRC food composition tables, 1986.
- A specially formulated vitamin-mineral mixture from the Roche7 Laboratory will be added to the TMR.

b) The total consumption of dry matter will be assigned for the first time based on a mean production of 40 L/cow/day, with 3.6% GB [raw fat] and 3.3% PB in the milk, and they will be adjusted based on the determinations of the adaptation period, with the average value plus 10% of the remainder.

c) The ration will be adjusted during the adaptation period whenever necessary according to:

- The percentage of dry matter [Ms] in the moist ingredient (corn silage), as follows:

(% of the previous MS) / (% of the current MS) = numerical index

(Numerical index) X (kg of silage supplied) = kg of silage to be supplied.

- Milk production and chemical composition.

Attachment 1b

Daily Feeding Program Procedure

pp. 32-33

ATTACHMENT 1b

Daily Feeding Program Procedure

Procedure for supplying Total Mixed Ration (TMR) mixture and cottonseed (transgenic cotton assessment trial with the Monsanto Company)

1. The TMR mixture is supplied individually to each animal twice a day, half AM and PM and after each milking.

2. The cottonseed is weighed previously in individual bags perfectly identified for each half of the daily supply, that is 1.375 kg AM and PM, a total of 2.750 kg/animal/day. Each supply is placed on top of the TMR mixer mixture in each feeding manger and the components are mixed manually by hand.

3. From week 1 to 3, that is the period of adaptation to the diet, an amount of feed is supplied per cow per day that would allow an average 10% rejection or refusal.

4. During the sampling week or experimental period, the dietary supply is adjusted for an average 5% rejection. This value is calculated based on the offer and rejection occurring the week prior to each data gathering week, that is, during the third week of the period of adaptation to each diet.

5. Rejection is measured daily before the morning supply, corresponding to an AM PM sequence offer (important for milk sampling).

6. MS [dry matter] consumption during each sampling period is calculated based on the daily dry matter offered and rejected; therefore TMR mixer mixture and cottonseed samples will be taken every day to correct the offers by their corresponding MS.

7. The MS of the remainders will be corrected according to the MS of the supply, proportionally to the participation of the feed mixture (mixer) and cottonseed.

8. The total consumption of MS, the consumption of MS in the mixer mixture and the consumption of MS in the cottonseed will be analyzed.

Attachment 1c

Feed Ingredient Sampling Procedures

pp. 34-35

ATTACHMENT 1c

Feed and Ingredient Sampling Procedures

Feed sampling for complete chemical analysis (individual ingredients, TMR mixer mixture and cottonseed)

1. Before the beginning of the trial or before the beginning of the adaptation period, complete chemical analyses will be performed on every ingredient, thus establishing the diet and its corresponding mixture of ingredients according to the objectives of each trial. The incorporation or change (purchase) of any ingredient must be accompanied by a new complete analysis and, if necessary, an adjustment of the diet. A retainer sample or "C" sample of each ingredient is kept in the freezer (see the sample designations at the end).

2. During the sampling week, that is, on each one of the 7 days of the experimental period, a daily sample of each cottonseed or treatment must be taken for each trial (~250 g/sample/day). The mixed feed sample, with the various ingredients, must be taken from different samples (8 to 10 manual samples) while the mixer is being unloaded until a total of approximately ~450 g per day is achieved. Both samples (different cottonseeds and mixer mixture) must be perfectly identified with: (a) trial name and number, (b) sampling date and day (1 to 7), and (c) experimental period, finally placing them in nylon bags in the freezer.

3. Once each experimental period is over, on the Monday or Tuesday following the sampling week, coinciding with the beginning of the new experimental period, the samples are removed from the freezer, left standing a few hours, mixed well manually and subdivided into three sub-samples designated A, B, C. The three samples must be properly identified (according to point 2 of this protocol) and differentiated by the corresponding letter. Sample "C", packaged and identified, is returned to the freezer as a safety sample. Samples "A" and "B" are weighed individually for dry matter, then they are ground dry and stored for chemical analysis with proper identification (proximal analysis Rafaela EEA laboratory and minerals Universidad del Litoral).

4. The chemical analyses must be performed during the corresponding adaptation periods, so that once the trial is over the information is available to begin preparing the first report.

Attachment 2a

Milk Sampling Collection and Handling for Analysis (EEA Rafaela, Argentina)

pp. 36-37

ATTACHMENT 2a.

Milk Sampling, Collection and Handling for Analysis

1. Milking routine is done with an AFIKIM S.A.E. equipment (from Israel). This equipment include electronic identification (ID) of the cows, AM&PM milk yield per animal, ID of cows with mastitis by electronic conductivity in milk, automatic cluster removal, milking time, cows activity for heat detection, etc.
2. The first step for milk samples collection is to clean the automatic milking recorder in each milking cluster. This is an automatic procedure pushing the corresponding button.
3. Then, the bottles or flasks (250ml) sterilized previously identify with the number of the cow, date, AM or PM and treatment, are inserted in the sampling collectors of each automatic milking recorder. The system derives a 5% sample in the bottle from the AM or PM milk yield in each cow.
4. Ones finished, the bottles are closed with metal paper and maintained in a refrigerator (4-7°C).
5. To prepare the samples, the bottles with the milk are taken out from the refrigerator are cooled in a Maria bath (36-38°C).
6. Milk samples for milk composition analysis were composited for 2ml /kg milk produced in each cow for AM & PM daily milking respectively. Example: a cow producing 10 kg of milk PM and 10 kg of milk AM (total 20 kg of milk/d), the sample for analysis were composited by 20 ml of AM and 20 ml of PM milk, giving total daily sample of 40 ml of milk.
7. The individual daily composited (AM&PM) milk sample for milk composition analysis were maintained in refrigerator (4-7°C) with a pill of Potassium Dichromate and daily sent to Sancor where the analysis were carried out.
8. For other analysis in milk (ADN, proteins, etc), were taken directly 15 ml from AM and 15 ml from PM of milk respectively, giving a composited daily sample of 30 ml. These samples were maintained in freezer (-20°C).
9. All the samples were identified with the date, the number of the cow, the treatment and AM or PM if correspond.
10. Milking routine was done from 06:30 to 07:00 in the morning and 16:30 to 17:00 in the evening or AM and PM respectively.

Attachment 2b

Daily Cow Management and Exercise Procedures

pp. 38-39

ATTACHMENT 2b

Daily Cow Management and Exercise Procedures

1) Daily animal exercise or movement:

- 5:15-5:30 a.m. All the cows are removed from the individual pens (roofed shed) to a dry lot and natural shade
- 6:30-7:00 a.m. The animals are taken to the milking room
- 7:30 a.m. The cows return to dry lot
- 9:15-9:30 a.m. The animals return to the individual pens in the shed
- 2:30 p.m. They go out to the dry lot again
- 4:30-5:00 p.m. The afternoon milking takes place
- 5:30-5:45 p.m. They return to the individual pens in the shed

The daily mean time for staying outside the individual pens or in the dry lot with natural shade is 7 (seven) hours per day. The individual pens are washed with abundant water twice per day.

2) Daily observation of the animals

The cows are observed every day individually. In addition, the individual AM and PM milk register of each cow, as well as the daily ingestion behavior of the animals (food offer and rejection) are used to detect and/or corroborate possible health or handling problems.

3) Special situations.

- Heat stress. During stressful situations, the cows are bathed or refreshed with water individually during the mid-day hours.
- Rain. When there is a strong rain or storm, the animals remain under cover in the shed. Once the storm is over, the normal activities indicated in point 1 of this procedure are reinitiated.
- Foot problems. Animals with foot problems are passed through a foot-wash with copper sulfate and sodium hypochlorite.

Attachment 3

Detection of Intramammary Infection

pp. 40-41

ATTACHMENT 3.

Detection of intramammary infection

Mastitis was detected using a computerized milking system that combines changes in milk production and electrical resistance (ER) of composite milk to warn o possible mastitis episodes (Afimilk®). The system indicates presence of combined falls in ER and milk production that deviate ⩾20% from the average of the nine previous days for the current lactation.

Data were obtained on a daily basis and cows showing a deviation on these parameters were clinically examined to detect abnormal milk or inflammatory signs. When abnormal milk or inflammatory signs were observed, individual quarter samples were taken using aseptic procedures and the affected quarter/s was/were treated with intramammary antibiotics (spiramicyn 0.2 g, neomycin 0.2 g, and flumetasone 0.25 mg in a 10-ml volume) for three milking. When no clinical signs were detected composite milk samples were taken. Either normal or abnormal milk samples were submitted for bacteriological culture. Milk samples were cultured and antibiotic susceptibility tests (AST) were performed for the isolated bacterial strains following standard procedures.

Treated cows were examined during treatment period to assess the outcome of antibiotic therapy. If no improvement was observed following administration of antibiotic therapy, and alternative drug was used (cloxacillin 0.5 g in 10-ml volume) for the next three milking. In case of failure of the second treatment course bacteriological culture data and results of in vitro AST were used to select an alternative drug or drug combination.

NOTE: composite milk samples were taken from all cows previous to the initiation of the study. Milk samples were cultured and those cows showing intramammary infection (IMI) by *Streptococcus* species were treated with antibiotic. Cows in which IMI by *Staphylococcus aureus* was detected were not included in the study.

Attachment 4

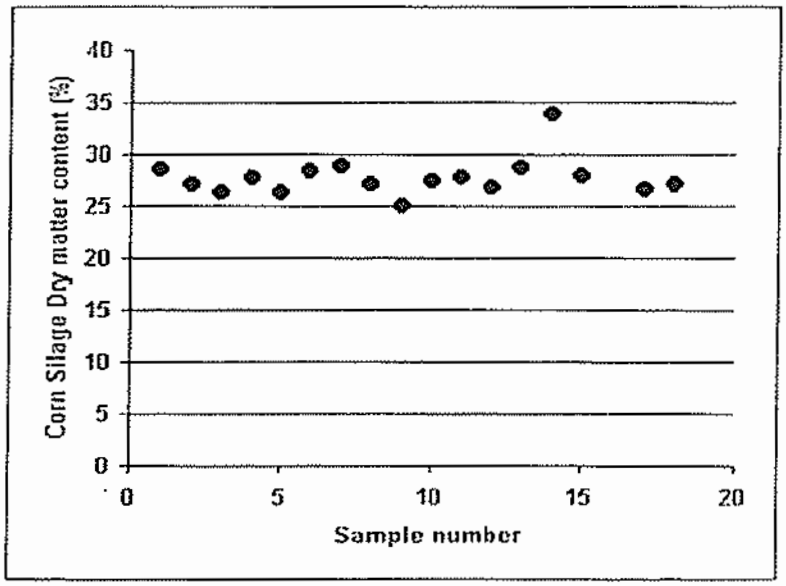
Corn Silage Dry Matter Determinations

pp. 42-43

ATTACHMENT 4.

Corn Silage Dry Matter Determinations

date		%DM	
5/30/00	1	28.60	
9/13/00	2	27.06	
9/20/00	3	26.32	
10/4/00	4	27.80	
10/11/00	5	26.22	
10/18/00	6	28.44	
10/26/00	7	28.85	
11/1/00	8	27.15	
11/7/00	9	25.07	
11/15/00	10	27.42	
11/22/00	11	27.82	
11/29/00	12	26.78	
12/6/00	13	28.66	
12/13/00	14	33.90	
12/20/00	15	27.94	
12/27/00	16	lost	
1/3/01	17	26.69	
1/10/01	18	27.05	
AVERAGE		27.75	
de		1.87	
max +2de		31.50valor max	33.90
min -2de		24.00valor min	25.07



Silage samples were initially taken the end of May for analysis for use in formulating diets. Feeding of the silage started in September, 2001.

Attachment 5

Daily Dry Matter Intakes

pp. 44-53

ATTACHMENT 6

DAILY DRY MATTER INTAKES experiment 00 04 36 04

per	day	trat	vaca	oferta mezcla mixer kg AM	oferta mezcla mixer kg PM	oferta mezcla mixer %MS	oferta mezcla mixer kgMS/dia (a)	oferta semilla de algodon kg AM+PM	oferta semilla de algodon %MS	oferta semilla de algodon kgMS/dia (b)	remanen total kg AM+PM	remanen total %MS	remanen total kgMS/dia (c)	consumo kg MS vaca/dia (a+b)-(c)	remanente total % (MS)	participac algodón % (MS)	consumo algodón kgMS/d	consumo mezcla kgMS/d
1	1	E	13	24	24	49.95	23.98	2.75	89	2.45	10.8	50.78	5.48	20.94	20.76	9.26	1.94	19.00
1	1	F	14	24	24	49.95	23.98	2.75	89.76	2.47	1	50.78	0.51	25.94	1.92	9.33	2.42	23.52
1	1	G	15	24	24	49.95	23.98	2.75	89.02	2.45	9.5	50.78	4.82	21.60	18.26	9.26	2.00	19.60
1	1	H	16	24	24	49.95	23.98	2.75	86.6	2.38	5.6	50.78	2.84	23.51	10.79	9.04	2.12	21.39
1	1	E	17	24	24	49.95	23.98	2.75	89	2.45	2	50.78	1.02	25.41	3.84	9.26	2.35	23.05
1	1	F	18	24	24	49.95	23.98	2.75	89.76	2.47	11.1	50.78	5.64	20.81	21.31	9.33	1.94	18.87
1	1	G	19	24	24	49.95	23.98	2.75	89.02	2.45	4.1	50.78	2.08	24.34	7.88	9.26	2.26	22.09
1	1	H	20	24	24	49.95	23.98	2.75	86.6	2.38	1.5	50.78	0.76	25.60	2.89	9.04	2.51	23.28
1	1	E	21	24	24	49.95	23.98	2.75	89	2.45	6.5	50.78	3.30	23.12	12.49	9.26	2.14	20.98
1	1	F	22	24	24	49.95	23.98	2.75	89.76	2.47	1	50.78	0.51	25.94	1.92	9.33	2.42	23.52
1	1	G	23	24	24	49.95	23.98	2.75	89.02	2.45	0.4	50.78	0.20	26.22	0.77	9.26	2.43	23.79
1	1	H	24	24	24	49.95	23.98	2.75	86.6	2.38	3.2	50.78	1.62	24.73	6.17	9.04	2.23	22.50
												promedios		24.01	9.08	9.22	2.21	21.80
1	2	E	13	24	24	51.66	24.80	2.75	89	2.45	10.8	50.78	5.48	21.76	20.13	8.98	1.95	19.81
1	2	F	14	24	24	51.66	24.80	2.75	89.76	2.47	1.5	50.78	0.76	26.50	2.79	9.05	2.40	24.10
1	2	G	15	24	24	51.66	24.80	2.75	89.02	2.45	7.7	50.78	3.91	23.33	14.35	8.99	2.10	21.24
1	2	H	16	24	24	51.66	24.80	2.75	86.6	2.38	5.4	50.78	2.74	24.44	10.09	8.76	2.14	22.29
1	2	E	17	24	24	51.66	24.80	2.75	89	2.45	2.1	50.78	1.07	26.18	3.91	8.98	2.35	23.83
1	2	F	18	24	24	51.66	24.80	2.75	89.76	2.47	11.0	50.78	5.74	21.53	21.05	9.05	1.95	19.58
1	2	G	19	24	24	51.66	24.80	2.75	89.02	2.45	3.6	50.78	1.83	25.42	6.71	8.99	2.28	23.10
1	2	H	20	24	24	51.66	24.80	2.75	86.6	2.38	3.2	50.78	1.62	25.55	5.98	8.76	2.24	23.31
1	2	E	21	24	24	51.66	24.80	2.75	89	2.45	9.8	50.78	4.98	22.27	18.27	8.98	2.00	20.27
1	2	F	22	24	24	51.66	24.80	2.75	89.76	2.47	1.5	50.78	0.76	26.50	2.79	9.05	2.40	24.10
1	2	G	23	24	24	51.66	24.80	2.75	89.02	2.45	0.2	50.78	0.10	27.14	0.37	8.99	2.44	24.70
1	2	H	24	24	24	51.66	24.80	2.75	86.6	2.38	2.2	50.78	1.12	26.06	4.11	8.76	2.28	23.78
												promedios		24.72	9.21	8.95	2.21	22.51
1	3	E	13	24	24	53.99	25.92	2.75	89	2.45	24.7	50.78	12.54	15.82	44.22	8.63	1.37	14.45
1	3	F	14	24	24	53.99	25.92	2.75	89.76	2.47	1.3	50.78	0.66	27.72	2.33	8.70	2.41	25.31
1	3	G	15	24	24	53.99	25.92	2.75	89.02	2.45	8.9	50.78	4.52	23.84	15.93	8.63	2.06	21.79
1	3	H	16	24	24	53.99	25.92	2.75	86.6	2.38	4	50.78	2.03	26.27	7.18	8.42	2.21	24.05
1	3	E	17	24	24	53.99	25.92	2.75	89	2.45	1.5	50.78	0.76	27.60	2.69	8.63	2.38	25.22
1	3	F	18	24	24	53.99	25.92	2.75	89.76	2.47	3	50.78	1.52	26.86	5.37	8.70	2.34	24.52
1	3	G	19	24	24	53.99	25.92	2.75	89.02	2.45	2.6	50.78	1.32	27.04	4.65	8.63	2.33	24.71
1	3	H	20	24	24	53.99	25.92	2.75	86.6	2.38	2	50.78	1.02	27.28	3.59	8.42	2.30	24.99
1	3	E	21	24	24	53.99	25.92	2.75	89	2.45	7.8	50.78	3.96	24.40	13.95	8.63	2.11	22.30
1	3	F	22	24	24	53.99	25.92	2.75	89.76	2.47	1.9	50.78	0.96	27.42	3.40	8.70	2.38	25.03
1	3	G	23	24	24	53.99	25.92	2.75	89.02	2.45	0.1	50.78	0.05	28.31	0.18	8.63	2.44	25.87
1	3	H	24	24	24	53.99	25.92	2.75	86.6	2.38	3.4	50.78	1.73	26.57	6.10	8.42	2.24	24.33
												promedios		25.76	9.13	8.59	2.21	23.55

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1	4	E	13	24	24	53.64	25.75	2.75	89	2.45	12.8	50.78	6.50	21.69	23.05	8.65	1.88	19.81
1	4	F	14	24	24	53.64	25.75	2.75	89.76	2.47	5.5	50.78	2.79	25.42	9.90	8.75	2.22	23.20
1	4	G	15	24	24	53.64	25.75	2.75	89.02	2.45	11.7	50.78	5.94	22.25	21.07	8.68	1.93	20.32
1	4	H	16	24	24	53.64	25.75	2.75	86.6	2.38	6.9	50.78	3.00	25.13	10.65	8.47	2.13	23.00
1	4	E	17	24	24	53.64	25.75	2.75	89	2.45	7.6	50.78	3.86	24.34	13.69	8.68	2.11	22.22
1	4	F	18	24	24	53.64	25.75	2.75	89.76	2.47	9.6	50.78	4.87	23.34	17.28	8.75	2.04	21.30
1	4	G	19	24	24	53.64	25.75	2.75	89.02	2.45	8.3	50.78	4.21	23.98	14.95	8.68	2.08	21.90
1	4	H	20	24	24	53.64	25.75	2.75	86.6	2.38	2.6	50.78	1.32	26.81	4.69	8.47	2.27	24.54
1	4	E	21	24	24	53.64	25.75	2.75	89	2.45	9	50.78	4.57	23.62	16.21	8.68	2.05	21.57
1	4	F	22	24	24	53.64	25.75	2.75	89.76	2.47	1	50.78	0.51	27.71	1.80	8.75	2.42	25.28
1	4	G	23	24	24	53.64	25.75	2.75	89.02	2.45	0.3	50.78	0.15	28.04	0.54	8.68	2.43	25.61
1	4	H	24	24	24	53.64	25.75	2.75	86.6	2.38	0	50.78	1.52	26.61	5.42	8.47	2.25	24.35
												promedios		24.91	11.60	8.64	2.15	22.76
1	5	E	13	24	24	52.69	25.29	2.75	89	2.45	8.7	50.78	4.42	23.32	15.93	8.82	2.06	21.26
1	5	F	14	24	24	52.69	25.29	2.75	89.76	2.47	3	50.78	1.52	26.24	5.49	8.89	2.33	23.90
1	5	G	15	24	24	52.69	25.29	2.75	89.02	2.45	4.5	50.78	2.29	25.45	8.24	8.83	2.25	23.21
1	5	H	16	24	24	52.69	25.29	2.75	86.6	2.38	7.9	50.78	4.01	23.66	14.50	8.61	2.04	21.62
1	5	E	17	24	24	52.69	25.29	2.75	89	2.45	2.9	50.78	1.47	26.27	6.31	8.82	2.32	23.95
1	5	F	18	24	24	52.69	25.29	2.75	89.76	2.47	6.6	50.78	3.35	24.41	12.07	8.89	2.17	22.24
1	5	G	19	24	24	52.69	25.29	2.75	89.02	2.45	4.3	50.78	2.18	25.56	7.87	8.83	2.26	23.30
1	5	H	20	24	24	52.69	25.29	2.75	86.6	2.38	0	50.78	3.05	24.63	11.01	8.61	2.12	22.51
1	5	E	21	24	24	52.69	25.29	2.75	89	2.45	7.9	50.78	4.01	23.73	14.46	8.82	2.09	21.63
1	5	F	22	24	24	52.69	25.29	2.75	89.76	2.47	1.1	50.78	0.56	27.20	2.01	8.89	2.42	24.78
1	5	G	23	24	24	52.69	25.29	2.75	89.02	2.45	0.4	50.78	0.20	27.54	0.73	8.83	2.43	25.11
1	5	H	24	24	24	52.69	25.29	2.75	86.6	2.38	1.8	50.78	0.91	26.76	3.30	8.61	2.30	24.46
												promedios		25.40	8.41	8.79	2.20	23.16
1	6	E	13	23	23	53.53	24.62	2.75	89	2.45	11.8	50.78	5.99	21.08	22.13	9.04	1.91	19.17
1	6	F	14	23	23	53.53	24.62	2.75	89.76	2.47	8.1	50.78	4.11	22.98	15.16	9.11	2.09	20.89
1	6	G	15	23	23	53.53	24.62	2.75	89.02	2.45	7.1	50.78	3.61	23.47	13.32	9.04	2.12	21.34
1	6	H	16	23	23	53.53	24.62	2.75	86.6	2.38	9.1	50.78	4.62	22.38	17.11	8.82	1.97	20.41
1	6	E	17	23	23	53.53	24.62	2.75	89	2.45	6.9	50.78	3.50	23.57	12.94	9.04	2.13	21.44
1	6	F	18	23	23	53.53	24.62	2.75	89.76	2.47	10.4	50.78	5.28	21.81	19.49	9.11	1.99	19.82
1	6	G	19	23	23	53.53	24.62	2.75	89.02	2.45	6.1	50.78	3.10	23.97	11.44	9.04	2.17	21.81
1	6	H	20	23	23	53.53	24.62	2.75	86.6	2.38	14.4	50.78	7.31	19.69	27.08	8.82	1.74	17.96
1	6	E	21	23	23	53.53	24.62	2.75	89	2.45	10.3	50.78	5.23	21.84	19.32	9.04	1.97	19.87
1	6	F	22	23	23	53.53	24.62	2.75	89.76	2.47	1.4	50.78	0.71	26.38	2.62	9.11	2.40	23.98
1	6	G	23	23	23	53.53	24.62	2.75	89.02	2.45	2.3	50.78	1.17	25.90	4.31	9.04	2.34	23.56
1	6	H	24	23	23	53.53	24.62	2.75	86.6	2.38	2.2	50.78	1.12	25.89	4.14	8.82	2.28	23.61
												promedios		23.26	14.09	9.00	2.09	21.15
1	7	E	13	23	23	55.22	25.40	2.75	89	2.45	10	50.78	5.08	22.77	18.23	8.79	2.00	20.77
1	7	F	14	23	23	55.22	25.40	2.75	89.76	2.47	1	50.78	0.51	27.36	1.82	8.86	2.42	24.94
1	7	G	15	23	23	55.22	25.40	2.75	89.02	2.45	2.9	50.78	1.47	26.38	5.29	8.79	2.32	24.06
1	7	H	16	23	23	55.22	25.40	2.75	86.6	2.38	2.4	50.78	1.22	26.56	4.39	8.57	2.28	24.29
1	7	E	17	23	23	55.22	25.40	2.75	89	2.45	1.7	50.78	0.86	26.99	3.10	8.79	2.37	24.61
1	7	F	18	23	23	55.22	25.40	2.75	89.76	2.47	2.3	50.78	1.17	26.70	4.19	8.86	2.36	24.34
1	7	G	19	23	23	55.22	25.40	2.75	89.02	2.45	7.6	50.78	3.86	23.99	13.86	8.79	2.11	21.88
1	7	H	20	23	23	55.22	25.40	2.75	86.6	2.38	2.5	50.78	1.27	26.51	4.57	8.57	2.27	24.24
1	7	E	21	23	23	55.22	25.40	2.75	89	2.45	3.3	50.78	1.68	26.17	6.02	8.79	2.30	23.87
1	7	F	22	23	23	55.22	25.40	2.75	89.76	2.47	1.6	50.78	0.81	27.06	2.92	8.86	2.40	24.66
1	7	G	23	23	23	55.22	25.40	2.75	89.02	2.45	0.8	50.78	0.41	27.44	1.46	8.79	2.41	25.03

1	7	H	24	23	23	55.22	25.40	2.75	86.6	2.38	1.7	50.75	0.86	26.92	3.11	8.57	2.31	24.61
												promedios		26.24	5.75	8.75	2.30	20.94

per	day	trat	vaca	oferta mezcla mixer kg AM	oferta mezcla mixer kg PM	oferta mezcla mixer %MS	oferta mezcla mixer kgMS/día (a)	oferta semilla de algodon kg AM+PM	oferta semilla de algodon %MS	oferta semilla de algodon kgMS/día (b)	remanen total kg AM+PM	remanen total %MS	remanen total kgMS/día (c)	consumo kg MS vaca/día (a+b)-(c)	remanente total % (MS)	participac algodón % (MS)	consumo algodón kgMS/d	consumo mezcla kgMS/d
2	1	F	13	22	23	51.36	23.11	2.75	91.53	2.52	5.8	46.4	2.69	22.94	10.50	9.82	2.25	20.69
2	1	G	14	22	23	51.36	23.11	2.75	91.59	2.52	0.3	46.4	0.14	25.49	0.54	9.83	2.51	22.99
2	1	H	15	22	23	51.36	23.11	2.75	90.63	2.49	11.1	46.4	5.15	20.45	20.12	9.73	1.99	18.46
2	1	E	16	22	23	51.36	23.11	2.75	92.25	2.54	1.8	46.4	0.84	24.81	3.26	9.89	2.45	22.36
2	1	G	17	22	23	51.36	23.11	2.75	91.59	2.52	1	46.4	0.46	25.17	1.81	9.83	2.47	22.69
2	1	E	18	22	23	51.36	23.11	2.75	92.25	2.54	3.9	46.4	1.81	23.84	7.06	9.89	2.36	21.48
2	1	H	19	22	23	51.36	23.11	2.75	90.63	2.49	4.7	46.4	2.18	23.42	8.52	9.73	2.28	21.14
2	1	F	20	22	23	51.36	23.11	2.75	91.53	2.52	1.5	46.4	0.70	24.93	2.72	9.82	2.45	22.48
2	1	F	21	22	23	51.36	23.11	2.75	91.53	2.52	6.3	46.4	2.92	22.71	11.41	9.82	2.29	20.48
2	1	H	22	22	23	51.36	23.11	2.75	90.63	2.49	3.7	46.4	1.72	23.89	6.71	9.73	2.33	21.56
2	1	E	23	22	23	51.36	23.11	2.75	92.25	2.54	0	46.4	0.00	25.65	0.00	9.89	2.54	23.11
2	1	G	24	22	23	51.36	23.11	2.75	91.59	2.52	1.4	46.4	0.65	24.98	2.53	9.83	2.45	22.53
												promedios		24.02	6.26	9.82	2.36	21.66

2	2	F	13	22	23	53.12	23.90	2.75	91.53	2.52	5.3	46.4	2.92	23.50	11.06	9.53	2.24	21.26
2	2	G	14	22	23	53.12	23.90	2.75	91.59	2.52	0.3	46.4	0.14	26.28	0.53	9.53	2.51	23.78
2	2	H	15	22	23	53.12	23.90	2.75	90.63	2.49	6	46.4	2.78	23.61	10.55	9.44	2.23	21.39
2	2	E	16	22	23	53.12	23.90	2.75	92.25	2.54	1.5	46.4	0.70	25.74	2.63	9.59	2.47	23.27
2	2	G	17	22	23	53.12	23.90	2.75	91.59	2.52	1.1	46.4	0.51	25.91	1.93	9.53	2.47	23.44
2	2	E	18	22	23	53.12	23.90	2.75	92.25	2.54	2.7	46.4	1.25	25.19	4.74	9.59	2.42	22.77
2	2	H	19	22	23	53.12	23.90	2.75	90.63	2.49	1.9	46.4	0.88	25.61	3.34	9.44	2.41	23.11
2	2	F	20	22	23	53.12	23.90	2.75	91.53	2.52	2.2	46.4	1.02	25.40	3.86	9.53	2.42	22.98
2	2	F	21	22	23	53.12	23.90	2.75	91.53	2.52	3.8	46.4	1.76	24.65	6.67	9.53	2.35	22.31
2	2	H	22	22	23	53.12	23.90	2.75	90.63	2.49	1.3	46.4	0.60	25.79	2.29	9.44	2.44	23.36
2	2	E	23	22	23	53.12	23.90	2.75	92.25	2.54	0.1	46.4	0.05	26.39	0.18	9.59	2.53	23.86
2	2	G	24	22	23	53.12	23.90	2.75	91.59	2.52	0.7	46.4	0.32	26.10	1.23	9.53	2.49	23.61
												promedios		25.34	4.09	9.52	2.41	22.93
2	3	F	13	22	23	53.26	23.97	2.75	91.53	2.52	5.6	46.4	2.55	23.93	9.64	9.50	2.27	21.66
2	3	G	14	22	23	53.26	23.97	2.75	91.59	2.52	0.8	46.4	0.37	26.11	1.40	9.51	2.48	23.63
2	3	H	15	22	23	53.26	23.97	2.75	90.63	2.49	6.8	46.4	3.16	23.30	11.92	9.42	2.20	21.11
2	3	E	16	22	23	53.26	23.97	2.75	92.25	2.54	2.2	46.4	1.02	25.48	3.85	9.57	2.44	23.04
2	3	G	17	22	23	53.26	23.97	2.75	91.59	2.52	1.2	46.4	0.56	25.93	2.10	9.51	2.47	23.46
2	3	E	18	22	23	53.26	23.97	2.75	92.25	2.54	2.9	46.4	1.35	25.16	5.08	9.57	2.41	22.75
2	3	H	19	22	23	53.26	23.97	2.75	90.63	2.49	2.2	46.4	1.02	25.44	3.86	9.42	2.40	23.04
2	3	F	20	22	23	53.26	23.97	2.75	91.53	2.52	2.6	46.4	1.21	25.28	4.56	9.50	2.40	22.88
2	3	F	21	22	23	53.26	23.97	2.75	91.53	2.52	7.4	46.4	3.43	23.05	12.95	9.50	2.19	20.86
2	3	H	22	22	23	53.26	23.97	2.75	90.63	2.49	0.7	46.4	0.32	26.13	1.23	9.42	2.46	23.67
2	3	E	23	22	23	53.26	23.97	2.75	92.25	2.54	0	46.4	0.00	26.50	0.00	9.57	2.54	23.97

2	3	G	24	22	23	53.26	23.97	2.75	91.59	2.52	0.6	46.4	0.37	26.11	1.40	9.51	2.48	23.63
												promedios		25.20	4.83	9.50	2.39	22.81
2	4	F	13	22	23	56.12	25.25	2.75	91.59	2.52	7.7	46.4	3.57	24.20	12.87	9.06	2.19	22.01
2	4	G	14	22	23	56.12	25.25	2.75	91.59	2.52	0.4	46.4	0.19	27.59	0.67	9.07	2.50	25.09
2	4	H	15	22	23	56.12	25.25	2.75	90.63	2.49	6.7	46.4	3.11	24.64	11.20	8.98	2.21	22.42
2	4	E	16	22	23	56.12	25.25	2.75	92.25	2.54	1.3	46.4	0.60	27.19	2.17	9.13	2.48	24.71
2	4	G	17	22	23	56.12	25.25	2.75	91.59	2.52	0.7	46.4	0.32	27.46	1.17	9.07	2.49	24.56
2	4	E	18	22	23	56.12	25.25	2.75	92.25	2.54	2.6	46.4	1.30	26.49	4.67	9.13	2.42	24.07
2	4	H	19	22	23	56.12	25.25	2.75	90.63	2.49	1.7	46.4	0.79	26.96	2.84	8.98	2.42	24.54
2	4	F	20	22	23	56.12	25.25	2.75	91.53	2.52	3.2	46.4	1.48	26.29	5.35	9.06	2.38	23.90
2	4	F	21	22	23	56.12	25.25	2.75	91.53	2.52	4.8	46.4	2.23	25.54	8.02	9.06	2.32	23.23
2	4	H	22	22	23	56.12	25.25	2.75	90.63	2.49	1.4	46.4	0.65	27.10	2.34	8.98	2.43	24.66
2	4	E	23	22	23	56.12	25.25	2.75	92.25	2.54	0	46.4	0.00	27.79	0.00	9.13	2.54	25.25
2	4	G	24	22	23	56.12	25.25	2.75	91.59	2.52	0.6	46.4	0.28	27.49	1.00	9.07	2.49	25.00
												promedios		26.56	4.36	9.06	2.41	24.15
2	5	F	13	22	23	53.23	23.95	2.75	91.53	2.52	5.2	46.4	2.41	24.06	9.12	9.51	2.29	21.77
2	5	G	14	22	23	53.23	23.95	2.75	91.59	2.52	0.6	46.4	0.28	26.19	1.05	9.51	2.49	23.70
2	5	H	15	22	23	53.23	23.95	2.75	90.63	2.49	7.6	46.4	3.53	22.92	13.33	9.42	2.16	20.76
2	5	E	16	22	23	53.23	23.95	2.75	92.25	2.54	2.4	46.4	1.11	25.38	4.20	9.58	2.43	22.95
2	5	G	17	22	23	53.23	23.95	2.75	91.59	2.52	0.7	46.4	0.32	26.15	1.23	9.51	2.49	23.66
2	5	E	18	22	23	53.23	23.95	2.75	92.25	2.54	10.1	46.4	4.69	21.80	17.69	9.58	2.09	19.72
2	5	H	19	22	23	53.23	23.95	2.75	90.63	2.49	2.0	46.4	1.07	25.35	4.04	9.42	2.39	22.99
2	5	F	20	22	23	53.23	23.95	2.75	91.53	2.52	1.9	46.4	0.88	25.59	3.33	9.51	2.43	23.16
2	5	F	21	22	23	53.23	23.95	2.75	91.53	2.52	4.5	46.4	2.09	24.38	7.89	9.51	2.32	22.06
2	5	H	22	22	23	53.23	23.95	2.75	90.63	2.49	1.2	46.4	0.56	25.89	2.11	9.42	2.44	23.45
2	5	E	23	22	23	53.23	23.95	2.75	92.25	2.54	0.2	46.4	0.09	26.40	0.35	9.58	2.53	23.87
2	5	G	24	22	23	53.23	23.95	2.75	91.59	2.52	1.7	46.4	0.79	25.68	2.98	9.51	2.44	23.24
												promedios		24.98	5.61	9.51	2.38	22.61
2	6	F	13	22	23	53.91	24.26	2.75	91.53	2.52	6.8	46.4	4.06	22.69	15.25	9.40	2.13	20.56
2	6	G	14	22	23	53.91	24.26	2.75	91.59	2.52	0.4	46.4	0.19	26.59	0.69	9.41	2.50	24.09
2	6	H	15	22	23	53.91	24.26	2.75	90.63	2.49	8.4	46.4	3.90	22.85	14.57	9.32	2.13	20.73
2	6	E	16	22	23	53.91	24.26	2.75	92.25	2.54	1.4	46.4	0.65	26.15	2.42	9.47	2.48	23.67
2	6	G	17	22	23	53.91	24.26	2.75	91.59	2.52	0.7	46.4	0.32	26.45	1.21	9.41	2.49	23.97
2	6	E	18	22	23	53.91	24.26	2.75	92.25	2.54	3.7	46.4	1.72	25.08	6.41	9.47	2.37	22.71
2	6	H	19	22	23	53.91	24.26	2.75	90.63	2.49	1.9	46.4	0.88	25.87	3.30	9.32	2.41	23.46
2	6	F	20	22	23	53.91	24.26	2.75	91.53	2.52	3.2	46.4	1.48	25.29	5.55	9.40	2.38	22.91
2	6	F	21	22	23	53.91	24.26	2.75	91.53	2.52	2.7	46.4	1.25	25.52	4.68	9.40	2.40	23.12
2	6	H	22	22	23	53.91	24.26	2.75	90.63	2.49	1.2	46.4	0.56	26.20	2.08	9.32	2.44	23.75
2	6	E	23	22	23	53.91	24.26	2.75	92.25	2.54	0.1	46.4	0.05	26.75	0.17	9.47	2.53	24.22
2	6	G	24	22	23	53.91	24.26	2.75	91.59	2.52	1.3	46.4	0.60	26.18	2.25	9.41	2.46	23.71
												promedios		25.47	4.88	9.40	2.39	23.08

2	7	F	13	22	23	52.62	23.68	2.75	91.53	2.52	7.7	46.4	3.57	22.62	13.64	9.61	2.17	20.45
2	7	G	14	22	23	52.62	23.68	2.75	91.59	2.52	0.6	46.4	0.28	25.92	1.06	9.61	2.49	23.43
2	7	H	15	22	23	52.62	23.68	2.75	90.63	2.49	5.1	46.4	2.37	23.60	9.04	9.62	2.27	21.34
2	7	E	16	22	23	52.62	23.68	2.75	92.25	2.54	3.1	46.4	1.44	24.78	5.49	9.65	2.40	22.38
2	7	G	17	22	23	52.62	23.68	2.75	91.59	2.52	0.9	46.4	0.42	25.78	1.59	9.61	2.48	23.30
2	7	E	18	22	23	52.62	23.68	2.75	92.25	2.54	6.6	46.4	3.02	23.20	11.50	9.68	2.25	20.95
2	7	H	19	22	23	52.62	23.68	2.75	90.63	2.49	2.9	46.4	1.35	24.83	5.14	9.62	2.38	22.46
2	7	F	20	22	23	52.62	23.68	2.75	91.53	2.52	2	46.4	0.93	25.27	3.54	9.61	2.43	22.84
2	7	F	21	22	23	52.62	23.68	2.75	91.53	2.52	4.3	46.4	2.00	24.20	7.62	9.61	2.33	21.88
2	7	H	22	22	23	52.62	23.68	2.75	90.63	2.49	2.1	46.4	0.97	25.20	3.72	9.52	2.40	22.80
2	7	E	23	22	23	52.62	23.68	2.75	92.25	2.54	0.1	46.4	0.05	26.17	0.18	9.68	2.53	23.64
2	7	G	24	22	23	52.62	23.68	2.75	91.59	2.52	1.7	46.4	0.79	25.41	3.01	9.61	2.44	22.97
												promedios		24.76	5.46	9.61	2.36	22.39

per	day	trat	vaca	oferta mezcla mixer kg AM	oferta mezcla mixer kg PM	oferta mezcla mixer %MS	oferta mezcla mixer kgMS/día (a)	oferta semilla de algodon kg AM+PM	oferta semilla de algodon %MS	oferta semilla de algodon kgMS/día (b)	remanen total kg AM+PM	remanen total %MS	remanen total kgMS/día (c)	consumo kg MS vaca/día (a+b)-(c)	remanente total % (MS)	participac algodón % (MS)	consumo algodón kgMS/d	consumo mezcla kgMS/d
3	1	H	13	22	22	64.45	23.96	2.75	92.28	2.54	12.7	55.37	7.03	19.46	26.54	9.58	1.86	17.60
3	1	E	14	22	22	64.45	23.96	2.75	92.12	2.53	9.5	55.37	5.26	21.23	19.86	9.56	2.03	19.20
3	1	F	15	22	22	64.45	23.96	2.75	92.46	2.54	17.7	55.37	9.80	16.70	36.98	9.59	1.69	15.10
3	1	G	16	22	22	64.45	23.96	2.75	91.66	2.52	16.4	55.37	8.53	17.95	32.20	9.52	1.71	16.24
3	1	F	17	22	22	64.45	23.96	2.75	92.46	2.54	5.8	55.37	3.21	23.29	12.12	9.59	2.23	21.05
3	1	H	18	22	22	64.45	23.96	2.75	92.28	2.54	8.7	55.37	4.82	21.68	18.18	9.58	2.08	19.60
3	1	E	19	22	22	64.45	23.96	2.75	92.12	2.53	12.1	55.37	6.70	19.79	25.29	9.56	1.89	17.90
3	1	G	20	22	22	64.45	23.96	2.75	91.66	2.52	17.3	55.37	9.58	16.90	36.18	9.52	1.61	15.29
3	1	G	21	22	22	64.45	23.96	2.75	91.66	2.52	7.5	55.37	4.15	22.33	15.65	9.52	2.13	20.20
3	1	E	22	22	22	64.45	23.96	2.75	92.12	2.53	2.6	55.37	1.44	25.05	5.43	9.56	2.40	22.66
3	1	H	23	22	22	64.45	23.96	2.75	92.28	2.54	10.4	55.37	5.76	20.74	21.73	9.58	1.99	18.75
3	1	F	24	22	22	64.45	23.96	2.75	92.46	2.54	2.1	55.37	1.16	25.34	4.39	9.59	2.43	22.91
											promedios		5.62	20.87	21.22	9.56	2.00	18.88
3	2	H	13	22	22	60.93	26.81	2.75	92.28	2.54	14.7	55.37	8.14	21.21	27.74	8.65	1.83	19.37
3	2	E	14	22	22	60.93	26.81	2.75	92.12	2.53	13.1	55.37	7.25	22.09	24.72	8.63	1.91	20.18
3	2	F	15	22	22	60.93	26.81	2.75	92.46	2.54	13.6	55.37	7.53	21.82	25.66	8.66	1.89	19.93
3	2	G	16	22	22	60.93	26.81	2.75	91.66	2.52	15.8	55.37	8.75	20.58	29.83	8.59	1.77	18.81
3	2	F	17	22	22	60.93	26.81	2.75	92.46	2.54	7.2	55.37	3.99	25.37	13.58	8.66	2.20	23.17
3	2	H	18	22	22	60.93	26.81	2.75	92.28	2.54	9.2	55.37	5.09	24.25	17.36	8.65	2.10	22.16
3	2	E	19	22	22	60.93	26.81	2.75	92.12	2.53	13.1	55.37	7.25	22.09	24.72	8.63	1.91	20.18
3	2	G	20	22	22	60.93	26.81	2.75	91.66	2.52	16.5	55.37	9.14	20.19	31.15	8.59	1.74	18.46
3	2	G	21	22	22	60.93	26.81	2.75	91.66	2.52	15.1	55.37	8.36	20.97	28.51	8.59	1.80	19.17
3	2	E	22	22	22	60.93	26.81	2.75	92.12	2.53	3	55.37	1.66	27.68	5.66	8.63	2.39	25.29
3	2	H	23	22	22	60.93	26.81	2.75	92.28	2.54	14.3	55.37	7.92	21.43	26.99	8.65	1.85	19.58
3	2	F	24	22	22	60.93	26.81	2.75	92.46	2.54	1.6	55.37	0.89	28.47	3.02	8.66	2.47	26.00
											promedios		6.03	23.01	21.58	8.63	1.99	21.02
3	3	H	13	22	22	64.27	28.23	2.75	92.28	2.54	10.6	39.72	4.21	26.61	13.66	8.23	2.19	24.42
3	3	E	14	22	22	64.27	28.23	2.75	92.12	2.53	7	39.72	2.78	28.03	9.02	8.22	2.30	25.73
3	3	F	15	22	22	64.27	28.23	2.75	92.46	2.54	15.6	39.72	6.20	24.63	20.10	8.25	2.03	22.69
3	3	G	16	22	22	64.27	28.23	2.75	91.66	2.52	17.1	39.72	6.79	24.01	22.05	8.16	1.95	22.04

3	3	F	17	22	22	64.27	28.28	2.75	92.46	2.54	8.7	39.72	3.46	27.37	11.21	8.25	2.26	25.11
3	3	H	18	22	22	64.27	28.28	2.75	92.28	2.54	12.2	39.72	4.85	25.97	15.72	8.23	2.14	23.83
3	3	E	19	22	22	64.27	28.28	2.75	92.12	2.53	14	39.72	5.56	25.25	18.05	8.22	2.08	23.16
3	3	G	20	22	22	64.27	28.28	2.75	91.66	2.52	10.1	39.72	4.01	26.79	13.03	8.18	2.19	24.60
3	3	G	21	22	22	64.27	28.28	2.75	91.66	2.52	4.4	39.72	1.75	29.05	5.67	8.18	2.38	26.67
3	3	E	22	22	22	64.27	28.28	2.75	92.12	2.53	2.4	39.72	0.95	29.86	3.09	8.22	2.45	27.40
3	3	H	23	22	22	64.27	28.28	2.75	92.28	2.54	6.5	39.72	2.58	28.23	8.38	8.23	2.33	25.91
3	3	F	24	22	22	64.27	28.28	2.75	92.46	2.54	5.9	39.72	2.34	28.48	7.60	8.25	2.35	26.13
											promedios		3.79	27.02	12.30	8.22	2.22	24.80
3	4	H	10	22	22	60.71	26.71	2.75	92.28	2.54	8.1	45.5	3.69	25.66	12.60	8.68	2.22	23.35
3	4	E	14	22	22	60.71	26.71	2.75	92.12	2.53	3	45.5	1.37	27.83	4.67	8.66	2.42	25.47
3	4	F	15	22	22	60.71	26.71	2.75	92.46	2.54	9.6	45.5	4.37	24.89	14.93	8.69	2.16	22.72
3	4	G	16	22	22	60.71	26.71	2.75	91.66	2.52	6.8	45.5	3.09	26.14	10.58	8.62	2.25	23.89
3	4	F	17	22	22	60.71	26.71	2.75	92.46	2.54	10.6	45.5	4.82	24.43	16.49	8.69	2.12	22.31
3	4	H	18	22	22	60.71	26.71	2.75	92.28	2.54	11.1	45.5	5.05	24.20	17.27	8.68	2.10	22.10
3	4	E	19	22	22	60.71	26.71	2.75	92.12	2.53	9.3	45.5	4.23	25.01	14.47	8.66	2.17	22.85
3	4	G	20	22	22	60.71	26.71	2.75	91.66	2.52	10.1	45.5	4.60	24.64	15.72	8.62	2.12	22.51
3	4	G	21	22	22	60.71	26.71	2.75	91.66	2.52	6.9	45.5	3.14	26.09	10.74	8.62	2.25	23.84
3	4	E	22	22	22	60.71	26.71	2.75	92.12	2.53	3.2	45.5	1.46	27.79	4.98	8.66	2.41	25.38
3	4	H	23	22	22	60.71	26.71	2.75	92.28	2.54	9	45.5	4.10	25.16	14.00	8.68	2.18	22.97
3	4	F	24	22	22	60.71	26.71	2.75	92.46	2.54	2.3	45.5	1.05	28.21	3.58	8.69	2.45	25.76
											promedios		3.41	25.83	11.67	8.66	2.24	23.60
3	5	H	13	22	22	55.72	24.52	2.75	92.28	2.54	8.5	55.37	4.71	22.35	17.40	9.38	2.10	20.25
3	5	E	14	22	22	55.72	24.52	2.75	92.12	2.53	0.6	55.37	0.33	26.72	1.23	9.37	2.50	24.22
3	5	F	15	22	22	55.72	24.52	2.75	92.46	2.54	9.7	55.37	5.37	21.69	19.85	9.40	3.04	19.65
3	5	G	16	22	22	55.72	24.52	2.75	91.66	2.52	13.3	55.37	7.36	19.67	27.24	9.32	1.83	17.84
3	5	F	17	22	22	55.72	24.52	2.75	92.46	2.54	2.5	55.37	1.38	25.68	5.12	9.40	2.41	23.26
3	5	H	18	22	22	55.72	24.52	2.75	92.28	2.54	6.9	55.37	3.82	23.23	14.12	9.38	2.18	21.05
3	5	E	19	22	22	55.72	24.52	2.75	92.12	2.53	8.6	55.37	4.76	22.29	17.60	9.37	2.09	20.20
3	5	G	20	22	22	55.72	24.52	2.75	91.66	2.52	5.8	55.37	3.21	23.83	11.88	9.32	2.22	21.60
3	5	G	21	22	22	55.72	24.52	2.75	91.66	2.52	7.4	55.37	4.10	22.94	15.15	9.32	2.14	20.80
3	5	E	22	22	22	55.72	24.52	2.75	92.12	2.53	1	55.37	0.55	26.50	2.05	9.37	2.48	24.01
3	5	H	23	22	22	55.72	24.52	2.75	92.28	2.54	0.6	55.37	0.33	26.72	1.23	9.38	2.51	24.22
3	5	F	24	22	22	55.72	24.52	2.75	92.46	2.54	3.4	55.37	1.88	25.18	6.96	9.40	2.37	22.81
											promedios		3.15	23.90	11.66	9.37	2.24	21.66
3	6	H	13	22	22	52.25	22.99	2.75	92.28	2.54	21.5	55.37	11.90	13.62	46.60	9.94	1.05	12.27
3	6	E	14	22	22	52.25	22.99	2.75	92.12	2.53	3	55.37	1.66	23.86	6.51	9.93	2.37	21.49
3	6	F	15	22	22	52.25	22.99	2.75	92.46	2.54	12.1	55.37	6.70	18.83	26.24	9.96	1.88	16.96
3	6	G	16	22	22	52.25	22.99	2.75	91.66	2.52	9.4	55.37	5.20	20.31	20.40	9.86	2.01	18.30
3	6	F	17	22	22	52.25	22.99	2.75	92.46	2.54	6.8	55.37	3.77	21.77	14.75	9.96	2.17	19.60
3	6	H	18	22	22	52.25	22.99	2.75	92.28	2.54	8.1	55.37	4.48	21.04	17.57	9.94	2.09	18.95
3	6	E	19	22	22	52.25	22.99	2.75	92.12	2.53	6.8	55.37	3.77	21.76	14.75	9.93	2.16	19.60
3	6	G	20	22	22	52.25	22.99	2.75	91.66	2.52	44	55.37	24.36	1.15	95.50	9.88	0.11	1.03
3	6	G	21	22	22	52.25	22.99	2.75	91.66	2.52	5.8	55.37	3.21	22.30	12.59	9.85	2.20	20.10
3	6	E	22	22	22	52.25	22.99	2.75	92.12	2.53	1.2	55.37	0.66	24.86	2.60	9.93	2.47	22.39
3	6	H	23	22	22	52.25	22.99	2.75	92.28	2.54	4.6	55.37	2.55	22.98	9.98	9.94	2.28	20.70
3	6	F	24	22	22	52.25	22.99	2.75	92.46	2.54	3.5	55.37	1.94	23.69	7.59	9.96	2.35	21.25
											promedios		5.85	19.67	22.93	9.93	1.95	17.72
3	7	H	13	22	22	50.29	20.45	2.75	92.28	2.54	6.1	55.37	3.38	22.61	13.00	9.77	2.21	20.40
3	7	E	14	22	22	50.29	20.45	2.75	92.12	2.53	0.9	55.37	0.50	25.48	1.92	9.75	2.48	23.00

3	7	F	16	22	22	53.29	23.45	2.75	92.46	2.54	7.5	56.37	4.16	21.84	15.98	9.78	2.14	19.70
3	7	G	16	22	22	53.29	23.45	2.75	91.66	2.52	3.5	56.37	1.94	24.03	7.46	9.71	2.09	21.70
3	7	F	17	22	22	53.29	23.45	2.75	92.46	2.54	2.5	56.37	1.38	24.61	5.39	9.78	2.41	22.20
3	7	H	16	22	22	53.29	23.45	2.76	92.28	2.54	3.3	56.37	1.83	24.16	7.03	9.77	2.36	21.80
3	7	E	19	22	22	53.29	23.45	2.75	92.12	2.53	4.3	56.37	2.38	23.60	9.16	9.75	2.30	21.30
3	7	G	20	22	22	53.29	23.45	2.75	91.66	2.52	14.6	56.37	8.08	17.88	31.13	9.71	1.74	16.15
3	7	G	21	22	22	53.29	23.45	2.75	91.66	2.52	2.7	56.37	1.49	24.47	5.76	9.71	2.36	22.10
3	7	E	22	22	22	53.29	23.45	2.75	92.12	2.53	0.9	56.37	0.50	25.46	1.92	9.75	2.48	23.00
3	7	H	23	22	22	53.29	23.45	2.75	92.28	2.54	0.1	56.37	0.06	25.90	0.21	9.77	2.53	23.40
3	7	F	24	22	22	53.29	23.45	2.75	92.46	2.54	2.3	56.37	1.27	24.72	4.90	9.78	2.42	22.30
											promedios		2.25	23.73	8.65	9.75	2.31	21.42

per	day	trat	vaca	oferta mezcla mixer kg AM	oferta mezcla mixer kg PM	oferta mezcla mixer %MS	oferta mezcla mixer kgMS/día (a)	oferta semilla de algodon kg AM+PM	oferta semilla de algodon %MS	oferta semilla de algodon kgMS/día (b)	remanen total kg AM+PM	remanen total %MS	remanen total kgMS/día [c]	consumo kg MS vaca/día (a+b)-[c]	remanente total % (MS)	participac algodón % (MS)	consumo algodón kgMS/d	consumo mezcla kgMS/d
4	1	g	13	17	17	57.4	19.52	2.75	91.71	2.52	2.7	55.47	1.50	20.54	6.80	11.44	2.35	18.19
4	1	h	14	17	17	57.4	19.52	2.75	92.03	2.53	3.1	55.47	1.72	20.33	7.80	11.48	2.33	17.99
4	1	e	15	17	17	57.4	19.52	2.76	92.28	2.54	7.5	55.47	4.16	17.89	16.86	11.51	2.06	15.83
4	1	f	16	17	17	57.4	19.52	2.75	92.51	2.54	6.9	55.47	3.83	18.23	17.35	11.53	2.10	16.13
4	1	h	17	17	17	57.4	19.52	2.75	92.03	2.53	2.2	55.47	1.22	20.83	5.54	11.48	2.39	18.44
4	1	g	18	17	17	57.4	19.52	2.75	91.71	2.52	2.7	55.47	1.50	20.54	6.80	11.44	2.35	18.19
4	1	f	19	17	17	57.4	19.52	2.75	92.51	2.54	3.4	55.47	1.89	20.17	8.55	11.53	2.30	17.85
4	1	e	20	17	17	57.4	19.52	2.75	92.28	2.54	9.8	55.47	5.44	16.62	24.65	11.51	1.91	14.71
4	1	h	21	17	17	57.4	19.52	2.75	92.03	2.53	2	55.47	1.11	20.94	5.03	11.48	2.40	18.53
4	1	g	22	17	17	57.4	19.52	2.75	91.71	2.52	1.4	55.47	0.78	21.26	3.52	11.44	2.40	18.63
4	1	f	23	17	17	57.4	19.52	2.75	92.51	2.54	2.7	55.47	1.50	20.56	6.79	11.53	2.37	18.19
4	1	e	24	17	17	57.4	19.52	2.75	92.28	2.54	2.2	55.47	1.22	20.83	5.53	11.51	2.40	18.44
												promedios		19.90	9.77	11.49	2.29	17.61
4	2	g	13	17	17	60.34	20.62	2.75	91.71	2.52	4.3	43.74	1.88	21.16	8.16	10.95	2.32	18.84
4	2	h	14	17	17	60.34	20.62	2.75	92.03	2.53	3.5	43.74	1.53	21.52	6.64	10.98	2.36	19.15
4	2	e	15	17	17	60.34	20.62	2.76	92.28	2.54	15.3	43.74	6.69	16.36	29.03	11.01	1.80	14.56
4	2	f	16	17	17	60.34	20.62	2.75	92.51	2.54	8.7	43.74	3.81	19.25	16.50	11.03	2.12	17.13
4	2	h	17	17	17	60.34	20.62	2.75	92.03	2.53	6.9	43.74	3.02	20.03	13.10	10.98	2.20	17.83
4	2	g	18	17	17	60.34	20.62	2.75	91.71	2.52	5.7	43.74	2.49	20.54	10.82	10.95	2.25	18.30
4	2	f	19	17	17	60.34	20.62	2.75	92.51	2.54	5.4	43.74	2.36	20.70	10.24	11.03	2.28	18.41
4	2	e	20	17	17	60.34	20.62	2.76	92.28	2.54	8.1	43.74	3.54	19.51	15.37	11.01	2.15	17.36
4	2	h	21	17	17	60.34	20.62	2.75	92.03	2.53	2.7	43.74	1.18	21.87	5.12	10.98	2.40	19.46
4	2	g	22	17	17	60.34	20.62	2.75	91.71	2.52	2.8	43.74	1.22	21.81	5.32	10.95	2.39	19.42
4	2	f	23	17	17	60.34	20.62	2.76	92.51	2.54	12.8	43.74	5.60	17.46	24.26	11.03	1.93	15.53
4	2	e	24	17	17	60.34	20.62	2.76	92.28	2.54	3.6	43.74	1.57	21.48	6.83	11.01	2.38	19.11
												promedios		20.14	12.62	10.99	2.21	17.93
4	3	g	13	17	17	61.14	20.79	2.75	91.71	2.52	1.2	55.47	0.67	22.64	2.66	10.82	2.45	20.19
4	3	h	14	17	17	61.14	20.79	2.75	92.03	2.53	0.3	55.47	0.17	23.16	0.71	10.85	2.51	20.64
4	3	e	15	17	17	61.14	20.79	2.75	92.28	2.54	18.1	55.47	10.04	13.29	43.04	10.89	1.45	11.84
4	3	f	16	17	17	61.14	20.79	2.75	92.51	2.54	6.3	55.47	3.49	19.84	14.98	10.90	2.16	17.67
4	3	h	17	17	17	61.14	20.79	2.75	92.03	2.53	1.4	55.47	0.78	22.54	3.33	10.86	2.45	20.10
4	3	g	18	17	17	61.14	20.79	2.75	91.71	2.52	2.7	55.47	1.50	21.81	6.43	10.82	2.36	19.45
4	3	f	19	17	17	61.14	20.79	2.75	92.51	2.54	1.6	55.47	0.89	22.44	3.80	10.90	2.45	20.00
4	3	e	20	17	17	61.14	20.79	2.75	92.28	2.54	1.1	55.47	0.61	22.72	2.62	10.88	2.47	20.24

4	3	h	21	17	17	61.14	20.79	2.75	92.03	2.53	1.2	55.47	0.67	22.65	2.85	10.85	2.46	20.19
4	3	g	22	17	17	61.14	20.79	2.75	91.71	2.52	0.6	55.47	0.28	23.03	1.19	10.82	2.49	20.54
4	3	f	23	17	17	61.14	20.79	2.75	92.51	2.54	0.6	55.47	0.33	23.00	1.40	10.90	2.51	20.49
4	3	o	24	17	17	61.14	20.79	2.75	92.28	2.54	1.4	55.47	0.78	22.55	3.33	10.85	2.45	20.10
											promedios			21.64	7.21	10.85	2.35	19.29
4	4	g	13	17	17	57.09	19.41	2.75	91.71	2.52	0.8	55.47	0.44	21.49	2.02	11.50	2.47	19.02
4	4	h	14	17	17	57.09	19.41	2.75	92.03	2.53	0.6	55.47	0.33	21.61	1.52	11.53	2.49	19.12
4	4	o	15	17	17	57.09	19.41	2.75	92.28	2.54	10.5	55.47	5.82	16.12	26.54	11.56	1.88	14.25
4	4	f	16	17	17	57.09	19.41	2.75	92.51	2.54	1.1	55.47	0.61	21.34	2.78	11.59	2.47	18.87
4	4	h	17	17	17	57.09	19.41	2.75	92.03	2.53	5	55.47	2.77	19.17	12.64	11.53	2.21	16.96
4	4	g	18	17	17	57.09	19.41	2.75	91.71	2.52	1.1	55.47	0.61	21.32	2.78	11.50	2.45	18.87
4	4	f	19	17	17	57.09	19.41	2.75	92.51	2.54	0.8	55.47	0.44	21.61	2.02	11.59	2.49	19.02
4	4	o	20	17	17	57.09	19.41	2.75	92.28	2.54	2.8	55.47	1.55	20.40	7.08	11.66	2.36	18.04
4	4	h	21	17	17	57.09	19.41	2.75	92.03	2.53	0.7	55.47	0.39	21.55	1.77	11.53	2.49	19.07
4	4	g	22	17	17	57.09	19.41	2.75	91.71	2.52	1	55.47	0.55	21.38	2.53	11.50	2.46	18.92
4	4	f	23	17	17	57.09	19.41	2.75	92.51	2.54	0.2	55.47	0.11	21.84	0.51	11.59	2.53	19.31
4	4	o	24	17	17	57.09	19.41	2.75	92.28	2.54	0.4	55.47	0.22	21.73	1.01	11.56	2.51	19.21
											promedios			20.79	5.27	11.55	2.40	18.39
4	5	g	13	17	17	59.16	20.11	2.75	91.71	2.52	0.6	55.47	0.33	22.30	1.47	11.14	2.48	19.82
4	5	h	14	17	17	59.16	20.11	2.75	92.03	2.53	0.5	55.47	0.28	22.37	1.22	11.18	2.50	19.87
4	5	o	15	17	17	59.16	20.11	2.75	92.28	2.54	11.7	55.47	6.49	16.16	28.65	11.20	1.81	14.35
4	5	f	16	17	17	59.16	20.11	2.75	92.51	2.54	2.6	55.47	1.44	21.22	6.37	11.23	2.38	18.83
4	5	h	17	17	17	59.16	20.11	2.75	92.03	2.53	1.1	55.47	0.61	22.04	2.69	11.18	2.46	19.57
4	5	g	18	17	17	59.16	20.11	2.75	91.71	2.52	1.8	55.47	1.00	21.64	4.41	11.14	2.41	19.23
4	5	f	19	17	17	59.16	20.11	2.75	92.51	2.54	0.5	55.47	0.28	22.38	1.22	11.23	2.51	19.87
4	5	o	20	17	17	59.16	20.11	2.75	92.28	2.54	2.3	55.47	1.28	21.35	5.63	11.20	2.39	18.98
4	5	h	21	17	17	59.16	20.11	2.75	92.03	2.53	0.8	55.47	0.44	22.20	1.96	11.18	2.48	19.72
4	5	g	22	17	17	59.16	20.11	2.75	91.71	2.52	0.9	55.47	0.50	22.14	2.21	11.14	2.47	19.67
4	5	f	23	17	17	59.16	20.11	2.75	92.51	2.54	0.4	55.47	0.22	22.44	0.98	11.23	2.52	19.92
4	5	o	24	17	17	59.16	20.11	2.75	92.28	2.54	0.5	55.47	0.28	22.37	1.22	11.20	2.51	19.87
											promedios			21.55	4.84	11.19	2.41	19.14
4	6	g	13	17	17	59.22	20.13	2.75	91.71	2.52	0.8	55.47	0.44	22.21	1.96	11.13	2.47	19.74
4	6	h	14	17	17	59.22	20.13	2.75	92.03	2.53	0.7	55.47	0.39	22.28	1.71	11.17	2.49	19.79
4	6	e	15	17	17	59.22	20.13	2.75	92.28	2.54	7.8	55.47	4.33	18.35	19.08	11.19	2.05	16.29
4	6	f	16	17	17	59.22	20.13	2.75	92.51	2.54	5	55.47	2.77	19.91	12.23	11.22	2.23	17.67
4	6	h	17	17	17	59.22	20.13	2.75	92.03	2.53	1	55.47	0.55	22.11	2.45	11.17	2.47	19.64
4	6	g	18	17	17	59.22	20.13	2.75	91.71	2.52	0.9	55.47	0.50	22.16	2.20	11.13	2.47	19.69
4	6	f	19	17	17	59.22	20.13	2.75	92.51	2.54	0.3	55.47	0.17	22.51	0.73	11.22	2.53	19.99
4	6	e	20	17	17	59.22	20.13	2.75	92.28	2.54	1.1	55.47	0.61	22.06	2.69	11.19	2.47	19.59
4	6	h	21	17	17	59.22	20.13	2.75	92.03	2.53	0.6	55.47	0.33	22.33	1.47	11.17	2.49	19.84
4	6	g	22	17	17	59.22	20.13	2.75	91.71	2.52	0.5	55.47	0.28	22.38	1.22	11.13	2.49	19.89
4	6	f	23	17	17	59.22	20.13	2.75	92.51	2.54	0	55.47	0.00	22.68	0.00	11.22	2.54	20.13
4	6	o	24	17	17	59.22	20.13	2.75	92.28	2.54	0.2	55.47	0.11	22.56	0.49	11.19	2.53	20.04
											promedios			21.79	3.85	11.18	2.44	19.36
4	7	g	13	17	17	59.9	20.37	2.75	91.71	2.52	2.7	55.47	1.50	21.39	6.54	11.02	2.36	19.03
4	7	h	14	17	17	59.9	20.37	2.75	92.03	2.53	1.7	55.47	0.94	21.95	4.12	11.05	2.43	19.53
4	7	o	15	17	17	59.9	20.37	2.75	92.28	2.54	9.8	55.47	5.44	17.47	23.73	11.08	1.94	15.53
4	7	f	16	17	17	59.9	20.37	2.75	92.51	2.54	6	55.47	3.33	19.58	14.53	11.10	2.17	17.41
4	7	h	17	17	17	59.9	20.37	2.75	92.03	2.53	0.2	55.47	0.11	22.79	0.48	11.05	2.52	20.27

4	7	g	18	17	17	59.9	20.37	2.75	91.71	2.52	0.2	55.47	0.11	22.78	0.48	11.02	2.51	20.27
4	7	f	19	17	17	59.9	20.37	2.75	92.51	2.54	0.2	55.47	0.11	22.80	0.48	11.10	2.50	20.27
4	7	e	20	17	17	59.9	20.37	2.75	92.28	2.54	5.3	55.47	2.94	19.96	12.84	11.08	2.21	17.75
4	7	h	21	17	17	59.9	20.37	2.76	92.03	2.50	0.7	65.47	0.39	22.51	1.70	11.05	2.49	20.02
4	7	g	22	17	17	69.9	20.37	2.76	91.71	2.62	0.2	55.47	0.11	22.78	0.48	11.02	2.51	20.27
4	7	f	23	17	17	59.9	20.37	2.75	92.51	2.54	0.7	55.47	0.39	22.52	1.69	11.10	2.50	20.02
4	7	e	24	17	17	59.9	20.37	2.75	92.28	2.54	0	55.47	0.00	22.90	0.00	11.03	2.54	20.37
												promedios		21.62	5.59	11.06	2.39	19.23

Attachment 6

Daily Milk Yield Data

pp. 54-56

ATTACHMENT 6.

Daily Milk Yield Data (kg/cow/day)

						milk yield kg/d							
						day							
box	color	period	cow	square	treatment	1	2	3	4	5	6	7	average
13	rosa	1	2670	1	e	23.20	22.80	22.00	24.00	26.40	.	23.60	23.67
14	verde	1	2442	1	f	33.80	36.40	26.20	32.00	31.20	26.80	30.40	30.97
15	gris	1	2671	1	g	30.40	28.00	29.60	29.60	28.80	28.40	29.00	29.11
16	naranja	1	2584	1	h	35.40	35.80	30.60	34.80	31.60	31.40	32.00	33.09
17	rosa	1	2574	2	e	34.80	31.80	32.60	32.80	29.20	26.80	30.00	31.14
18	verde	1	2591	2	f	29.60	25.00	31.00	22.40	28.80	31.00	30.80	28.37
19	gris	1	2663	2	g	28.40	27.40	29.20	28.80	27.00	27.60	25.20	27.66
20	naranja	1	2586	2	h	32.00	30.40	32.00	29.60	30.60	21.60	28.10	29.19
21	rosa	1	2579	3	e	32.40	31.80	30.40	33.80	31.60	29.80	31.00	31.54
22	verde	1	2401	3	f	36.60	32.60	35.00	33.80	35.40	32.00	33.40	34.11
23	gris	1	2416	3	g	41.80	39.60	42.20	40.80	37.80	38.40	36.40	39.57
24	naranja	1	2555	3	h	39.80	38.20	38.00	36.00	40.00	37.60	37.40	38.14
						33.18	31.65	31.57	31.53	31.53	30.13	30.61	31.38

						milk yield kg/d							
						day							
box	color	period	cow	square	treatment	1	2	3	4	5	6	7	average
13	verde	2	2670	1	f	24.80	24.00	26.20	19.80	24.20	24.60	25.80	24.20
14	gris	2	2442	1	g	32.60	35.60	34.20	31.40	30.00	37.80	31.80	33.34
15	naranja	2	2671	1	h	24.60	24.40	25.60	25.40	26.60	25.20	.	25.30
16	rosa	2	2584	1	e	30.80	35.60	31.80	35.60	33.00	33.40	30.20	32.91
17	gris	2	2574	2	g	30.00	28.20	30.00	29.40	29.80	31.40	26.80	29.37
18	rosa	2	2591	2	e	30.20	25.60	30.40	28.20	28.80	29.00	27.80	28.57
19	naranja	2	2663	2	h	27.80	27.20	26.80	28.20	27.00	28.60	27.40	27.57
20	verde	2	2586	2	f	27.10	28.20	27.60	29.60	28.60	26.60	27.60	27.90
21	verde	2	2579	3	f	31.40	30.00	32.60	33.00	31.40	31.80	29.20	31.34
22	naranja	2	2401	3	h	28.40	28.60	27.80	26.60	29.20	34.00	31.20	29.40
23	rosa	2	2416	3	e	38.80	.	37.60	40.80	37.40	35.80	35.60	37.67
24	gris	2	2555	3	g	36.60	37.40	34.40	35.20	31.60	33.60	32.00	34.40
						30.26	29.53	30.42	30.27	29.80	30.98	29.58	30.17

						milk yield kg/d							
						day							
box	color	period	cow	square	treatment	1	2	3	4	5	6	7	average
13	naranja	3	2670	1	h	22.80	21.60	22.00	22.60	22.60	17.20	14.20	20.43
14	rosa	3	2442	1	e	24.20	25.00	24.20	27.80	27.80	30.80	30.80	27.23
15	verde	3	2671	1	f	19.00	19.20	20.40	19.60	18.80	18.60	19.40	19.29
16	gris	3	2584	1	g	20.00	18.80	20.80	25.20	23.60	25.60	27.20	23.03
17	verde	3	2574	2	f	25.40	22.80	21.60	23.20	24.60	24.00	26.20	23.97
18	naranja	3	2591	2	h	25.80	24.40	24.20	21.40	20.80	22.00	23.20	23.11
19	rosa	3	2663	2	e	22.20	21.60	21.20	22.80	21.20	23.60	24.00	22.37

20	gris	3	2586	2	g	25.00	21.40	22.00	24.00	.	.	.	23.10
21	gris	3	2579	3	g	26.80	26.20	26.00	26.60	26.40	26.60	26.80	26.49
22	rosa	3	2401	3	e	28.80	26.20	28.00	28.00	25.40	25.00	24.40	26.54
23	naranja	3	2416	3	h	31.20	25.80	25.40	30.60	27.40	32.00	34.40	29.54
24	verde	3	2555	3	f	32.60	32.40	31.40	30.80	30.00	30.40	31.80	31.34
						25.32	23.78	23.93	25.22	24.42	25.07	25.67	24.70

milk yield kg/d

box	color	period	cow	square	treatment	day 1	2	3	4	5	6	7	average
13	gris	4	2670	1	g	21.40	20.00	21.20	17.20	20.00	17.40	23.80	20.14
14	naranja	4	2442	1	h	19.20	19.00	21.40	25.20	25.20	24.20	28.00	23.17
15	rosa	4	2671	1	e	14.80	13.40	11.20	13.00	11.80	12.60	13.40	12.89
16	verde	4	2584	1	f	18.80	19.20	21.60	21.40	24.80	24.00	24.60	22.06
17	naranja	4	2574	2	h	23.20	23.40	23.60	24.20	24.20	19.20	23.40	23.03
18	gris	4	2591	2	g	20.80	22.80	21.40	22.40	20.80	27.00	23.40	22.66
19	verde	4	2663	2	f	21.30	19.00	21.80	22.60	23.00	24.00	25.20	22.41
20	rosa	4	2586	2	e	18.20	18.20	18.00	17.60	17.00	16.40	22.60	18.29
21	naranja	4	2579	3	h	24.60	26.40	26.80	27.60	27.00	26.20	30.80	27.06
22	gris	4	2401	3	g	22.20	20.80	20.60	21.00	19.60	23.60	25.20	21.86
23	verde	4	2416	3	f	20.40	19.20	24.20	28.00	25.40	29.80	,	24.50
24	rosa	4	2555	3	e	30.60	29.80	28.60	29.20	27.80	31.80	30.00	29.69
						21.29	20.93	21.70	22.45	22.22	23.02	24.58	22.31

Attachment 7

Milk Composition Data

pp. 57-60

ATTACHMENT 7.

Milk Composition Data

period	cow	square	treatm	milk days 1	2	3	4	fat * 5	6	7	average	milk days 1	2	3	4	protein 5	6	7	average	days 1	2	3	4	milk factor
1	2670	1	e	3.91	4	4.51	4.41	4.87	4.37	4.51	4.37	3.47	3.58	3.49	3.26	3.35	3.28	3.32	3.41	4.9	4.84	4.88	4.79	
1	2442	1	l	3.02	2.72	3.29	3.68	3.27	3.81	3.44	3.32	3.16	3.07	3.13	3.15	3.11	3.14	3.03	3.11	5.25	5.21	5.21	5.27	
1	2671	1	g	2.88	3.16	2.54	3.92	3.72	3.17	3.33	3.25	2.91	2.82	2.83	2.8	2.76	2.76	2.73	2.80	5.23	5.21	5.22	5.22	
1	2584	1	h	4.35	3.51	3.34	4.16	3.88	3.41	4.78	3.92	2.93	2.87	2.89	2.76	2.81	2.77	2.69	2.82	5.23	5.25	5.24	5.1	
1	2574	2	e	3.68	3.38	4.04	4.38	.	3.88	3.74	3.85	3.14	3.18	3.16	3.07	.	3.07	3.04	3.11	5.28	5.31	5.21	5.24	
1	2591	2	l	2.75	3.75	2.89	2.71	2.68	2.98	2.44	2.89	2.89	3.06	2.97	2.96	2.99	2.97	3.02	2.98	4.87	4.75	4.78	4.87	
1	2663	2	g	3.94	3.58	3.53	3.47	3.39	4.42	4.54	3.84	3.53	3.56	3.51	3.5	3.45	3.39	3.5	3.49	5.25	5.33	5.31	5.34	
1	2586	2	h	3.25	2.62	3.1	3.18	5.04	3.47	4.03	3.53	3.31	2.95	2.92	2.93	2.93	2.77	2.75	2.92	4.91	4.79	4.73	4.82	
1	2579	3	e	2.91	3.64	3.31	3.88	2.67	2.17	4.11	3.27	3.1	3.09	3.05	3.04	3.02	2.99	3.07	3.05	5.2	5.21	5.21	5.15	
1	2401	3	f	3.13	2.57	3.89	3.67	3.03	3.75	3.18	3.43	3.21	3.5	3.46	3.37	3.31	3.31	3.42	3.37	4.85	5.13	4.98	5.03	
1	2416	3	g	3.15	2.96	3.27	3.95	3.86	3.26	3.37	3.40	3.13	3.11	3.08	3	2.96	2.86	2.89	3.00	5.01	4.96	4.99	5.01	
1	2555	3	h	3.15	2.65	2.93	3.24	3.1	2.47	3.21	2.96	2.93	2.92	2.88	2.9	2.85	2.81	2.91	2.89	4.89	4.87	4.92	4.9	
				3.34	**3.21**	**3.39**	**3.72**	**3.68**	**3.43**	**3.72**	**3.50**	**3.15**	**3.14**	**3.11**	**3.07**	**3.04**	**3.01**	**3.03**	**3.08**	**5.07**	**5.07**	**5.05**	**5.06**	

period	cow	square	treatme	1	2	3	4	5	6 *	7 *	average	1	2	3	4	5	6	7	average	1	2	3	4
2	2670	1	f	3.33	2.65	3.80	4.38	6.29		.	4.09	3.13	3.24	3.10	3.05	2.99	3.08	3.09	3.10	5.08	5.18	5.10	5.08	
2	2442	1	g	2.75	2.94	3.39	3.98	2.95	.	.	3.20	3.21	3.27	3.20	3.12	3.14	3.16	3.06	3.17	5.17	5.15	5.11	5.08	
2	2671	1	h	3.57	2.95	2.90	3.69	2.99	.	.	3.22	2.72	2.87	2.85	2.82	2.83	2.88	2.91	2.84	5.30	5.28	5.28	5.22	
2	2584	1	e	4.71	4.07	4.68	4.42	2.16	.	.	4.05	2.86	2.96	2.85	2.87	2.93	2.90	2.92	2.90	5.21	5.17	5.15	5.14	
2	2574	2	g	4.01	3.56	4.21	3.38	3.46		.	3.80	3.21	3.17	3.16	3.20	3.15	3.18	3.19	3.18	5.23	5.19	5.19	5.16	
2	2591	2	e	3.26	3.02	2.84	2.68	2.94			2.95	3.20	3.07	3.18	3.12	3.25	3.08	3.10	3.14	4.76	4.81	4.91	4.87	
2	2663	2	h	4.39	3.51	4.22	3.34	3.33		.	3.76	3.42	3.46	3.42	3.48	3.43	3.50	3.49	3.46	5.29	5.23	5.26	5.29	
2	2586	2	l	2.18	2.31	4.39	3.23	3.13	.		3.05	2.95	3.00	2.93	2.97	2.94	2.99	2.97	2.96	4.74	4.71	4.65	4.69	
2	2579	3	f	3.54	4.24	3.16	3.67	3.31	.	.	3.58	3.13	3.13	3.19	3.18	2.91	3.23	3.18	3.14	5.12	5.17	5.25	5.20	
2	2401	3	h	2.15	5.65	3.01	3.49	2.24	.		3.31	3.31	3.20	3.25	3.16	3.17	3.39	3.32	3.26	5.03	4.91	5.06	4.78	
2	2416	3	e	2.88	4.20	3.78	2.61	3.65			3.42	3.08	2.98	2.99	3.03	2.97	3.02	2.98	3.01	5.04	4.97	5.02	5.06	
2	2555	3	g	2.54	2.61	2.70	2.13	2.61	.	.	2.52	3.05	3.00	3.00	2.99	2.95	2.97	2.91	2.98	4.91	4.88	4.85	4.85	
				3.28	**3.51**	**3.61**	**3.42**	**3.26**			**3.41**	**3.11**	**3.11**	**3.09**	**3.08**	**3.06**	**3.12**	**3.09**	**3.09**	**5.08**	**5.05**	**5.07**	**5.04**	

period	cow	square	treatme	1 *	2	3	4	5	6	7	average	1	2	3	4	5	6	7	average	1	2	3	4
3	2670	1	h		3.94	3.90	3.82	3.73	3.98	4.27	3.94	3.09	3.11	3.01	3.05	3.28	3.22	2.95	3.10	5.09	5.01	4.97	4.9	
3	2442	1	e	.	4.12	2.89	3.70	2.88	3.17	2.54	3.22	3.01	3.08	2.91	2.90	3.05	3.14	3.05	3.03	5.05	5.05	5.09	5	
3	2671	1	l		3.30	3.50	3.80	3.87	3.76	3.68	3.67	2.95	2.93	2.90	2.87	2.99	3.03	3.03	2.96	5.06	5.09	5.09	5.17	
3	2584	1	g	.	4.15	4.24	3.74	3.98		3.56	3.93	2.78	2.83	2.71	2.82	2.92	.	2.77	2.81	5.28	5.32	5.27	5.28	
3	2574	2	f		3.52	3.71	5.02	3.63	3.59	4.05	3.93	3.17	3.29	3.24	3.16	3.26	2.88	3.31	3.19	5.2	5.25	5.26	5.21	
3	2591	2	h	.	3.19	2.95	3.96	3.91	3.79	.	3.56	3.10	3.18	3.00	3.29	3.43	3.28	.	3.23	4.9	4.9	4.63	4.65	
3	2663	2	e	.	4.32	4.04	4.61	3.85	3.10	3.47	3.90	3.38	3.37	3.27	3.33	3.47	3.40	3.56	3.40	5.42	5.35	5.28	5.22	
3	2586	2	g		3.92	2.84	3.63	3.35	4.07	.	3.56	2.85	2.91	2.98	2.90	2.92	3.54	.	3.02	4.71	4.7	4.74	4.64	
3	2579	3	g	.	3.75	2.73	3.60	3.01	3.00	3.03	3.19	3.17	3.28	3.21	3.23	3.37	3.40	3.36	3.29	5.12	5.09	5.09	5.02	
3	2401	3	e	.	3.44	2.77	4.17	3.00	4.05	3.18	3.44	3.28	3.38	3.31	3.32	3.31	3.40	3.32	3.33	5.15	5.14	5.11	5.04	
3	2416	3	h		4.81	3.17	3.35	3.18	3.29	2.48	3.33	2.96	2.98	2.77	2.86	2.90	3.09	3.05	2.94	4.97	4.91	4.95	4.93	
3	2555	3	f	.	3.30	2.21	2.26	2.97	3.09	2.61	2.74	3.03	3.09	2.95	2.96	2.97	3.02	2.94	2.99	4.79	5.06	4.76	4.75	
				.	**3.81**	**3.25**	**3.81**	**3.45**	**3.54**	**3.29**	**3.54**	**3.07**	**3.12**	**3.02**	**3.06**	**3.16**	**3.22**	**3.14**	**3.11**	**5.06**	**5.03**	**5.02**	**4.98**	

period	cow	square	treatme	1	2	3	4	5	6	7	average	1	2	3	4	5	6	7	average	1	2	3	4
4	2670	1	g	4.05	4.45	4.10	4.79	4.58	3.31	5.86	4.45	3.36	3.24	3.30	3.38	3.44	3.31	3.43	3.35	5.03	4.89	4.86	4.92	
4	2442	1	h	4.14	4.24	3.87	4.54	3.49	3.54	4.45	4.04	3.27	3.33	3.33	3.52	3.45	3.44	3.26	3.37	5.06	5.12	5.07	5.07	
4	2671	1	e	3.83	4.27	4.51	4.02	4.29	4.48	5.51	4.42	3.04	3.05	2.92	2.82	3.04	3.18	3.32	3.05	4.95	5.05	5.07	5.06	
4	2584	1	f	4.09	4.12	4.63	4.70	4.27	4.00	4.86	4.38	2.96	3.06	2.98	3.02	3.11	3.17	3.09	3.06	5.19	5.19	5.12	5.13	
4	2574	2	h	3.41	3.90	4.00	4.39	3.49	3.75	4.43	3.91	3.34	3.41	3.37	3.39	3.32	3.36	3.55	3.39	5.05	5.01	5.01	4.99	
4	2591	2	g	3.24	3.10	3.29	5.31	3.48	3.39	2.71	3.50	3.22	3.33	3.28	3.15	3.29	3.34	3.35	3.28	4.75	4.78	4.79	4.72	
4	2663	2	f	4.01	4.21	4.57	4.26	3.84	3.46	3.45	3.97	3.57	3.58	3.56	3.57	3.62	3.66	3.67	3.60	5.12	5.00	5.09	5.09	
4	2586	2	e	3.44	3.89	3.81	4.14	2.77	2.34	3.26	3.38	3.22	3.29	3.24	3.25	3.30	3.31	3.33	3.28	4.64	4.64	4.62	4.60	
4	2579	3	h	3.38	2.82	4.21	4.03	3.32	3.04	3.60	3.49	3.40	3.43	3.43	3.37	3.37	3.42	3.37	3.40	4.94	4.94	4.96	4.95	
4	2401	3	g	3.56	3.29	3.75	3.98	3.52	3.94	3.01	3.57	3.33	3.36	3.33	3.34	3.33	3.32	3.30	3.33	5.07	5.10	5.02	5.06	
4	2416	3	f	4.01	3.18	5.35	3.88	3.68	3.92	4.70	4.10	3.19	3.12	3.03	3.19	3.35	3.30	3.36	3.22	4.78	4.98	4.92	4.92	
4	2555	3	e	2.58	3.19	3.48	2.88	2.23	2.63	2.82	2.83	3.11	3.12	3.09	3.15	3.17	3.19	3.19	3.15	4.65	4.61	4.63	4.63	
				3.65	**3.71**	**4.13**	**4.24**	**3.58**	**3.48**	**4.06**	**3.84**	**3.25**	**3.28**	**3.24**	**3.26**	**3.31**	**3.33**	**3.35**	**3.30**	**4.94**	**4.92**	**4.91**	**4.90**	

* Due to milk samples were not cooled (by a human error) days 6 and 7, and day 1 in periods 2 and 3 respectively, the % of fat was eliminated

a	%					milk	NFS		%					milk	urea		%				
5	6	7	average	1	days 2	3	4	5	6	7	averag	1	days 2	3	4	5	6	7	average	1	days 2
4.83	4.97	4.92	4.87	9.12	9.16	9.1	8.68	8.92	9.01	8.99	9.03	0.004	0.004	0.014	0.025	0.022	0.030	0.018	0.017	1.87	1.87
5.28	5.27	5.27	5.25	9.23	9.09	9.15	9.23	9.2	9.23	9.11	9.18	0.038	0.053	0.049	0.062	0.067	0.065	0.056	0.056	17.75	24.75
5.19	5.2	5.17	5.20	8.95	8.84	8.87	8.83	8.75	8.77	8.71	8.82	0.042	0.047	0.045	0.049	0.052	0.055	0.051	0.049	19.61	21.95
5.23	5.3	5.19	5.22	8.97	8.94	8.94	8.65	8.85	8.9	8.80	8.85	0.041	0.048	0.044	0.059	0.055	0.058	0.054	0.051	13.15	22.42
	5.31	5.25	5.27	9.25	9.31	9.18	9.12		9.2	9.1	9.19	0.035	0.042	0.037	0.050	.	0.054	0.043	0.044	16.35	19.61
4.89	4.84	4.87	4.84	8.52	8.55	8.5	8.58	8.62	8.56	8.64	8.57	0.023	0.022	0.013	0.033	0.032	0.024	0.028	0.025	10.74	19.27
5.33	5.27	5.24	5.30	9.59	9.71	9.64	9.66	9.6	9.48	9.53	9.60	0.036	0.041	0.043	0.054	0.057	0.053	0.053	0.048	16.81	19.15
4.73	4.83	4.75	4.79	8.98	8.49	8.38	8.5	8.3	8.34	8.24	8.46	0.028	0.034	0.031	0.049	0.055	0.054	0.038	0.041	13.08	15.88
5.23	5.16	5.25	5.20	9.1	9.11	9.07	8.99	9.07	8.95	9.13	9.06	0.038	0.045	0.041	0.055	0.057	0.053	0.050	0.048	17.75	21.02
4.94	4.91	4.96	4.97	8.81	9.42	9.21	9.17	9.01	8.97	9.13	9.10	0.027	0.033	0.027	0.042	0.039	0.039	0.036	0.035	12.61	15.41
5.01	5.03	5.03	5.01	8.92	8.87	8.83	8.78	8.75	8.67	8.7	8.79	0.028	0.037	0.025	0.051	0.048	0.058	0.045	0.043	13.08	17.28
4.82	4.83	4.86	4.87	8.63	8.54	8.57	8.55	8.42	8.29	8.53	8.52	0.030	0.037	0.036	0.048	0.042	0.046	0.046	0.041	15.41	17.28
5.04	**5.08**	**5.06**	**5.07**	**9.01**	**9.00**	**8.95**	**8.91**	**8.86**	**8.87**	**8.88**	**8.93**	**0.03**	**0.04**	**0.03**	**0.05**	**0.05**	**0.05**	**0.04**	**0.041**	**14.52**	**17.24**

5	6	7	averag	1	2	3	4	5	6	7	averag	1	2	3	4	5	6	7	average		
4.99	5.15	5.12	5.10	8.99	9.20	8.98	8.91	8.74	9.02	9.00	8.98	0.031	0.027	0.035	0.037	0.038	0.033	0.033	0.033	14.48	12.61
5.13	5.15	5.07	5.12	9.16	9.21	9.08	8.97	9.05	9.09	8.91	9.07	0.043	0.041	0.046	0.045	0.047	0.048	0.050	0.046	20.08	19.15
5.29	5.21	5.29	5.26	8.87	8.96	8.93	8.85	8.89	8.89	8.99	8.91	0.053	0.047	0.045	0.049	0.050	0.048	0.052	0.049	24.75	21.95
2.82	5.19	5.23	4.84	8.86	8.92	8.79	8.80	2.84	8.83	8.96	8.01	0.047	0.044	0.050	0.049	0.050	0.049	0.049	0.047	21.95	20.55
5.20	5.26	5.22	5.21	9.26	9.15	9.15	9.14	9.24	9.21	9.19	9.19	0.047	0.046	0.046	0.043	0.042	0.039	0.039	0.043	21.95	21.48
4.88	4.89	4.85	4.85	8.68	8.60	8.64	8.87	8.87	8.71	8.86	8.78	0.025	0.018	0.028	0.026	0.025	0.024	0.022	0.024	11.68	8.41
5.29	5.34	5.30	5.29	9.52	9.59	9.48	9.57	9.52	9.65	9.50	9.56	0.051	0.052	0.050	0.049	0.048	0.056	0.051	0.051	23.82	24.28
4.64	4.72	4.70	4.69	8.41	8.42	8.29	8.37	8.28	8.43	8.38	8.37	0.044	0.036	0.035	0.021	0.038	0.041	0.035	0.036	20.55	16.81
5.19	5.28	5.24	5.21	9.03	9.08	9.20	9.18	8.89	9.01	9.21	9.13	0.048	0.045	0.053	0.050	0.050	0.050	0.052	0.050	22.42	21.02
4.97	4.95	4.96	4.95	9.11	8.85	9.07	8.66	8.89	9.08	9.03	8.96	0.039	0.042	0.040	0.037	0.044	0.037	0.019	0.037	17.75	19.61
5.01	5.02	4.98	5.01	8.68	8.71	8.77	8.66	8.75	8.60	8.72	8.78	0.041	0.042	0.045	0.041	0.047	0.046	0.045	0.044	19.15	19.61
4.83	4.85	4.81	4.85	8.71	8.63	8.59	8.58	8.51	8.55	8.45	8.57	0.045	0.046	0.046	0.048	0.043	0.047	0.046	0.046	21.48	21.48
4.85	**5.03**	**5.06**	**5.03**	**8.96**	**8.94**	**8.93**	**8.90**	**8.36**	**8.97**	**8.94**	**8.85**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.04**	**0.042**	**20.00**	**18.91**

5	6	7	averag	1	2	3	4	5	6	7	averag	1	2	3	4	5	6	7	average		
4.94	4.81	4.86	4.94	8.93	8.92	8.73	8.68	8.95	8.75	8.55	8.79	0.038	0.048	0.041	0.038	0.034	0.031	0.020	0.04	17.75	22.42
5.02	5.01	4.95	5.02	8.84	8.91	8.74	8.71	8.81	8.90	8.75	8.81	0.062	0.060	0.060	0.054	0.047	0.051	0.050	0.06	28.95	32.22
5.25	5.18	5.11	5.14	8.79	8.81	8.75	8.81	8.90	8.99	8.91	8.86	0.048	0.065	0.060	0.051	0.050	0.051	0.050	0.05	22.42	30.36
5.32	.	5.36	5.31	8.87	8.97	8.78	8.80	9.04		8.94	8.92	0.059	0.078	0.067	0.060	0.058		0.047	0.06	27.55	36.43
5.26	5.33	5.17	5.24	9.17	9.35	9.30	9.18	9.31	9.01	9.25	9.22	0.050	0.056	0.057	0.059	0.044	0.056	0.048	0.05	23.35	30.82
4.55	5.18	.	4.86	8.83	8.84	8.32	8.63	8.65	9.23	.	8.75	0.031	0.045	0.034	0.030	0.030	0.047	.	0.04	14.48	21.02
5.25	4.76	5.2	5.21	9.64	9.54	9.34	9.33	9.51	8.86	9.54	9.39	0.057	0.073	0.068	0.063	0.055	0.029	0.055	0.06	26.02	34.09
4.37	5.17	.	4.72	8.29	8.33	8.33	8.21	7.93	9.48	.	8.43	0.037	0.052	0.045	0.045	0.036	0.054		0.04	17.28	24.28
5.11	5.07	5.07	5.08	9.08	9.15	9.06	9.00	9.25	9.23	9.19	9.14	0.050	0.054	0.061	0.053	0.055	0.049	0.048	0.05	23.35	29.83
5.07	4.98	5.07	5.03	9.22	9.31	9.19	9.11	9.14	9.13	9.14	9.18	0.051	0.053	0.057	0.057	0.057	0.052	0.047	0.05	23.82	29.42
4.89	4.89	4.86	4.91	8.69	8.64	8.46	8.53	8.51	8.72	8.64	8.59	0.051	0.070	0.056	0.051	0.047	0.042	0.042	0.05	23.82	32.69
4.73	4.78	4.74	4.80	8.55	8.92	8.42	8.42	8.41	8.48	8.39	8.51	0.044	0.053	0.045	0.046	0.049	0.050	0.047	0.05	20.55	24.75
4.98	**5.01**	**5.04**	**5.02**	**8.91**	**8.97**	**8.79**	**8.79**	**8.88**	**8.98**	**8.93**	**8.88**	**0.06**	**0.06**	**0.05**	**0.05**	**0.05**	**0.05**	**0.05**	**0.051**	**22.49**	**29.03**

5	6	7	averag	1	2	3	4	5	6	7	averag	1	2	3	4	5	6	7	average		
4.96	4.89	4.99	4.93	9.16	8.89	8.91	9.06	9.16	8.95	9.18	9.04	0.031	0.032	0.023	0.017	0.012	0.023	0.031	0.024	14.48	14.94
5.01	5.06	5.05	5.06	9.12	9.24	9.18	9.36	9.23	9.28	9.06	9.21	0.049	0.057	0.049	0.049	0.034	0.040	0.048	0.047	22.68	26.62
5.09	4.92	4.90	5.01	8.75	8.90	8.78	8.67	8.93	8.86	8.98	8.84	0.041	0.046	0.043	0.037	0.029	0.024	0.033	0.036	19.15	21.48
5.07	5.06	5.06	5.12	8.95	9.06	8.90	8.96	8.97	9.00	8.95	8.97	0.050	0.052	0.046	0.044	0.032	0.043	0.052	0.046	23.35	24.28
4.82	4.97	4.94	4.97	9.17	9.18	9.15	9.14	8.87	9.09	9.24	9.12	0.043	0.054	0.039	0.035	0.009	0.026	0.025	0.033	20.08	25.22
4.70	4.71	4.60	4.75	8.69	8.64	8.60	8.59	8.67	8.77	8.89	8.75	0.026	0.032	0.023	0.024	0.008	0.013	0.019	0.021	12.14	14.94
5.05	5.04	5.10	5.08	9.48	9.41	9.42	9.44	9.43	9.46	9.55	9.46	0.048	0.049	0.045	0.037	0.029	0.033	0.038	0.040	22.42	22.88
4.54	4.58	4.64	4.61	8.56	8.63	8.55	8.55	8.52	8.59	8.68	8.58	0.025	0.028	0.020	0.016	0.010	0.013	0.023	0.019	12.14	13.08
4.94	4.85	4.93	4.90	9.09	9.12	9.14	9.09	9.06	9.01	9.06	9.08	0.048	0.048	0.047	0.043	0.029	0.032	0.039	0.041	22.42	22.42
5.09	5.09	5.06	5.08	9.19	9.24	9.12	9.18	9.20	9.09	9.14	9.17	0.061	0.063	0.051	0.047	0.041	0.049	0.040	0.050	28.43	29.42
4.73	4.72	4.73	4.70	8.70	8.52	8.36	8.52	8.60	8.74	8.80	8.63	0.048	0.050	0.032	0.018	0.010	0.018	0.027	0.026	21.48	14.01
4.64	4.61	4.63	4.63	8.46	8.43	8.42	8.49	8.51	8.50	8.52	8.48	0.044	0.042	0.036	0.034	0.025	0.033	0.030	0.035	20.55	19.61
4.89	**4.87**	**4.90**	**4.90**	**8.94**	**8.96**	**8.89**	**8.92**	**8.96**	**8.95**	**9.01**	**8.94**	**0.04**	**0.04**	**0.04**	**0.03**	**0.02**	**0.03**	**0.03**	**0.035**	**19.95**	**20.74**

N- urea in milk mg/100ml							N- urea in milk g/d							
						days								
3	4	5	6	7	average		1	2	3	4	5	6	7	average
6.54	11.68	10.27	14.01	8.41	7.81	23.67	0.4421	0.4421	1.5473	2.7631	2.4315	3.3157	1.9804	1.85
22.88	28.95	31.29	32.36	26.15	26.02	30.07	5.4062	7.6657	7.0872	8.9575	9.6007	9.4014	8.0966	8.06
21.02	22.88	24.28	25.69	23.82	22.75	29.11	5.7105	6.3003	5.1184	6.0622	7.0701	7.478	6.9341	6.52
20.55	27.55	25.69	27.09	25.22	23.95	33.09	6.3349	7.4165	6.7985	9.1161	8.4381	8.9616	8.3436	7.92
17.28	23.35	.	25.22	20.08	20.31	31.14	5.0003	6.1084	5.3812	7.2719	.	7.8536	6.2538	6.33
6.07	15.41	14.94	11.21	12.14	11.54	28.37	3.0474	2.9149	1.7224	4.3723	4.2308	3.1709	3.4449	3.27
20.08	25.22	26.62	24.75	24.75	22.48	27.66	4.6497	5.2955	5.5538	6.9746	7.3621	6.8454	6.8454	6.22
14.48	22.88	25.69	25.22	17.75	19.28	29.19	3.8163	4.6341	4.2252	6.6786	7.4964	7.3601	5.1703	5.63
19.15	25.69	26.62	24.75	23.35	22.62	31.54	5.5976	6.6287	6.0385	8.1018	8.3964	7.8072	7.3653	7.13
12.61	19.61	18.21	18.21	16.81	16.21	34.11	4.3015	5.2574	4.3015	6.6312	6.2132	6.2132	5.7353	5.53
16.35	23.82	22.42	27.09	21.02	20.15	33.57	5.1744	6.8375	6.468	9.4247	8.8703	10.718	8.3159	7.97
16.81	22.42	19.61	21.48	21.48	19.21	38.14	5.8782	6.5907	5.4126	8.5501	7.4813	8.1938	8.1938	7.33
16.15	**22.45**	**22.33**	**22.92**	**20.08**	**19.36**		**4.63**	**5.52**	**5.14**	**7.13**	**7.07**	**7.28**	**6.39**	**6.16**
16.35	17.28	17.75	15.41	15.41	15.61	24.20	3.5034	3.0514	3.9555	4.1815	4.2945	3.7295	3.7295	3.78
21.48	21.02	21.95	22.42	23.35	21.35	33.34	6.6956	6.3842	7.1027	7.007	7.3184	7.4741	7.7856	7.12
21.02	22.88	23.35	22.42	24.28	22.95	25.30	6.262	5.5531	5.3168	5.7894	5.9076	5.6712	6.1439	5.81
20.15	22.88	16.81	22.88	22.88	22.02	32.91	7.2244	6.7632	8.0077	7.5318	7.5318	7.5318	7.5318	7.25
22.42	20.08	19.61	18.21	18.21	20.28	29.37	6.4467	6.0096	6.5839	6.0981	5.7609	5.3494	5.3494	5.96
13.08	12.14	11.68	11.21	10.27	11.21	29.57	3.3357	2.4017	3.736	3.4691	3.3357	3.2023	2.9354	3.20
23.35	22.88	22.42	26.15	23.82	23.82	27.57	6.5667	6.6054	6.4379	6.3092	6.1804	7.2105	6.5667	6.57
16.35	9.81	17.75	19.15	16.35	16.68	27.90	5.7329	4.6905	4.5603	2.7362	4.9511	5.342	4.5603	4.65
24.75	23.35	23.35	23.35	24.28	23.22	31.34	7.0258	6.5867	7.7577	7.3186	7.3186	7.3186	7.6113	7.28
18.68	17.28	20.55	17.28	8.87	17.15	29.40	5.2173	5.7665	5.4919	5.08	6.0411	5.08	2.6087	5.04
21.02	19.15	21.95	21.48	21.02	20.48	37.67	7.212	7.3879	7.9157	7.212	8.2575	8.0915	7.9157	7.71
21.48	22.42	20.08	21.95	21.48	21.48	34.40	7.3898	7.3898	7.3898	7.7111	8.9079	7.5505	7.3898	7.39
20.51	**19.26**	**19.77**	**20.16**	**19.19**	**19.69**		**6.05**	**5.75**	**6.24**	**5.85**	**5.98**	**6.13**	**5.84**	**5.98**
19.15	17.75	15.68	14.48	9.34	16.68	20.43	3.6253	4.5793	3.9115	3.6253	3.2436	2.9574	1.908	3.41
26.02	25.22	21.95	23.82	23.35	26.22	27.23	7.8838	8.7739	7.6294	6.8665	5.9764	6.485	6.3579	7.14
28.02	23.82	23.35	23.82	23.35	25.02	19.29	4.3231	5.8542	5.4033	4.5933	4.5032	4.5933	4.5032	4.82
31.29	28.02	27.09	.	21.95	28.72	23.03	6.3451	8.3884	7.2054	6.4526	5.2375	.	5.0545	6.61
26.62	27.55	20.55	26.15	22.42	25.35	23.97	5.5073	7.3885	6.381	6.9048	4.9256	6.269	5.3734	6.08
15.88	14.01	14.01	21.95	.	16.89	23.11	3.3463	4.8575	3.6701	3.2383	3.2383	5.0734	.	3.90
31.76	29.42	25.69	19.54	25.69	26.69	22.37	5.9551	7.6256	7.1043	6.5819	5.7461	3.0298	5.7461	5.97
21.02	21.02	16.81	25.22	.	20.94	23.10	3.6914	5.6096	4.8545	4.8545	3.8836	5.8254	.	4.84
28.49	24.75	25.69	22.88	22.42	25.35	29.49	6.1844	7.9161	7.545	6.5555	6.8029	6.0607	5.937	6.71
26.62	25.02	26.62	24.28	21.95	25.62	26.54	6.3217	7.8092	7.0654	7.0654	7.0654	6.4457	5.8259	6.80
26.15	23.82	21.95	19.61	19.61	23.95	29.54	7.0362	9.6576	7.726	7.0362	6.4844	5.7945	5.7945	7.08
21.02	21.48	22.88	23.35	21.95	22.28	31.34	6.4403	7.7577	6.5867	6.7231	7.1722	7.3186	6.8794	6.98
25.33	**23.62**	**21.87**	**21.74**	**21.20**	**23.64**		**5.59**	**7.18**	**6.26**	**5.85**	**5.44**	**5.44**	**5.34**	**5.86**
10.74	7.94	5.60	10.74	14.48	11.27	20.14	2.9161	3.0101	2.1635	1.5991	1.1288	2.1635	2.9161	2.27
22.88	22.88	15.88	18.68	22.42	21.75	23.17	5.3023	6.168	5.3023	5.3023	3.6792	4.3264	5.1041	5.04
20.08	17.28	13.54	11.21	15.41	16.88	12.89	2.4672	2.7681	2.5976	2.2265	1.7451	1.4442	1.9058	2.17
21.48	20.55	14.94	20.08	24.28	21.28	22.06	5.1503	5.3564	4.7383	4.5323	3.2962	4.4283	5.3564	4.69
18.21	16.35	4.20	12.14	11.68	15.41	23.03	4.6244	5.6073	4.1942	3.764	0.9679	2.7961	2.5886	3.55
10.74	11.21	3.74	6.07	8.87	9.67	22.66	2.751	3.3859	2.4336	2.5334	0.8465	1.3755	2.0104	2.19
21.02	17.28	13.54	15.41	17.75	18.61	22.41	5.0244	5.1291	4.7104	3.873	3.0356	3.4543	3.9776	4.17
9.34	7.47	4.67	5.07	10.74	9.07	18.29	2.2203	2.391	1.7079	1.3663	0.8539	1.1101	1.9641	1.66
21.95	20.08	13.54	14.94	18.21	19.05	27.06	6.0651	6.0651	5.9388	5.4333	3.6543	4.0434	4.9279	5.16
23.82	21.95	19.15	22.88	18.68	23.48	21.85	6.2264	6.4306	5.2057	4.7974	4.185	5.0016	4.0829	5.13
14.94	8.41	4.67	8.41	12.61	12.08	24.50	5.2931	3.4225	3.6513	2.0595	1.1442	2.0595	3.0892	2.96
16.81	15.88	11.68	15.41	14.01	16.28	29.69	6.0998	5.0226	4.9008	4.7135	3.4556	4.5742	4.159	4.83
17.67	**15.61**	**10.43**	**13.50**	**15.76**	**16.24**		**4.51**	**4.65**	**3.97**	**3.52**	**2.39**	**3.07**	**3.53**	**3.65**

Attachment 8

Nutrient Composition of Feeds Used for Mixed Rations

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ATTACHMENT 8.

Nutrient Composition of Feeds Used for Mixed Rations

	DM	CP	NDF	ADF	LDA	EE	Ash	ADIN
				%				
Corn silage	27.8±1.87	8.6±0.50	48.3±0.87	27.4±1.20	2.63±0.13	ND	8.3±0.50	12.1±3.14
Alfalfa hay	84.2±6.65	19.6±1.83	40.8±4.34	31.0±4.02	8.3±1.52	2.9±1.00	11.5±1.03	ND
Ground corn	89.1±1.05	9.7±0.75	18.6±1.45	5.63±1.27	1.35±0.49	4.95±0.91	3.33±0.18	ND
Soybean meal	90.1±1.28	38.9±9.77	18.6±6.19	11.0±3.57	1.4±1.124	4.6±0.12	6.7±0.57	ND

ND = not determined

TMR Composition

FreshWeight	1st. period		2nd period		3th period		4th period		average	sd
	kg/d	%	kg/d	%	kg/d	%	kg/d	%	%	
alfalfa hay	250	0.21	240	0.21	240	0.21	200	0.20	0.21	0.005
soybean meal	135	0.11	125	0.11	105	0.09	85	0.09	0.10	0.013
corn grain	125	0.10	115	0.10	135	0.12	225	0.23	0.14	0.059
corn silage	680	0.57	645	0.57	645	0.57	480	0.48	0.55	0.044
minerals&vitamins	10	0.01	10	0.01	10	0.01	10	0.01	0.01	0.001
totals	1200	1.00	1135	1.00	1135	1.00	1000	1.00	1.00	
kg offered/cow/d	50.00		47.29		47.29		43.48		47.02	2.68
total cows/period	(24 cows)		(24 cows)		(24 cows)		(23 cows)			

DryMatterBased	average DM	1st. period		2nd period		3th period		4th period		average	sd
		kg/d	%	kg/d	%	kg/d	%	kg/d	%	%	
alfalfa hay	84.2	210.5	0.33	202.08	0.33	202.08	0.33	168.4	0.29	0.32	0.023
soybean meal	90.1	121.635	0.19	112.625	0.19	94.605	0.16	76.585	0.13	0.17	0.028
corn grain	89.1	111.375	0.17	102.465	0.17	120.285	0.20	200.475	0.34	0.22	0.081
corn silage	27.8	189.04	0.29	179.31	0.30	179.31	0.30	133.44	0.23	0.28	0.034
minerals&vitamins	100	10	0.02	10	0.02	10	0.02	10	0.02	0.018	0.001
totals		642.55	1.00	606.48	1.00	606.28	1.00	588.9	1.00	1.00	
mixer DM estimated		53.55		53.43		53.42		58.89		54.82	
kg offered/cow/d		26.77		25.27		25.26		25.60		25.73	0.72
total cows/period		(24 cows)		(24 cows)		(24 cows)		(23 cows)			

Twenty four cows are shown in this table because two studies (12 cows each) were in progress simultaneously that used the same TMR mix.

Attachment 9

Chemical Composition of Cottonseeds From DHI Forage Testing Lab (Dairy One) and Laboratory of Forage Analysis, Experimental Station Rafaela

pp. 63-64

ATTACHMENT 9.

Chemical composition of cottonseeds from DHI Forage Testing Lab (Dairy One) and Laboratory of Forage Analysis, Experimental Station Rafaela, INTA. (Values expressed on Dry Matter Basis)

Cottonseeds / Laboratories		DP50		DP50B		DP50BII		DP50RR	
Analyses		Dairy One	INTA	Dairy One	INTA	Dairy One	INTA	Dairy One	INTA
Dry Matter	%	89.1	91.4	90.2	91.6	89.2	91.2	89.6	91.2
Crude Protein	%	22.3	17.6	23.3	18.1	24.9	16.7	27.1	19.6
NDF	%	60.3	52.3	66.4	52.7	68.4	52.8	49.3	48.8
ADF	%	53.9	37.3	55.7	37.5	54.9	39.4	40.7	34.9
ADL	%	16.1	8.0	13.8	8.5	15.0	7.7	9.0	8.3
Crude Fat	%	14.5	15.9	13.9	15.4	11.8	14.2	20.8	17.3
Ash	%	4.9	4.3	3.7	4.2	3.8	3.9	5.0	4.3
NFC §	%	*-2.0*	*9.9*	*-7.3*	*9.6*	*-8.9*	*12.4*	*-2.2*	*10*
TDN ϒ	%	64	66	69	65	65	65	83	67
NEL ϒ	Mcal/kg	1.61	1.73	1.72	1.66	1.61	1.64	2.16	1.73
NEM ϒ	Mcal/kg	1.54	1.74	1.65	1.72	1.50	1.70	2.25	1.80
NEG ϒ	Mcal/kg	0.95	1.12	1.06	1.10	0.90	1.09	1.57	1.18
DNDF ϒ	%	22.7	42.8	45.7	41.6	42.7	43.5	32.8	40.2
Calcium	%	0.12	0.14	0.12	0.15	0.19	0.15	0.18	0.14
Phosphorus	%	0.55	0.64	0.36	0.61	0.43	0.58	0.87	0.61
Magnesium	%	0.25	0.38	0.16	0.34	0.24	0.36	0.40	0.36
Potassium	%	0.81	1.40	0.63	1.45	1.30	1.51	1.20	1.41
Sodium	%	0	0.02	0	0.02	0	0.03	0	0.02
Sulfur	%	0.22	ND	0.22	ND	0.16	ND	0.23	ND
Iron	ppm	33	38	29	37	20	37	36	38
Zinc	ppm	16	34	11	38	14	36	26	35
Copper	ppm	<1	4.6	<1	4.5	1	4.5	2	4.7
Manganese	ppm	8	14	7	13	10	13	12	14
Molybdenum	ppm	<1	ND	<1	ND	<1	ND	<1	ND

§: NFC (NonFibrous Carbohydrates): 100-(Crude Protein+NDF+Crude Fat+Ash)
ϒ: Data from INTA Lab are based on ecuations of NRC 2001
ND: not determined

Attachment 10

Gossypol, Pesticide and Mycotoxin Contents

pp. 65-66

ATTACHMENT 10.

Gossypol, Pesticide and Mycotoxin contents (as is basis)

Cottonseeds Analysis (as is basis)		Det-Lim §	DP50	DP50B	DP50BX	DP50RR
Gossypol						
Free	%	...	0.374	0.330	0.262	0.384
Total	%	...	0.447	0.428	0.291	0.525
General Pesticide Screen						
Organophosphates	ppm	0.050	ND	ND	ND	ND
Organonitrogens	ppm	0.500	ND	ND	ND	ND
Organochlorinated	ppm	0.200	ND	ND	ND	ND
N-Methilcarbamates	ppm	0.100	ND	ND	ND	ND
Mycotoxins						
Aflatoxin B1	ppb	1.0	ND	ND	ND	ND
Aflatoxin B2	ppb	1.0	ND	ND	ND	ND
Aflatoxin G1	ppb	1.0	ND	ND	ND	ND
Aflatoxin G1	ppb	1.0	ND	ND	ND	ND
Ochratoxin A	ppb	5.0	ND	ND	ND	ND
Citrinin	ppm	0.2	**	**	**	**
T-2 Toxin	ppm	0.1	ND	ND	ND	ND
HT-2 Toxin	ppm	0.1	ND	ND	ND	ND
Diacetoxyscirpenol	ppm	0.3	**	**	**	**
Neosolaniol	ppm	0.5	ND	ND	ND	ND
Fusarenon X	ppm	0.5	ND	ND	ND	ND
Deoxynivalenol	ppm	0.1	ND	ND	ND	ND
15-Acetil-DON	ppm	0.1	ND	ND	ND	ND
3-Acetil-DON	ppm	0.1	ND	ND	ND	ND
Nivalenol	ppm	0.5	ND	ND	ND	ND
Zearelenone	ppb	100	464	140	414	130
Zearelenone *	ppb	100	271	ND	168	ND
Fumonisin B1	ppm	0.2	ND	ND	ND	ND
Fumonisin B2	ppm	0.2	ND	ND	ND	ND
Fumonisin B3	ppm	0.2	ND	ND	ND	ND

§ Detection Limits
* Zearelenone corrected for hull content
** Laboratory was unable to analyze using their normal methods due to complex sample matrix.

Attachment 11

Body Weights and Body Condition Scores

pp. 67-69

ATTACHMENT 11.

Body Weights & Body condition Scores

box	color	period	cow	square	treatment	condition score	BW (kg)
13	rosa	1	2670	1	e	2.125	488
14	verde	1	2442	1	f	2.125	583
15	gris	1	2671	1	g	2.25	565
16	naranja	1	2584	1	h	2.25	525
17	rosa	1	2574	2	e	2	564
18	verde	1	2591	2	f	2.125	491
19	gris	1	2663	2	g	2.5	576
20	naranja	1	2586	2	h	2.25	597
21	rosa	1	2579	3	e	2.5	600
22	verde	1	2401	3	f	2.5	678
23	gris	1	2416	3	g	2.25	618
24	naranja	1	2555	3	h	2.375	593
13	verde	2	2670	1	f	2.25	489
14	gris	2	2442	1	g	2.125	585
15	naranja	2	2671	1	h	2.5	564
16	rosa	2	2584	1	e	2.375	528
17	gris	2	2574	2	g	2.25	561
18	rosa	2	2591	2	e	2.25	500
19	naranja	2	2663	2	h	2.675	583
20	verde	2	2586	2	f	2.25	587
21	verde	2	2579	3	f	2.75	595
22	naranja	2	2401	3	h	2.675	685
23	rosa	2	2416	3	e	2.25	593
24	gris	2	2555	3	g	2.5	600
13	naranja	3	2670	1	h	2	485
14	rosa	3	2442	1	e	2	581
15	verde	3	2671	1	f	2.25	555
16	gris	3	2584	1	g	2.25	524
17	verde	3	2574	2	f	2	565
18	naranja	3	2591	2	h	2.25	493
19	rosa	3	2663	2	e	2.5	571
20	gris	3	2586	2	g	2.375	559
21	gris	3	2579	3	g	2.5	603
22	rosa	3	2401	3	e	2.5	665
23	naranja	3	2416	3	h	2.125	589
24	verde	3	2555	3	f	2.25	596
13	gris	4	2670	1	g	2	475
14	naranja	4	2442	1	h	2	549
15	rosa	4	2671	1	e	2.375	543
16	verde	4	2584	1	f	2.25	503

17	naranja	4	2574	2	h	2.25	561
18	gris	4	2591	2	g	2.25	496
19	verde	4	2663	2	f	2.75	568
20	rosa	4	2586	2	e	2.375	578
21	naranja	4	2579	3	h	2.75	600
22	gris	4	2401	3	g	2.375	639
23	verde	4	2416	3	f	2.125	597
24	rosa	4	2555	3	e	2.375	598

Attachment 12

Statistics (SAS Outputs)

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ATTACHMENT 12.

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	205.83018977	
1	2	153.14895963	0.00001096
2	1	153.14810829	0.00000001

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	8.42411046
PERIOD	18.44025849
COW(SQUARE)	6.23227210
SQUARE*PERIOD	0.00000000
Residual	4.11483223

Model Fitting Information for MILK

Description	Value
Observations	48.0000
Res Log Likelihood	-117.007
Akaike's Information Criterion	-122.007
Schwarz's Bayesian Criterion	-126.468
-2 Res Log Likelihood	234.0147

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.50	0.6860

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	26.87583333	2.87756149	24	9.34	0.0001
TREAT	f	26.70500000	2.87756149	24	9.28	0.0001
TREAT	g	27.56083333	2.87756149	24	9.58	0.0001
TREAT	h	27.41916667	2.87756149	24	9.53	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	0.17083333	0.82813367	24	0.21	0.8383	Tukey-Kramer	0.9968
TREAT	e	g	-0.68500000	0.82813367	24	-0.83	0.4163	Tukey-Kramer	0.8411
TREAT	e	h	-0.54333333	0.82813367	24	-0.66	0.5180	Tukey-Kramer	0.9124
TREAT	f	g	-0.85583333	0.82813367	24	-1.03	0.3117	Tukey-Kramer	0.7318
TREAT	f	h	-0.71416667	0.82813367	24	-0.86	0.3970	Tukey-Kramer	0.8240
TREAT	g	h	0.14166667	0.82813367	24	0.17	0.8656	Tukey-Kramer	0.9982

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-12.36503197	
1	1	-43.51808201	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.04239376
PERIOD	0.02586372
COW(SQUARE)	0.11238815
SQUARE*PERIOD	0.01274388
Residual	0.04446545

Model Fitting Information for FAT

Description	Value
Observations	48.0000
Res Log Likelihood	-18.6743
Akaike's Information Criterion	-23.6743
Schwarz's Bayesian Criterion	-28.1347
-2 Res Log Likelihood	37.3485

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.37	0.7766

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	3.59166667	0.18636069	24	19.27	0.0001
TREAT	f	3.59583333	0.18636069	24	19.30	0.0001
TREAT	g	3.51750000	0.18636069	24	18.87	0.0001
TREAT	h	3.58500000	0.18636069	24	19.24	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	-0.00416667	0.08608663	24	-0.05	0.9618	Tukey-Kramer	1.0000
TREAT	e	g	0.07416667	0.08608663	24	0.86	0.3975	Tukey-Kramer	0.8244
TREAT	e	h	0.00666667	0.08608663	24	0.08	0.9389	Tukey-Kramer	0.9998
TREAT	f	g	0.07833333	0.08608663	24	0.91	0.3719	Tukey-Kramer	0.7997
TREAT	f	h	0.01083333	0.08608663	24	0.13	0.9009	Tukey-Kramer	0.9993
TREAT	g	h	-0.06750000	0.08608663	24	-0.78	0.4407	Tukey-Kramer	0.8609

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-84.83422586	
1	4	-123.0309365	0.00003986
2	1	-123.0333226	.
3	1	-123.0333398	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00002991
PERIOD	0.00904889
COW(SQUARE)	0.02783106
SQUARE*PERIOD	0.00000000
Residual	0.00742654

Model Fitting Information for PROTEIN

Description	Value
Observations	48.0000
Res Log Likelihood	21.0834
Akaike's Information Criterion	16.0834
Schwarz's Bayesian Criterion	11.6229
-2 Res Log Likelihood	-42.1667

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.12	0.9470

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	3.15500000	0.07218260	24	43.71	0.0001
TREAT	f	3.13916667	0.07218260	24	43.49	0.0001
TREAT	g	3.14166667	0.07218260	24	43.52	0.0001
TREAT	h	3.13500000	0.07218260	24	43.43	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	0.01583333	0.03518177	24	0.45	0.6567	Tukey-Kramer	0.9690
TREAT	e	g	0.01333333	0.03518177	24	0.38	0.7080	Tukey-Kramer	0.9810
TREAT	e	h	0.02000000	0.03518177	24	0.57	0.5750	Tukey-Kramer	0.9405
TREAT	f	g	-0.00250000	0.03518177	24	-0.07	0.9439	Tukey-Kramer	0.9999
TREAT	f	h	0.00416667	0.03518177	24	0.12	0.9067	Tukey-Kramer	0.9994
TREAT	g	h	0.00666667	0.03518177	24	0.19	0.8513	Tukey-Kramer	0.9975

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-88.35527525	
1	2	-118.8324588	0.00000667
2	1	-118.8328595	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00000000
PERIOD	0.00417553
COW(SQUARE)	0.02677289
SQUARE*PERIOD	0.00000000
Residual	0.00920804

Model Fitting Information for LACTOSE

Description	Value
Observations	48.0000
Res Log Likelihood	18.9831
Akaike's Information Criterion	13.9831
Schwarz's Bayesian Criterion	9.5227
-2 Res Log Likelihood	-37.9663

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	1.37	0.2768

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	4.96666667	0.06357903	24	78.12	0.0001
TREAT	f	5.01166667	0.06357903	24	78.83	0.0001
TREAT	g	5.04500000	0.06357903	24	79.35	0.0001
TREAT	h	4.99916667	0.06357903	24	78.63	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	-0.04500000	0.03917492	24	-1.15	0.2620	Tukey-Kramer	0.6638
TREAT	e	g	-0.07833333	0.03917492	24	-2.00	0.0570	Tukey-Kramer	0.2161
TREAT	e	h	-0.03250000	0.03917492	24	-0.83	0.4149	Tukey-Kramer	0.8399
TREAT	f	g	-0.03333333	0.03917492	24	-0.85	0.4032	Tukey-Kramer	0.8296
TREAT	f	h	0.01250000	0.03917492	24	0.32	0.7524	Tukey-Kramer	0.9885
TREAT	g	h	0.04583333	0.03917492	24	1.17	0.2535	Tukey-Kramer	0.6510

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-42.66254498	
1	3	-78.26011919	0.00007774
2	1	-78.26325175	0.00000017
3	1	-78.26325832	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00000000
PERIOD	0.00000000
COW(SQUARE)	0.08592493
SQUARE*PERIOD	0.00000000
Residual	0.02537735

Model Fitting Information for NFS

Description	Value
Observations	48.0000
Res Log Likelihood	-1.3017
Akaike's Information Criterion	-6.3017
Schwarz's Bayesian Criterion	-10.7621
-2 Res Log Likelihood	2.6033

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	1.04	0.3944

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	8.84416667	0.09630779	24	91.83	0.0001
TREAT	f	8.91500000	0.09630779	24	92.57	0.0001
TREAT	g	8.95750000	0.09630779	24	93.01	0.0001
TREAT	h	8.90083333	0.09630779	24	92.42	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	-0.07083333	0.06503506	24	-1.09	0.2869	Tukey-Kramer	0.6994
TREAT	e	g	-0.11333333	0.06503506	24	-1.74	0.0942	Tukey-Kramer	0.3247
TREAT	e	h	-0.05666667	0.06503506	24	-0.87	0.3922	Tukey-Kramer	0.8195
TREAT	f	g	-0.04250000	0.06503506	24	-0.65	0.5197	Tukey-Kramer	0.9133
TREAT	f	h	0.01416667	0.06503506	24	0.22	0.8294	Tukey-Kramer	0.9962
TREAT	g	h	0.05666667	0.06503506	24	0.87	0.3922	Tukey-Kramer	0.8195

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	198.83172364	
1	2	164.67568038	0.00000230
2	1	164.67548821	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00000000
PERIOD	8.75223499
COW(SQUARE)	14.26447498
SQUARE*PERIOD	0.00000000
Residual	5.49657664

Model Fitting Information for NUREA

Description	Value
Observations	48.0000
Res Log Likelihood	-122.771
Akaike's Information Criterion	-127.771
Schwarz's Bayesian Criterion	-132.232
-2 Res Log Likelihood	245.5421

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	1.40	0.2665

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	18.76750000	1.95826787	24	9.58	0.0001
TREAT	f	19.49166667	1.95826787	24	9.95	0.0001
TREAT	g	20.66000000	1.95826787	24	10.55	0.0001
TREAT	h	20.01000000	1.95826787	24	10.22	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	-0.72416667	0.95712910	24	-0.76	0.4567	Tukey-Kramer	0.8729
TREAT	e	g	-1.89250000	0.95712910	24	-1.98	0.0596	Tukey-Kramer	0.2243
TREAT	e	h	-1.24250000	0.95712910	24	-1.30	0.2066	Tukey-Kramer	0.5729
TREAT	f	g	-1.16833333	0.95712910	24	-1.22	0.2341	Tukey-Kramer	0.6202
TREAT	f	h	-0.51833333	0.95712910	24	-0.54	0.5931	Tukey-Kramer	0.9479
TREAT	g	h	0.65000000	0.95712910	24	0.68	0.5036	Tukey-Kramer	0.9040

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-99.42806891	
1	2	-133.4905985	0.00000197
2	1	-133.4907314	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00000000
PERIOD	0.01400334
COW(SQUARE)	0.01258636
SQUARE*PERIOD	0.00000000
Residual	0.00658899

Model Fitting Information for FAT

Description	Value
Observations	48.0000
Res Log Likelihood	26.3121
Akaike's Information Criterion	21.3121
Schwarz's Bayesian Criterion	16.8516
-2 Res Log Likelihood	-52.6241

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.25	0.8582

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	0.95083333	0.07140574	24	13.32	0.0001
TREAT	f	0.94666667	0.07140574	24	13.26	0.0001
TREAT	g	0.95416667	0.07140574	24	13.36	0.0001
TREAT	h	0.97333333	0.07140574	24	13.63	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	0.00416667	0.03313856	24	0.13	0.9010	Tukey-Kramer	0.9993
TREAT	e	g	-0.00333333	0.03313856	24	-0.10	0.9207	Tukey-Kramer	0.9996
TREAT	e	h	-0.02250000	0.03313856	24	-0.68	0.5037	Tukey-Kramer	0.9041
TREAT	f	g	-0.00750000	0.03313856	24	-0.23	0.8229	Tukey-Kramer	0.9958
TREAT	f	h	-0.02666667	0.03313856	24	-0.80	0.4289	Tukey-Kramer	0.8516
TREAT	g	h	-0.01916667	0.03313856	24	-0.58	0.5684	Tukey-Kramer	0.9376

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-105.1884952	
1	2	-151.8527075	0.00001276
2	1	-151.8536915	0.00000001

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00924166
PERIOD	0.01283600
COW(SQUARE)	0.00540432
SQUARE*PERIOD	0.00000000
Residual	0.00424400

Model Fitting Information for PROTEIN

Description	Value
Observations	48.0000
Res Log Likelihood	35.4936
Akaike's Information Criterion	30.4936
Schwarz's Bayesian Criterion	26.0331
-2 Res Log Likelihood	-70.9871

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.32	0.8081

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	0.84308333	0.08422340	24	10.01	0.0001
TREAT	f	0.83850000	0.08422340	24	9.96	0.0001
TREAT	g	0.86216667	0.08422340	24	10.24	0.0001
TREAT	h	0.85408333	0.08422340	24	10.14	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	0.00458333	0.02659574	24	0.17	0.8646	Tukey-Kramer	0.9981
TREAT	e	g	-0.01908333	0.02659574	24	-0.72	0.4800	Tukey-Kramer	0.8891
TREAT	e	h	-0.01100000	0.02659574	24	-0.41	0.6828	Tukey-Kramer	0.9756
TREAT	f	g	-0.02366667	0.02659574	24	-0.89	0.3824	Tukey-Kramer	0.8101
TREAT	f	h	-0.01558333	0.02659574	24	-0.59	0.5634	Tukey-Kramer	0.9354
TREAT	g	h	0.00808333	0.02659574	24	0.30	0.7638	Tukey-Kramer	0.9900

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-55.78964828	
1	2	-111.0068388	0.00002976
2	1	-111.0085244	0.00000003
3	1	-111.0085261	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.01640079
PERIOD	0.05369924
COW(SQUARE)	0.01683112
SQUARE*PERIOD	0.00000000
Residual	0.00989689

Model Fitting Information for LACTOSE

Description	Value
Observations	48.0000
Res Log Likelihood	15.0710
Akaike's Information Criterion	10.0710
Schwarz's Bayesian Criterion	5.6105
-2 Res Log Likelihood	-30.1419

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.89	0.4606

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	1.33725000	0.14532403	24	9.20	0.0001
TREAT	f	1.33650000	0.14532403	24	9.20	0.0001
TREAT	g	1.39183333	0.14532403	24	9.58	0.0001
TREAT	h	1.37100000	0.14532403	24	9.43	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	0.00075000	0.04061381	24	0.02	0.9854	Tukey-Kramer	1.0000
TREAT	e	g	-0.05458333	0.04061381	24	-1.34	0.1915	Tukey-Kramer	0.5451
TREAT	e	h	-0.03375000	0.04061381	24	-0.83	0.4142	Tukey-Kramer	0.8392
TREAT	f	g	-0.05533333	0.04061381	24	-1.36	0.1857	Tukey-Kramer	0.5340
TREAT	f	h	-0.03450000	0.04061381	24	-0.85	0.4040	Tukey-Kramer	0.8303
TREAT	g	h	0.02083333	0.04061381	24	0.51	0.6127	Tukey-Kramer	0.9552

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-9.42210483	
1	2	-63.69854915	0.00006231
2	1	-63.70057777	0.00000007
3	1	-63.70058014	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.06055734
PERIOD	0.14333730
COW(SQUARE)	0.04618285
SQUARE*PERIOD	0.00000000
Residual	0.02941784

Model Fitting Information for NFS

Description	Value
Observations	48.0000
Res Log Likelihood	-8.5830
Akaike's Information Criterion	-13.5830
Schwarz's Bayesian Criterion	-18.0435
-2 Res Log Likelihood	17.1660

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.84	0.4858

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	2.37233333	0.24964007	24	9.50	0.0001
TREAT	f	2.37908333	0.24964007	24	9.53	0.0001
TREAT	g	2.46666667	0.24964007	24	9.88	0.0001
TREAT	h	2.43508333	0.24964007	24	9.75	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	-0.00675000	0.07002124	24	-0.10	0.9240	Tukey-Kramer	0.9997
TREAT	e	g	-0.09433333	0.07002124	24	-1.35	0.1905	Tukey-Kramer	0.5432
TREAT	e	h	-0.06275000	0.07002124	24	-0.90	0.3791	Tukey-Kramer	0.8069
TREAT	f	g	-0.08758333	0.07002124	24	-1.25	0.2231	Tukey-Kramer	0.6018
TREAT	f	h	-0.05600000	0.07002124	24	-0.80	0.4317	Tukey-Kramer	0.8538
TREAT	g	h	0.03158333	0.07002124	24	0.45	0.6560	Tukey-Kramer	0.9688

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	108.18171828	
1	2	66.22690006	0.00023324
2	1	66.21883932	0.00000115
3	1	66.21880120	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.28728501
PERIOD	1.34670085
COW(SQUARE)	1.57777964
SQUARE*PERIOD	0.00000000
Residual	0.54215675

Model Fitting Information for NUREA

Description	Value
Observations	48.0000
Res Log Likelihood	-73.5427
Akaike's Information Criterion	-78.5427
Schwarz's Bayesian Criterion	-83.0032
-2 Res Log Likelihood	147.0854

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	1.77	0.1800

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	5.17000000	0.78044747	24	6.62	0.0001
TREAT	f	5.18916667	0.78044747	24	6.65	0.0001
TREAT	g	5.75250000	0.78044747	24	7.37	0.0001
TREAT	h	5.53666667	0.78044747	24	7.09	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	-0.01916667	0.30059850	24	-0.06	0.9497	Tukey-Kramer	0.9999
TREAT	e	g	-0.58250000	0.30059850	24	-1.94	0.0645	Tukey-Kramer	0.2394
TREAT	e	h	-0.36666667	0.30059850	24	-1.22	0.2344	Tukey-Kramer	0.6207
TREAT	f	g	-0.56333333	0.30059850	24	-1.87	0.0732	Tukey-Kramer	0.2654
TREAT	f	h	-0.34750000	0.30059850	24	-1.16	0.2590	Tukey-Kramer	0.6594
TREAT	g	h	0.21583333	0.30059850	24	0.72	0.4797	Tukey-Kramer	0.8890

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	127.96901856	
1	2	89.44671393	0.00000216
2	1	89.44661678	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.69852853
PERIOD	3.44201548
COW(SQUARE)	0.88080074
SQUARE*PERIOD	0.00000000
Residual	1.17383697

Model Fitting Information for DMI

Description	Value
Observations	48.0000
Res Log Likelihood	-85.1566
Akaike's Information Criterion	-90.1566
Schwarz's Bayesian Criterion	-94.6171
-2 Res Log Likelihood	170.3132

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.56	0.6476

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	23.37916667	1.12452947	24	20.79	0.0001
TREAT	f	23.77583333	1.12452947	24	21.14	0.0001
TREAT	g	23.93083333	1.12452947	24	21.28	0.0001
TREAT	h	23.74666667	1.12452947	24	21.12	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	-0.39666667	0.44231154	24	-0.90	0.3787	Tukey-Kramer	0.8066
TREAT	e	g	-0.55166667	0.44231154	24	-1.25	0.2243	Tukey-Kramer	0.6040
TREAT	e	h	-0.36750000	0.44231154	24	-0.83	0.4142	Tukey-Kramer	0.8393
TREAT	f	g	-0.15500000	0.44231154	24	-0.35	0.7291	Tukey-Kramer	0.9849
TREAT	f	h	0.02916667	0.44231154	24	0.07	0.9480	Tukey-Kramer	0.9999
TREAT	g	h	0.18416667	0.44231154	24	0.42	0.6808	Tukey-Kramer	0.9751

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-87.57481299	
1	2	-106.0491013	0.00000026
2	1	-106.0491153	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00581912
PERIOD	0.01335991
COW(SQUARE)	0.00910326
SQUARE*PERIOD	0.00000000
Residual	0.01583947

Model Fitting Information for DMI

Description	Value
Observations	48.0000
Res Log Likelihood	12.5913
Akaike's Information Criterion	7.5913
Schwarz's Bayesian Criterion	3.1308
-2 Res Log Likelihood	-25.1825

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.26	0.8537

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	2.24666667	0.08578021	24	26.19	0.0001
TREAT	f	2.29083333	0.08578021	24	26.71	0.0001
TREAT	g	2.27666667	0.08578021	24	26.54	0.0001
TREAT	h	2.26750000	0.08578021	24	26.43	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	-0.04416667	0.05138007	24	-0.86	0.3985	Tukey-Kramer	0.8253
TREAT	e	g	-0.03000000	0.05138007	24	-0.58	0.5647	Tukey-Kramer	0.9360
TREAT	e	h	-0.02083333	0.05138007	24	-0.41	0.6887	Tukey-Kramer	0.9770
TREAT	f	g	0.01416667	0.05138007	24	0.28	0.7851	Tukey-Kramer	0.9925
TREAT	f	h	0.02333333	0.05138007	24	0.45	0.6538	Tukey-Kramer	0.9682
TREAT	g	h	0.00916667	0.05138007	24	0.18	0.8599	Tukey-Kramer	0.9979

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	400.06317215	
1	1	307.66107262	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	1462.0781250
PERIOD	36.31944444
COW(SQUARE)	1420.4305556
SQUARE*PERIOD	2.83333333
Residual	91.96527778

Model Fitting Information for BW

Description	Value
Observations	48.0000
Res Log Likelihood	-194.264
Akaike's Information Criterion	-199.264
Schwarz's Bayesian Criterion	-203.724
-2 Res Log Likelihood	388.5277

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.09	0.9667

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	567.41666667	24.95412458	24	22.74	0.0001
TREAT	f	567.25000000	24.95412458	24	22.73	0.0001
TREAT	g	566.75000000	24.95412458	24	22.71	0.0001
TREAT	h	568.66666667	24.95412458	24	22.79	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	0.16666667	3.91504103	24	0.04	0.9664	Tukey-Kramer	1.0000
TREAT	e	g	0.66666667	3.91504103	24	0.17	0.8662	Tukey-Kramer	0.9982
TREAT	e	h	-1.25000000	3.91504103	24	-0.32	0.7523	Tukey-Kramer	0.9885
TREAT	f	g	0.50000000	3.91504103	24	0.13	0.8994	Tukey-Kramer	0.9992
TREAT	f	h	-1.41666667	3.91504103	24	-0.36	0.7206	Tukey-Kramer	0.9834
TREAT	g	h	-1.91666667	3.91504103	24	-0.49	0.6289	Tukey-Kramer	0.9607

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-82.25382397	
1	1	-124.7073791	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00464508
PERIOD	0.00366267
COW(SQUARE)	0.03107522
SQUARE*PERIOD	0.00176671
Residual	0.00604427

Model Fitting Information for SCORE

Description	Value
Observations	48.0000
Res Log Likelihood	21.9204
Akaike's Information Criterion	16.9204
Schwarz's Bayesian Criterion	12.4599
-2 Res Log Likelihood	-43.8408

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
TREAT	3	24	0.78	0.5149

Least Squares Means

Effect	TREAT	LSMEAN	Std Error	DF	t	Pr > |t|
TREAT	e	2.30416667	0.07552845	24	30.51	0.0001
TREAT	f	2.30333333	0.07552845	24	30.50	0.0001
TREAT	g	2.30333333	0.07552845	24	30.50	0.0001
TREAT	h	2.34333333	0.07552845	24	31.03	0.0001

Differences of Least Squares Means

Effect	TREAT	_TREAT	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
TREAT	e	f	0.00083333	0.03173923	24	0.03	0.9793	Tukey-Kramer	1.0000
TREAT	e	g	0.00083333	0.03173923	24	0.03	0.9793	Tukey-Kramer	1.0000
TREAT	e	h	-0.03916667	0.03173923	24	-1.23	0.2291	Tukey-Kramer	0.6120
TREAT	f	g	0.00000000	0.03173923	24	0.00	1.0000	Tukey-Kramer	1.0000
TREAT	f	h	-0.04000000	0.03173923	24	-1.26	0.2197	Tukey-Kramer	0.5960
TREAT	g	h	-0.04000000	0.03173923	24	-1.26	0.2197	Tukey-Kramer	0.5960

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	94.73499665	
1	4	77.47199530	0.00305384
2	1	77.33687830	0.00020617
3	1	77.32851731	0.00000126
4	1	77.32846823	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.69852488
TREAT	-0.00000000
COW(SQUARE)	0.89259667
SQUARE*TREAT	0.00000000
Residual	1.12671245

Model Fitting Information for DMI

Description	Value
Observations	48.0000
Res Log Likelihood	-79.0975
Akaike's Information Criterion	-84.0975
Schwarz's Bayesian Criterion	-88.5580
-2 Res Log Likelihood	158.1951

08:11 Tuesday, June 19, 2001

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
PERIOD	3	24	37.70	0.0001

Least Squares Means

Effect	PERIOD	LSMEAN	Std Error	DF	t	Pr > |t|
PERIOD	1	24.89916667	0.63333829	24	39.31	0.0001
PERIOD	2	25.19166667	0.63333829	24	39.78	0.0001
PERIOD	3	23.67916667	0.63333829	24	37.39	0.0001
PERIOD	4	21.06250000	0.63333829	24	33.26	0.0001

Differences of Least Squares Means

Effect	PERIOD	_PERIOD	Difference	Std Error	DF	t	Pr > |t|	Adjustment
PERIOD	1	2	-0.29250000	0.43334214	24	-0.67	0.5061	Tukey-Kramer
PERIOD	1	3	1.22000000	0.43334214	24	2.82	0.0096	Tukey-Kramer
PERIOD	1	4	3.83666667	0.43334214	24	8.85	0.0001	Tukey-Kramer
PERIOD	2	3	1.51250000	0.43334214	24	3.49	0.0019	Tukey-Kramer
PERIOD	2	4	4.12916667	0.43334214	24	9.53	0.0001	Tukey-Kramer
PERIOD	3	4	2.61666667	0.43334214	24	6.04	0.0001	Tukey-Kramer

Differences of Least Squares Means

Adj P
0.9055
0.0443
0.0000
0.0095
0.0000
0.0000

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-102.7986454	
1	2	-115.5695456	0.00008780
2	1	-115.5748216	0.00000035
3	1	-115.5748420	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00581913
TREAT	0.00000000
COW(SQUARE)	0.00936971
SQUARE*TREAT	0.00000000
Residual	0.01477352

Model Fitting Information for DMI

Description	Value
Observations	48.0000
Res Log Likelihood	17.3541
Akaike's Information Criterion	12.3541
Schwarz's Bayesian Criterion	7.8937
-2 Res Log Likelihood	-34.7083

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
PERIOD	3	24	11.92	0.0001

Least Squares Means

Effect	PERIOD	LSMEAN	Std Error	DF	t	Pr > |t|
PERIOD	1	2.20166667	0.06286212	24	35.02	0.0001
PERIOD	2	2.38833333	0.06286212	24	37.99	0.0001
PERIOD	3	2.13583333	0.06286212	24	33.98	0.0001
PERIOD	4	2.35583333	0.06286212	24	37.48	0.0001

Differences of Least Squares Means

Effect	PERIOD	_PERIOD	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
PERIOD	1	2	-0.18666667	0.04962109	24	-3.76	0.0010	Tukey-Kramer	0.0050
PERIOD	1	3	0.06583333	0.04962109	24	1.33	0.1971	Tukey-Kramer	0.5556
PERIOD	1	4	-0.15416667	0.04962109	24	-3.11	0.0048	Tukey-Kramer	0.0232
PERIOD	2	3	0.25250000	0.04962109	24	5.09	0.0001	Tukey-Kramer	0.0002
PERIOD	2	4	0.03250000	0.04962109	24	0.65	0.5187	Tukey-Kramer	0.9128
PERIOD	3	4	-0.22000000	0.04962109	24	-4.43	0.0002	Tukey-Kramer	0.0009

monsanto experiment 00-04-36-04 40
BODY WEIGHT BY PERIODS (kg) 10:56 Monday, June 18, 2001

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	399.44795075	
1	2	299.60352926	0.00005921
2	1	299.59422631	0.00000036
3	1	299.59417221	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	1462.7636715
TREAT	0.00000000
COW(SQUARE)	1421.7993353
SQUARE*TREAT	0.00000000
Residual	86.39001227

Model Fitting Information for BW

Description	Value
Observations	48.0000
Res Log Likelihood	-190.230
Akaike's Information Criterion	-195.230
Schwarz's Bayesian Criterion	-199.691
-2 Res Log Likelihood	380.4608

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
PERIOD	3	24	6.24	0.0028

Least Squares Means

Effect	PERIOD	LSMEAN	Std Error	DF	t	Pr > |t|
PERIOD	1	573.16666667	24.76429559	24	23.14	0.0001
PERIOD	2	572.50000000	24.76429559	24	23.12	0.0001
PERIOD	3	565.50000000	24.76429559	24	22.84	0.0001
PERIOD	4	558.91666667	24.76429559	24	22.57	0.0001

Differences of Least Squares Means

Effect	PERIOD	_PERIOD	Difference	Std Error	DF	t	Pr > |t|	Adjustment
PERIOD	1	2	0.66666667	3.79451385	24	0.18	0.8620	Tukey-Kramer
PERIOD	1	3	7.66666667	3.79451385	24	2.02	0.0546	Tukey-Kramer
PERIOD	1	4	14.25000000	3.79451385	24	3.76	0.0010	Tukey-Kramer
PERIOD	2	3	7.00000000	3.79451385	24	1.84	0.0774	Tukey-Kramer
PERIOD	2	4	13.58333333	3.79451385	24	3.58	0.0015	Tukey-Kramer
PERIOD	3	4	6.58333333	3.79451385	24	1.73	0.0956	Tukey-Kramer

Differences of Least Squares Means

Adj P
0.9980
0.2086
0.0050
0.2779
0.0077
0.3284

The MIXED Procedure

Class Level Information

Class	Levels	Values
COW	12	2401 2416 2442 2555 2574 2579 2584 2586 2591 2663 2670 2671
SQUARE	3	1 2 3
PERIOD	4	1 2 3 4
TREAT	4	e f g h

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-85.86655097	
1	2	-130.2674744	0.00000873
2	1	-130.2680509	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
SQUARE	0.00508696
TREAT	0.00000000
COW(SQUARE)	0.03077726
SQUARE*TREAT	0.00000000
Residual	0.00721059

Model Fitting Information for SCORE

Description	Value
Observations	48.0000
Res Log Likelihood	24.7007
Akaike's Information Criterion	19.7007
Schwarz's Bayesian Criterion	15.2403
-2 Res Log Likelihood	-49.4015

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
PERIOD	3	24	7.91	0.0008

Least Squares Means

Effect	PERIOD	LSMEAN	Std Error	DF	t	Pr > |t|
PERIOD	1	2.27250000	0.06972309	24	32.59	0.0001
PERIOD	2	2.40583333	0.06972309	24	34.51	0.0001
PERIOD	3	2.25083333	0.06972309	24	32.28	0.0001
PERIOD	4	2.32500000	0.06972309	24	33.35	0.0001

Differences of Least Squares Means

Effect	PERIOD	_PERIOD	Difference	Std Error	DF	t	Pr > |t|	Adjustment	Adj P
PERIOD	1	2	-0.13333333	0.03466649	24	-3.85	0.0008	Tukey-Kramer	0.0040
PERIOD	1	3	0.02166667	0.03466649	24	0.63	0.5379	Tukey-Kramer	0.9230
PERIOD	1	4	-0.05250000	0.03466649	24	-1.51	0.1430	Tukey-Kramer	0.4448
PERIOD	2	3	0.15500000	0.03466649	24	4.47	0.0002	Tukey-Kramer	0.0009
PERIOD	2	4	0.08083333	0.03466649	24	2.33	0.0284	Tukey-Kramer	0.1188
PERIOD	3	4	-0.07416667	0.03466649	24	-2.14	0.0428	Tukey-Kramer	0.1695

Differences of Least Squares Means

Attachment 13

Animal Health and Reproduction

pp. 109-112

ATTACHMENT 13.

Animal Health and Reproduction

Animal Health

Cows ID	Diagnoses, Date and treatment	Reproductive status
2401	Mastitis (10/8/00: local Cloxacilyn-10/10/00: Spiramicyn, Neomycin, Flumetazone-10/20/00: Yeasts) Pododermatitis (11/13/00: Fluorphenicol)	Empty
2416		Empty, anoestrus
2442	Pododermatitis (12/4/00: local cooper sulfate)	Empty, anoestrus
2555	Mastitis (11/1/00: local Cloxacilyn, parenteral-Spiramicyn and Streptomycin) Pododermatitis (11/30/00: Fluorphenicol)	Pregnant
2574	Pododermatitis (9/27/00: local povidone iodine)	Empty, anoestrus
2579		Pregnant
2584	Pododermatitis (12/15/00: Tetraciclyne LA)	Empty, anoestrus
2586	Mastitis (12/16/00: Riphampicyn, Cephalosporyn)	Pregnant
2591	Mastitis (10/6/00: local Cloxacilyn-10/12/00: Yeasts-11/6/00: Spiramicyn, Neomycin, Flumetazone-11/14/00: Riphampicyn, Cephalosporyn-11/27/00: Riphampicyn, Cephalosporyn-12/9/00: local Cloxacilyn-12/14/00: Myconazole)	Empty, anoestrus
2663	Mastitis (9/18/00: subclinical, high conductivity)	Pregnant
2670	Mastitis (10/12/00: local Cloxacilyn-12/13/00: Riphampicyn, Cephalosporyn)	Empty, anoestrus
2671	Pododermatitis (10/27/00 and 11/13/00: local cooper sulfate, parenteral Fluorphenicol 11/16/00: Ceftiofur-11/30/00: local cooper sulfate, parenteral Fluorphenicol-12/13/00: Tetracycline LA)	Empty anoestrus

Reproductive Performance

The dates of parturitions were between the 07/21/2000 and the 08/27/2000 and the mating period between the 10/20/2000 and the 12/27/2000.

Calving

The calving type was classified as easy, assisted and difficult according to the degree of received help: any, smaller and bigger respectively. In the following chart the classification of the same ones is shown according to the group and total.

Exp. 00-04-36-04	Calving type		
	Easy	Assisted	Difficult
	7 (59%)	4 (33%)	1 (8%)

As it can be observed, the difficulty was minimum. The puerperium controls were carried out from 20 days after parturition. There were not alterations diagnosed that could affect the aptitude for the services.

Services

Artificial insemination (AI) was carried out using frozen semen in straws of six bulls coming from two national AI centers (CIAVT and CIALE). The heat detection was performed by means of observation of the sexual behavior at the time the animals were liberated of their boxes. Tail painting was used to help but the "tied up" stall system and the few moments out of the boxes, were decisive to implement a protocol of hormonal therapy to carry out the "time fixed AI" (TFAI). The method used was the "OVSYNC" that consisted in injecting, the 10/24/00, gonadotrophin releasing factor (GnRH), prostaglandins seven days later, in the day eight again GnRH and in the day nine, 11/2/00, all the treated cows were inseminated.

Exp. 00-04-36-04	Services/pregnancies and conception rate (%)			
	1st service	2nd service	3rd service	Total
	12/2 (17)	9/0 (0)	2/2 (100)	23/4 (17)

The return to the second service was characterized by the lack of demonstration of heat (anoestrus) besides a low conception rate. The following table shows the number of first, second, third and total of services, of pregnancies in each one of them and the rate of corresponding conception. The percentage of pregnancy was of 33% during the whole period of service.

Comments:

A total of ten cows had different health problems. Six cows suffered pododermatitis and the others six mastitis. Mainly environmental agents caused this. This stands out the importance of the feet afflictions that influenced directly on the reproductive performance causing absence of heat with the consequent destination to sale (dry empty).

All the cows used in this experiments were multiparous cows, which always were managed under grazing condition. Despite that the cows had seven hours/day out of the boxes (see Attachment 2, cows management & exercise). The scarce mobility, the hard and humid floor with abundant depositions and the weather characterized by high temperatures and humidity during the development of the experiment, were the most important factors of the observed pathologies. Also, limiting the recovery of the animals. These problems must be seriously considerate for controlled future experiment using multiparous dairy cows from grazing systems, not adapted to feedlot systems or indoor conditions. It is important to stand out that hot weather affected the productive and reproductive status in the dairy farms of the Argentinean central dairy basin, to which the Experimental Station Rafaela of the INTA belongs.

Attachment 14

Protocol, Amendments and Deviations

pp. 113-144

PROTOCOL

Study Number:

Monsanto 00-04-36-04
INTA (Convenio Monsanto-INTA)

Study Title:

Effects of Feeding Cottonseed Produced From Cotton containing Bollgard and CryX Genes on Feed Intake, Milk Production and Composition in Lactating Dairy Cows in Argentina

Sponsor:

Monsanto Company
700 Chesterfield Parkway North
St. Louis, MO 63198

Clinical Investigator:

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Study Monitor:

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Sponsor Representative:	James D. Astwood, Ph.D. Product Safety Center Monsanto Company 700 Chesterfield Village Parkway North St. Louis, MO 63198 Phone: (636) 737-6356 FAX: (636) 737-6189 e-mail: james.d.astwood@monsanto.com
Veterinarian:	Martín Maciel INTA (Instituto Nacional de Tecnología Agropecuaria) Estación Experimental Agropecuaria Rafaela C.C. 22 2300 Rafaela - Pcia. Santa Fe Argentina Phone: (34924) 40121/4 FAX: (34924) 40114 e-mail: mmaciel@rafaela.inta.gov.ar
Primary Testing Facility:	INTA Estación Experimental Agropecuaria Rafaela C.C. 22 2300 Rafaela Pcia. Santa Fe Argentina
Analytical Testing Facility:	Monsanto Company VIA, 800 N. Lindbergh Blvd. St. Louis, MO 63141 Contact: Jack Milligan Phone: (636) 694-6709 FAX: (636) 737-6189 e-mail: jack.l.milligan@monsanto.com

Romer Labs, Inc.
1301 Stylemaster Drive
Union, MO 63084
Phone: (636)583-8600

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730 Warren Road
Ithaca, NY 14850
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Forage Analysis Laboratory
EFA Rafaela INTA
Argentina and Faculty of Chemistry
Santa Fe
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Sancor Cul
Sunchales, Santa Fe
Argentina

Table of Contents

Section **Page**

1.0 Introduction

Cotton is modified to provide tolerance towards insect pests(BollGard® and Cry protein; BG x CryX). Cotton has also been modified to impart tolerance to the herbicide Roundup® (Roundup Ready®; RR). Utilization of this crop offers producers an alternative strategy for managing weed pressure and insect pests. Insect tolerance is inferred to the plant by inclusion of a gene for Cry protein derived from *Bacillus thurigenesis* (B.t.). Herbicide tolerance is inferred to the plant by inclusion of the gene for CP4EPSPS. Ginned cottonseed (linted) is utilized extensively in dairy cattle rations as an energy, fiber and protein source. This study is being conducted to confirm comparable performance of cottonseed from modified cotton (BG x CryX), BG, and RR with cottonseed from conventional variety without the transgenic traits in lactating dairy cows.

2.0 Purpose

The primary purpose of this experiment is to assess the effects of feeding cottonseed ginned from cotton containing both BollGard® and CryX and the individual genes for BollGard® and Roundup Ready® on feed intake, milk production and milk composition in lactating dairy cattle compared with cottonseed from a parental line grown under identical conditions and harvested at the same physiological maturity.

3.0 Time Lines

Animal Feeding Start Date:	September 25, 2000
Animal Feeding End Date:	January 14, 2001

4.0 Type Of Study

This is a nonpivotal clinical food chain study which will be conducted according to the concepts in CVM's Guideline: Good Target Animal Study Practices - Clinical Investigators and Monitors (December 1996). This study is not required for regulatory approval.

5.0 Quality Assurance

The Monsanto Ag Regulatory Quality Assurance Unit will provide quality assurance support such as protocol and final report reviews.

6.0 Materials and Methods

6.1 *Test Substances. Stacked and individual traits.* Cottonseed from cotton (DP50B x CryX; "G", Brown) genetically modified to contain two different B.t.'s, cottonseed from cotton (DP50B; "F", Green) genetically modified to contain BollGard and cottonseed from cotton (DP50RR; "H", Orange) genetically modified to tolerate Roundup® will be supplied by Monsanto. Information on growing conditions, harvest, storage and processing is available from Monsanto (Production plan 99-04-36-01) and archived. Sample (~400 g) of each cottonseed product will be sent to Monsanto in a properly labeled (study number, date collected, treatment letter and color, initials of the person performing the sampling) plastic bag on dry ice for glyphosate residue analysis. The samples will be sent to Jack Milligan, Monsanto Company – VIA, 800 N Lindbergh Blvd., St. Louis, MO 63141.

6.1 *Control. Parental.* . Cottonseed from non-genetically modified cotton (DP50; "E", Red) will be supplied by Monsanto (Production Plan 99-04-36-01).

Information on growing conditions, harvest, storage and processing is available from Monsanto (Production Plan 99-04-36-01) and archived. Sample (~400 g) of each cottonseed product will be sent to Monsanto in a properly labeled (study number, date collected, treatment letter and color, initials of the person performing the sampling) plastic bag on dry ice for glyphosate residue analysis. The samples will be sent to Jack Milligan, Monsanto Company – VIA, 800 N Lindbergh Blvd., St. Louis, MO 63141.

6.3 *Test and Control Substance Storage.* Test and control cottonseed will be stored at ambient temperature in paper bags on concrete and dry floor in a completely enclosed shed.

6.4 *Test and Control Substance Administration.* Cottonseed from test or control lines will be blended into total mixed rations daily and the respective treatment will be fed to dairy cows.

6.5 *Test and Control Substance Preparation and Accountability.* Test and control substance accountability will be maintained throughout the study. Amount of test and control product harvested, ginned, used and in inventory will be recorded. Samples of each substance will be taken and retained by the sponsor. Excess test and control product will be disposed of according to the sponsor's directions.

Prior to the start of study, test and control substances will be sampled (~800 g) and sent to Monsanto in a properly labeled plastic bag on dry ice with the study number, date collected, treatment letter "E","F","G", or "H" and color of bag, and the initials of the person doing the sampling.

Cottonseed samples (~200 g) will be characterized using PCR by Monsanto to confirm the test substance is actually the Roundup Ready, BollGard or BG x CryX product and the control is not. Also, representative samples of each cottonseed product (~200 g) will be sent by the Monitor to Romer Labs for mycotoxin, approximately 200 g to Covance for gossypol and approximately 200 g sent to Dairy One, DHI Forage Analysis Laboratory for nutrient content.

7.0 Test System

7.1 *Site.* This study will be conducted at INTA's research farm located at Rafaela, Santa Fe, Argentina. The site was selected based on a review of the record-keeping methods, herd health, cow availability, herd management, quality of investigator, storage capabilities for test and control articles, and feed processing capabilities. The record-keeping system will include individual milk weights at each milking (2 times per day), feed intake and refusals and all medications and therapies and other management information for each cow. A list of criteria for site selection is included in Attachment 1.

7.2 *Animals.* This study will include 12 mid-lactation multiparous dairy cattle (approximately 70 to 130 days in milk) selected from INTA's research herd and blocked by previous two week milk production and stage of lactation that meet the Criteria for Animal Selection (Attachment 2). Animals will be assigned to the study using an animal inventory form (Attachment 3). Animals will be randomly assigned to specific treatments between 21 and 28 days before initiation of treatment feeding.

7.3 *Identification.* All cows on study will be identified uniquely by two different means (ear tag and tattoo).

7.4 *Safety.* Safety procedures will be followed according to practices currently used at the site. Appropriate security measures will be in place to prevent tampering, destruction and stealing of the test and control substances.

7.5 *Animal Care and Facilities.* Established site practices will be followed for management, health care, reproduction, milking and feeding. The formulated feed will meet or exceed the Agricultural Food and Research Council (AFRC, 1993)

nutrient requirements for dairy cattle. Feed and water will be provided *ad libitum* throughout the trial so that milk yield is not limited. The study file will include documentation which describes the following practices:

a) Feeding program, including ration specifications and ingredients, feed and ingredient sampling. Feed will be offered such that a minimum of a 10% refusal is recorded and refusals will be disposed in a secure location and burned.

b) Milking program.

c) Mastitis identification and treatment. In addition, validation of the following systems will be included in the study file:
   1. Milk meter accuracy records comparing the periods one week before study initiation and once per 28 days during the study.
   2. Accuracy of electronic capture of milk weights.
   3. Accuracy of scales for measuring feed offered and refused, mixing, and weighing of cattle.

8.0 Experimental Design and Conduct

8.1 *Design.* This is a 4 x 4 Latin Square design with 4 lactating multiparous Holstein Freisian cows and four treatments. Each square will be replicated three times so a total of 12 animals will be used. Cows will be blocked into three groups based on days in milk and previous two week milk yield. Each block of four cows will assigned to a square. Cows in each square/block will be randomly assigned to treatments for the first period (Attachment 4). Thereafter, cows in each square will be assigned to the treatments for periods 2 through 4 in the sequence described in Table 1. Each of the four periods will be 28 days in duration. The first 21 days of each period will be used for adaptation to treatment, the next seven days will be used to determine treatment effects on feed intake, milk yield, and milk composition.

Table 1
4 X 4 Latin Square Design

Square 1

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
Cow 1	E	F	H	G
Cow 2	F	G	E	H
Cow 3	G	H	F	E
Cow 4	H	E	G	F

Square 2

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
Cow 5	E	G	F	H
Cow 6	F	E	H	G
Cow 7	G	H	E	F
Cow 8	H	F	G	E

Square 3

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
Cow 9	E	F	G	H
Cow 10	F	H	E	G
Cow 11	G	E	H	F
Cow12	H	G	F	E

Where: Letters designate individual treatment codes.

8.2 *Treatments.* Cows will be fed total mixed diets containing linted cottonseed. Actual treatment/descriptions for each code will not be made available to the research unit personnel during the course of study.

Treatments will be incorporated into a total mixed ration in a fixed percentage on a dry matter basis such that the percent of corn grain, protein supplement, mineral and vitamins, alfalfa hay, corn silage and cottonseed will be similar across treatment groups. Cottonseed will be incorporated in the diet to achieve about 2.5 kg (as is) consumption per cow per day. Diets will be fed for about a 10% refusal.

8.3 *Treatment Assignment.* Animals that meet the selection criteria (Attachment 2) will be randomly assigned to study between 21 and 28 days before the beginning of the treatment period using an Animal Inventory Form and Treatment Assignment Forms provided by the Monsanto Company.

8.4 *Study Duration.* The study includes four 28-day periods for a total of 112 days.

8.5 *Animal and Milk Disposition.* Animals will remain on study until the end of the fourth 28-day period. Animals may only be removed from the study for significant health reasons. Removal of an animal requires prior notification and

agreement between Clinical Investigator and Study Monitor or Study Co-Monitor. Animals removed from the study for health reasons must be examined by qualified personnel and the reason for removal recorded. Animals that die while on study or that require euthanasia because they are moribund will be necropsied to the extent necessary to determine cause of death or morbidity. The removal of animals either prematurely or at the end of the study will be recorded on an Animal Release form (Attachment 5). No milk from cows in this study will enter the food chain until 30 days after the completion of the study. The location and means of milk disposal will be documented.

9.0 Observations, Examinations and Tests

9.1 *Daily Observations.* Cows will be observed daily and noted as observed by designated individuals. All health-related observations and/or medicines administered will be recorded. The paper on which the observation is originally recorded will be sent to Monsanto and copies retained by the investigator. Electronic records may be used if this system for data collection has been validated and documentation is on file. All electronic data will be printed out on the day of observation and signed and dated by the individual responsible for those data collection.

9.2 *Feed Intake.* Quantity of feed offered and refused will be recorded daily throughout the treatment period.

9.3 *Diets.* Diets will be formulated by the Investigator to meet or exceed the United Kingdom's current requirements for lactating dairy cattle (AFRC, 1993). The same proportion of cottonseed, corn grain, protein supplement, alfalfa hay and corn silage will be used in the control and transgenic diets. Rations will be adjusted weekly to accommodate any variation in dry matter content of feeds.

9.4 *Feed Composition.* Prior to the start of study, all ingredients will be sampled and analyzed for but not limited to moisture, acid detergent fiber, neutral detergent fiber, crude protein, fat, calcium, phosphorus, magnesium, potassium, sodium and ash (Forage Analysis Laboratory, EFA Rafaela INTA, Argentina and Faculty of Chemistry, Santa Fe, Argentina). In addition, corn silage samples will be analyzed for in vitro digestibility, pH, and organic acids (Forage Analysis Laboratory, EFA Rafaela INTA, Argentina and Faculty of Chemistry, Santa Fe, Argentina). Total mixed rations will be sampled on an experimental period basis. A sample of the commingled feed will be analyzed for but not limited to moisture, acid detergent fiber, neutral detergent fiber, crude protein, fat, calcium,

phosphorus, magnesium, potassium, sodium and ash (Forage Analysis Laboratory, EFA Rafaela INTA, Argentina and Faculty of Chemistry, Santa Fe, Argentina).

Corn silage samples will be collected weekly for dry matter determination by the study site and rations adjusted weekly to accommodate any variation in feeds. In addition, samples of the dietary ingredients will be taken from each lot and/or batch before they are incorporated in the ration. These samples will be retained until the analytical results from the corresponding total mixed rations (TMRs) have been evaluated. If the nutrient values from the TMRs do not match what was expected from the formulations, the individual ingredient samples will be analyzed for the nutrient content.

9.5 *Body Weight and Body Condition Score.* One week prior to the initiation of the study and on Day 28 of each period, body weights will be measured and body condition scored using the 5 point system (quarter point basis). Body condition scoring will be done independently by the same two people over the course of the study.

9.6 *Milk Yield and Composition.* Individual milk yields will be recorded after each milking. Consecutive morning and evening milk samples collected daily during the last 7 days of each period will be analyzed for fat, protein, SNF (solids nonfat), SCC (somatic cell counts), MUN (milk urea nitrogen) and lactose. Milk Analysis will be carried out with a Milkoscan Model 4000, Foss Electric, DK DK-3400, Hillorod, Denmark (Sancor Cul, Sunchales, Argentina). Thirty ml samples of milk will be taken aseptically from each cow at each milking during the seven day collection period and stored in properly labelled (cow ID, date, study number, AM or PM milking, initials) containers at -5 to -20 degrees C for future analysis as requested by the sponsor.

9.7 *Reproduction.* Individual animal reproduction data will be collected. The sheets on which the information is originally recorded will be sent to Monsanto. The information will include breedings and medicines or reproductive aids used and results of reproduction examinations. The pregnancy status of the animal will be documented within two weeks of when the animal is removed from the study. These data may only be used to address unexpected variances in milk production.

9.8 *Adverse Experiences.* All adverse experiences or reactions that might be related to test or control substances will be reported immediately. An adverse experience is defined as any event that is unintended or unexpected in terms of duration or severity that may be associated with treatment administration.

9.9 *Data Handling.* Monsanto Study Monitor/Co-Monitors will review the raw data periodically and be responsible for transfer of the originals or exact copies to Monsanto's Biotechnology Regulatory Science Archive. All raw data will be transferred to Monsanto (via Study Monitor) within 2 months after the final report has been completed. The data will be stored in Monsanto's Biotechnology Regulatory Science Archive in Chesterfield for a minimum of two years after study completion. An exact copy of the data will remain at the test facility (accessible to the Investigator) for five years after study completion.

10.0 Data Analysis

Data will be analyzed using the MIXED procedure in SAS by INTA. The model will account for variation due to cow and period along with comparisons among the treatments. Treatment means will be reported as least squares means with the associated standard error. Assumptions, e.g., uniformity of error variance, will be evaluated and appropriate action taken if any assumptions are violated. Observations that are at least two standard deviations outside the treatment means will be investigated. If they are deemed to be outliers then they will be subject to elimination from the analysis. Only the following comparisons will be made: DP50B x CryX vs DP50; DP50B vs DP50; and DP50RR vs DP50.

11.0 Experimental Timeline

	2000												2001					
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	
Cotton production	XXXXXXXXXXXXX																	
Harvest & gin cotton					XXXX													
Transport cottonseed to site & analytical						XXXXX												
Feeding trial									XXXXXXXXX									
Data analysis													XXXX					
Data summary / report																XXXXX		

12.0 Record Requirements

A. Adverse experiences or reactions
B. Amendments, deviations, clarifications to the protocol and memos to file, etc.
C. Animal inventory
D. Animal release
E. Body weights and body condition scores
F. Breeding and rectal palpation data

G. Clinical observations
H. Contact reports
I. Descriptions of feeding, milking and mastitis programs
J. Electronic capture of milk weights accuracy check (if applicable)
K. Feed accountability (controls and treatment)
L. Feed formulations
M. Feed offered and refused
N. Feed sample collection and analysis results
O. Log entries
P. Medication records
Q. Milk meter accuracy check
R. Milk production and composition results
S. Necropsy results, if applicable
T. Statistical analysis and results
U. Treatment assignments

Attachment 1
Criteria for Selection of Farm

1. A sufficient number of multiparous Holstein dairy cows available for start on study at the same time (12 mid lactation cows) which meet Animal Eligibility criteria (Attachment 2).

2. Records of individual milk weights at each milking.

3. Records of individual daily feed intakes.

4. Reliable record-keeping of health records for individual cows.

5. Regular herd health practices, no major health problems in herd.

6. Adequate labor that is well trained.

7. Qualified Clinical Investigator.

8. GLP practices in place or approaching GLP standards.

9. Secure facilities for storing test and control substances and conduct of the study.

10. Adequate storage facilities for test and control substances.

12. Twice daily milking.

13. Feed total mixed ration to individual cows.

14. Good milking techniques practiced.

15. Milk equipment properly maintained.

16. Cows in adequate body condition.

17. Good nutrition program.
    a. Balanced rations
    b. Consistent and adequate feed and water supply

Attachment 2
Criteria for Selection of Animals for Study Eligibility

1. Lactating multiparous dairy animal (all animals of the same breed).

2. Between 70 and 130 days in milk and greater than 30 kg milk/day at the start of the first period.

3. Good general health (no recent history of reproductive, digestive or metabolic disorders or injury). Specific criteria include:
   a) Good conformation (feet, legs and udder);
   b) Good disposition, easy to handle and be acclimated to facility;
   c) Good body condition for the stage of lactation;
   d) Four functional quarters with no recent history of chronic or repeated mastitis;
   e) Vaccinated for brucellosis (strain 19), IBR, BVD and Rotacoli (Rotavirus and E. coli);
   f) Animals will not be on any other study.

Attachment 3
Animal Inventory Form

ID	ID	ID	ID	ID	ID	ID	ID	ID	ID

Signature of Investigator Date

Attachment 4
Treatment Assignment Form

SQUARE 1

Treatment	Cow ID
A	
B	
C	
D	

SQUARE 2

Treatment	Cow ID
A	
B	
C	
D	

SQUARE 3

Treatment	Cow ID
A	
B	
C	
D	

Signature of Investigator **Date**

Attachment 5
Animal Release Form

Date	Animal ID	Reason for removal from study	Initials

Signature of Investigator **Date**

Signatures

Approved by:

Gary F. Hartnell
Study Monitor
Monsanto Company

29AUG00
Date

Claudia Gianni
Study Co-Monitor
Monsanto Company

05 SEPT 2000
Date

Guillermo Videla
Study Co-Monitor
Monsanto Company

08SEP00
Date

Patrick T.Weston
Testing Facility Management Representative
Monsanto Company

Aug 31, 2000
Date

James D. Astwood
Sponsor Representative
Monsanto Company

Aug 30, 2000
Date

Alejandro R. Castillo
Clinical Investigator
INTA (Instituto Nacional de Technología Agropecuaria)

Sept 14, 2000
Date

Protocol Amendment Form **Amendment #1**

Monsanto Study: # 00-04-36-04

Date changes implemented: December 25, 2000

Page number(s) and section(s): Page 9, sections 8.4
Page 13, section 11

Protocol originally stated:

8.4 ***Study Duration.*** The study includes four 28-day periods for a total of 112 days.

11. ***Experimental Timelines***
Feeding trial: to be finished January 22, 2001

Protocol amended as follows:

Study Duration. The study includes four 28-day periods for a total of 112 days for squares 2 and 3. For Square 1, the study includes 3 28-day periods (1 through 3) and one 35-day period, for a total of 119 days. The cows in Square 1 will be on study for one additional week. During this 5th week, the same measurements will be collected as in the 4th week.

11. ***Experimental Timelines***
Feeding trial: to be finished January 29, 2001

Approved by:

Gary F. Hartnell
Study Monitor
Monsanto Company

1/30/01
Date

Protocol Amendment Form
Monsanto Study:

Amendment #1
 # 00-04-36-04

James D. Astwood
Sponsor Representative
Monsanto Company

Jan 15, 01
Date

Alejandro R. Castillo
Clinical Investigator
INTA (Instituto Nacional de Technología Agropecuaria)

Jan. 22/01
Date

Deviations to the protocol

- Deviation #1, Page 8, Section 7.2

Protocol states:
"*Animals*. This study will include 12 mid-lactation multiparous dairy cattle (approximately 70 to 130 days in milk) selected from INTA's research herd."

Deviation:

This study includes 12 mid-lactation dairy cattle (approximately 42 to 64 days in milk) selected from INTA's research herd.

Reason for the deviation:

The reasons for this deviation are: 1. there were some animals with health problems, mainly mastitis, 2. to avoid the summer and holidays of Inta staff, 3. the cows will have more than 70 days during the sampling week in the first experimental period, which means that the cows will be after the pick of lactation.

Impact on study results:

These deviations will have not no impact on the outcome of the study. 17/Oct. 00

17/Oct. 00

Signature of Investigator Date

- **Deviation #2. Page 8, Section 7.2 and Page 9, Section 8.1**

Protocol states

7.2 "*Animals.* This study will include 12 mid-lactation multiparous dairy cattle (approximately 70 to 130 days in milk) selected from INTA's research herd and blocked by previous two week milk production and stage of lactation, that meet the Criteria for Animal Selection (Attachment 2). ."

8.1 "*Design*: ... Cows will be blocked into three groups based on days in milk and previous two-week milk yield."

Attachment 2, Section 2- Criteria for Selection "Between 70 and 130 days in milk...."

Deviation:

The animals were not blocked on days in milk and previous two-week milk yield. They were randomly assigned to the treatments.

Reason for the deviation:

To avoid animal stress and to improved the management of the cows, during one week before the experiment the cows were managed to choose freely the box, after a frecuency analysis, they were assigned to one box.

Impact on study results:

These deviations will have not no impact on the outcome of the study 18/Oct./00

17/Oct./00

Signature of Investigator Date

- Deviation #3. Page 10, Section 8.2 and Page 11, Section 9.3

Protocol states

8.2 "Treatments will be incorporated into a total mixed ration in a fixed percentage on a dry matter basis such that the percent of corn grain, protein supplement, mineral and vitamins, alfalfa hay, corn silage and cottonseed will be similar across treatment groups. Cottonseed will be incorporated in the diet to achieve about 2.5-kg (as is) consumption per cow per day. Diets will be fed for about a 10% refusal.

9.3 "The same proportion of cottonseed, corn grain, protein supplement, alfalfa hay and corn silage will be used in the control and transgenic diets."

Deviation:

Treatments will be incorporated into a total mixed ration in a fixed amount on as fed basis such that the percent of corn grain, protein supplement, mineral and vitamins, alfalfa hay and corn silage will be similar across treatment groups. Cottonseed will be incorporated in the diet to achieve about 2.75 kg (as is) consumption per cow per day. Diets will be fed for about a 10% refusal from week 1 through 3 and for about 5% refusal during week 4, based on mean consumption during week 3.

Reason for the deviation:

To achieved an average of 2.5 kg of cotton seeds/cow/day and considering 10% of refusal, a total of 2.75 kg/cow/day will be used.
The size of the mixer wagon and quantity of cotton seed in each treatment is not enougth to prepare a mix per treatment

* Impact on study results:

These deviations will have not ~~no~~ impact on the outcome of the study. [signature] 17/Oct./00

* During week four a further reduction in refusal to about 5% was initiated to help maintain a high cottonseed intake during the week of data collection.
DAA
30Aug01

[signature] 17/Oct./00

Signature of Investigator — Date

- Deviation #4, Page 10, Section 8.3

Protocol states
8.3 "*Treatment Assignment:* animals that meet the selection criteria (Attachment 2) will be randomly assigned to study between 21 and 28 days before the beginning of the treatment period using an Animal Inventory Form and treatment Assignment Forms provided by the Monsanto Company."

Deviation:

Animals that met the selection criteria were randomly assigned to study 14 days before the beginning of thee treatment period.

Reason for the deviation:

There were some problems during the facilities construction, these were finished two weeks before the beginning of the experiment.

Impact on study results:

These deviations will have not no impact on the outcome of the study. 17/Oct. 00

17/ Oct.

Signature of Investigator Date

- Deviation #5, Page 20, Section 9.5, Body Weight and Body Condition Score

Protocol states
9.5 "One week prior to the initiation of the study and on day 28 of each period, body weights will be measured and body condition scored using the 5 point system..."

Deviation:

Body condition was recorded one week before and every two weeks after calving and in the day 2 during the period 1 of the study

Reason for the deviation:

There were a deviation (management problems) just with the first observation, the followings will be done on day 28 in each period as was planned

Impact on study results:

These deviations will have not no impact on the outcome of the study.

17/Oct./00

17/Oct./00

Signature of Investigator Date

Deviations to the protocol

- **Deviation #6, Page 2, Section 8.1**

Protocol states:

8.1 *Design.* This is a 4 x 4 Latin Square design with 4 lactating multiparous Holstein Friesian cows and four treatments. Each square will be replicated three times so a total of 12 animals will be used. Cows will be blocked into three groups based on days in milk and previous two week milk yield. Each block of four cows will assigned to a square. Cows in each square/block will be randomly assigned to treatments for the first period (Attachment 4). **Thereafter, cows in each square will be assigned to the treatments for periods 1 through 4 in the sequence described in Table 1. Each of the four periods will be 28 days in duration.** The first 21 days of each period will be used for adaptation to treatment, the next seven days will be used to determine treatment effects on feed intake, milk yield, and milk composition.

Table 1
4 X 4 Latin Square Design

Square 1

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
Cow 13 (2670)	E	F	H	G
Cow 14 (2442)	F	G	E	H
Cow 15 (2671)	G	H	F	E
Cow 16 (2584)	H	E	G	F

Square 2

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
Cow 5	E	G	F	H
Cow 6	F	E	H	G
Cow 7	G	H	E	F
Cow 8	H	F	G	E

August 20, 2001

Signature of Investigator Date

Square 3

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
Cow 9	E	F	G	H
Cow 10	F	H	E	G
Cow 11	G	E	H	F
Cow 12	H	G	F	E

Where. Letters designate individual treatment codes.

Deviation:
Cows in Square 1 were assigned to the treatments for Period 4 in a different sequence than described in Table 1. The four cows were fed incorrect diets the first 7 days of the fourth 28-day period (from December 18 through December 25) (*). The error was detected 7 days after the beginning of Period 4. Fourth period for Square 1 will be 28 days in duration, but the cows will remain in the study one extra week.

(*) Square 1 (Actual diet during the first week for Period 4 – Dec. 18 through Dec.25)

Cow	Period 1 (28 days)	Period 2 (28 days)	Period 3 (28 days)	Period 4 (28 days)
Cow 13 (2670)	E	F	H	H
Cow 14 (2442)	F	G	E	G
Cow 15 (2671)	G	H	F	F
Cow 16 (2584)	H	E	G	E

Reason for the deviation:
Treatment assignment for Square 1, fourth period was assumed to be similar to those for Squares 2 and 3.

Impact on study results:
According to previous experiences the deviation will not impact on study results. To avoid any impact on study results, Square 1 for the fourth period will be sampled and analyzed together with the other squares (two weeks of adaptation to the diet) and will also remain in the study for an extra week, to complete the four weeks under the same treatment (three weeks of adaptation

Signature of Investigator

August 30, 2001
Date

Monsanto Company
INTA
Biotechnology Regulatory Science

Study No. 00-04-36-04

Deviation to the protocol

Deviation #7, page 12, Section 9.6

Protocol states:

"Consecutive morning and evening milk samples collected daily during the last 7 days of each period will be analyzed for fat, protein, SNF (solid nonfat), SCC (somatic cell counts), MUN (milk urea nitrogen) and lactose."

Deviation:

Consecutive morning and evening milk samples collected daily during the last 7 days of each period were analyzed for fat, protein, SNF (solid nonfat), MUN (milk urea nitrogen) and lactose.

Reason for the deviation:

SCC (somatic cell counts) in milk were not analyzed because the equipment was out of order

Impact on study results:

These deviation will have not impact on the outcome of the study

August 30, 2001

Signature of Investigator **Date**

Monsanto Company
INTA
Biotechnology Regulatory Science

Study No. 00-04-36-04

Deviation to the protocol

Deviation #8, page 11, Section 9.3

Protocol states:

"Diets will be formulated by the Investigator to meet or exceed the United Kingdom's current requirement for lactating dairy cattle (AFRC, 1993)."

Deviation:

Diets were formulated by the Investigator to meet or exceed the current requirement for lactating dairy cattle with the CNCPS system from the University of Cornell.

Reason for the deviation:

To balance diets of dairy cows managed under controlled conditions, the CNCPS system from the University of Cornell offer more details related to feed digestion and animal requirements than the English system (AFRC, 1993).

Impact on study results:

These deviation will have no impact on the outcome of the study.

Signature of Investigator

August 30, 2001
Date

Attachment 15

Argentina Production of Bulk Seed from Insect-Protected Cotton Varieties DP50BX, DP50B, Control DP50 and Reference DP50RR in 1999/2000 Season

pp. 145-169

REPORT TITLE

Argentina Production of Bulk Seed from Insect-Protected Cotton Varieties DP50BX, DP50B, Control DP50 and Reference DP50RR in 1999/2000 Season

SPONSOR

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198

PRODUCTION COORDINATOR

Kathryn A. Hamilton
Monsanto Company

AUTHORS

Kathryn A. Hamilton
Guillermo Videla
Abdul Fabellar
James Colyer

REPORT DATE

March 15, 2001

REPORT NUMBER

MSL-17089

PRODUCTION PLAN NUMBER

99-04-36-01

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(l)(A), (B), or (C).

"We submit this material to the United States Environmental Protection Agency specifically under provisions contained in FIFRA as amended, and thereby consent to use and disclosure of this material by EPA according to FIFRA. In submitting this material to the EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection of rights involving this material that would have been claimed by the company if this material had not been submitted to the EPA."

Company: Monsanto Company

Company Agent: _____

Title: _____

Signature _____ Date: _____

STATEMENT OF COMPLIANCE

This production plan was not conducted in compliance with the 40 CFR Part 160 (EPA) for the following reasons:

This production plan report describes the synthesis of the test, control and reference substances, and is therefore not a study. However, quality control oversight and review were conducted to ensure the quality and integrity of the materials generated and the documentation of the synthesis records and agronomic data collected.

Submitter: _____ Date: _____

Sponsor Representative: _____ Date: Mar 15, 2001
Patrick T. Weston

Production Coordinator: _____ Date: March 15, 2001
Kathryn A. Hamilton

Production Supervisor: Jim Colyer Date: 15, March 2001
Jim Colyer

QUALITY CONTROL STATEMENT

This report was reviewed to assure that it accurately reflects the raw data of this production plan. The raw data was audited for compliance to the Monsanto Company Guidelines for Keeping Research Records (GRR 10/1/99), and where applicable, to Monsanto's SOPs.

Reviewed by:

Clinton J. Fletcher Date: 03-12-01

Clinton J. Fletcher
Quality Assurance
Monsanto Regulatory

SIGNATURES OF APPROVAL

Report Number:	MSL-17089
Plan Number:	99-04-36-01
Title:	Argentina Production of Bulk Seed from Insect-Protected Cotton Varieties DP50BX, DP50B, Control DP50 and Reference DP50RR in 1999/2000 Season
Facility:	Monsanto Company 700 Chesterfield Parkway North St. Louis, Missouri 63198
Sponsor Representative::	Patrick T. Weston
Production Coordinator:	Kathryn A. Hamilton
Production Supervisor:	Jim Colyer
Contributor(s):	Abdul Fabellar
Production Initiation Date:	December 21, 1999
Records Retention:	All production plan specific raw data, production plan document, final reports and facility records will be retained at Monsanto, St. Louis.
Sample Storage:	Any production plan tissue samples that are to be retained will be stored at Monsanto, St. Louis.

Signatures of Approval:

Sponsor Representative: _____ Date: Mar 15, 2001
Patrick T. Weston

Production Coordinator: _____ Date: March 15, 2001
Kathryn A. Hamilton

Production Supervisor: Jim Colyer Date: 15, March, 2001
Jim Colyer

CERTIFICATION OF AUTHENTICITY

This is an accurate and authentic representation of the conditions and results of the field production of the test, control and reference materials described in this report.

Production Coordinator: _____ Date: 15, March 2001
Kathryn A. Hamilton
700 Chesterfield Parkway North
St. Louis, Missouri 63198

Production Supervisor: _____ Date: 15, March, 2001
Jim Colyer
700 Chesterfield Parkway North
St. Louis, Missouri 63198

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INTRODUCTION AND SUMMARY

Purpose

The purpose of this production plan was to generate test, control and reference substances from field-grown plants for use in a feeding study. Bulk seed quantities of four cotton varieties, DP50BX (test variety), control variety DP50B and two reference varieties (DP50 and DP50RR) were produced in Argentina during the 1999/2000 growing season.

Summary of Results

Monsanto Company has developed an insect-protected cotton variety DP50BX which contains two insecticidal proteins making the plants tolerant to certain lepidopteran insect pests. Bollgard cotton variety (DP50B) was modified to express a second protein from *Bacillus thuringiensis* var. *kurstaki* HD-1 [CryX].

Cottonseed from one test event, one control and two reference varieties were planted at a single field location in Argentina to produce seed for a feeding study. The cotton was grown under agronomic and cultural practices that are typical of cotton production for that region of Argentina. Seed was planted in non-replicated plots in multiple rows that were spaced 38 inches (96 cm) apart. The total area planted per variety was approximately 13 acres. All production plots were established with acceptable isolation distances between plots (24 ft). Crop growth and development data such as date of emergence and plant stand count were observed and recorded. Plots were monitored at more than seven separate times for the presence of crop pests and diseases. Aphid infestations and the presence of a foliar disease were noted. While air temperatures were normal, precipitation was low in December, resulting in a stand reduction between 7 to 14 days. Seed yields per plot ranged from 3,720 kg to 5,840 kg.

EXPERIMENTAL DESIGN

Location

One field site was utilized for this production plan; Saenz Peña-Chaco, located within the cotton growing region of Argentina (Table 1). Jorge Bosch was the Field Investigator. Soil texture for the site is a sandy loam containing 2.0% organic matter. Cotton was planted for the two years prior to this growing season (1999/2000) and the field preparation and maintenance procedures followed normal agronomic practices for the region. Information regarding soil information and field history is in Tables 2 and 3.

Test Events and Control Varieties

Under this production plan one test event, one control and two reference varieties were planted. The test event was insect-protected (IP) cotton DP50BX (event 15985) expressing both Cry1Ac and Cry2Ab proteins. The control variety was the parental variety DP50B (event 531) and the reference varieties were DP50RR and DP50. Table 4 lists the starting seed for the field site. The control seed provides a background matrix for the analytical evaluation of the cottonseed collected from field grown plants.

The test and control starting seed materials for the site, were characterized at the molecular level to distinguish between the test event and control variety by extracting DNA and analyzing the DNA by PCR analysis. This analysis confirmed that test event and control seed materials generated results consistent with their expected identities. Based on these results, it was concluded that the cotton variety DP50BX contains the Cry2Ab2 transformation event and the control variety does not contain the Cry2Ab2 event.

Field Plot Design

A total of four plots were established by planting a single replicate per variety. Each plot was approximately 13 acres (2952ft by 480 ft). Each plot was separated by approximately 38ft of Sorghum. A diagram of the plot plan is in Appendix 1.

Seed Planting

The production was planted on Dec 28 and Dec 29, 1999. Cottonseed was planted in 38 inch (96 cm) rows at a seeding rate of 4-5 seeds/foot (15-18 seed/m). This resulted in a planted area of approximately 13 acres in size per plot. Table 5 contains a summary of seed bed preparation. Seed bed preparation followed normal agronomic practices for the region. Planting information is in Table 6.

SAMPLING

Samples were collected from all test events and control varieties, placed in uniquely labeled sample bags and maintained at ambient conditions during shipment and storage. For this Production Plan the following tissues were sampled:

Seed

Seed was harvested on July 5 to July 13, 2000 from all test, control and reference variety plots. All cotton plants in each plot were harvested, and the seedcotton samples were sent directly to the gin. Ginning occurred July 6th to July 14th for all samples. A sub sample of the seed from each variety was sent to St Louis on July 29th for variety confirmation. Between 1700 and 2350 kg of each line was also shipped from the Field Cooperator's facility to Instituto Nacional de Tenologia Agropecuaria, Santa Fe, Argentina on July 18, 2000 under

ambient temperature conditions. All transgenic seed was shipped via double containment and labeled in accordance with Argentina Biotech Commission regulations. Of this seed 60kg of each line were shipped to Monsanto St. Louis on November 6, 2000. Table 12 contains information regarding seed sample shipments.

Quality Control

The following quality control measures and precautions were taken to maintain the identity of each test event and control variety and to ensure the integrity of the samples collected:

1. Use of chain of custody forms to document receipt, storage, handling and disposition of tissue samples.
2. A unique number was assigned to each plot. In addition, at least one plot stake was labeled or coded to indicate Production Plan number, plot number, cotton variety planted, block number or any other parameters required to uniquely identify the plot.
3. Sample integrity and identity were maintained by collecting samples directly into uniquely labeled containers for each sample before proceeding with subsequent sampling.
4. A production plan field notebook was maintained by the Production Supervisor for documenting production plan activities.
5. The Quality Assurance Unit reviewed the production plan, field notebook and this report.
6. All raw data and original supporting documentation related to this Production Plan will be archived at the Biotechnology Regulation Science archive facility in Chesterfield, Missouri.

Deviations

The following deviations from the production plan occurred:

1. Calibration was not conducted within the specified 24 hours prior to application. Calibration was performed by farmers consultant and calibration data was not provided for input in the notebook.
2. Calibration was not validated on day of application. Validation of calibration was conducted by farmers consultant and data and calculations were not provided for input into the notebook
3. Calibration data and calculations were not provided for input into the notebook.
4. Target output for Roundup application was not between 10 and 20 gallons as specified in the production plan. Target output applied was 40 gallons per acre.

5. The entire plot was surrounded by 24 ft of Sorghum instead of 40 feet of nontransgenic cotton. The experimental area was isolated by at least 800 m from any other cotton.

None of the deviations listed has a significant effect on the production plan.

AGRONOMIC OBSERVATIONS AND PRACTICES

Crop Growth and Development

Plots were maintained according to normal production standards for cotton and were kept free of weeds and insects. At five to six days after planting (Jan 3, 2000), there was 50% emergence of the seed. A drought reduced plant stand from day 7 to 14 DAP. A summary of the crop growth observations are presented in Table 8.

Insect Pest and Disease Observations

The crop was observed for insect pests and disease infestation throughout the season. Aphid infestations and the presence of a foliar disease were noted. Information regarding insect and disease observations are in Table 9.

Chemical Treatments

One chemical treatment was applied to the field during seed bed preparation and 12 treatments were made during the growing season. Applications are listed in Tables 5 and 7. Sprayer calibration information and environmental conditions for Roundup applications are listed in Tables 10 and 11.

Fertilization

Fertilizer was not applied at the time of planting or throughout the growing season.

Crop Destruction

After harvest, all plots were destroyed on July 18, 2000 by shredding and plowing down. Information regarding crop destruction is in Table 13.

Weather

Precipitation was below average in December 1999 and March 2000. For the rest of the growing season average monthly precipitation was achieved. Temperatures at the site were average as compared to normal climate data. A summary of weather information is reported in Table 14.

USDA/APHIS Compliance

Only the test seed DP50BX was regulated and imported from Argentina under USDA notification 00-054-01n. Seed was shipped double contained and the notification number and identity of contents were on the outer container.

CONCLUSIONS

Cotton test event (containing Cry1Ac and Cry2Ab2 proteins for insect tolerance), control variety DP50B and reference varieties DP50RR and DP50 were grown at a representative location of the major Argentina cotton growing region. Agronomic and cultural practices were typical of commercial cotton growing practices. Samples taken from test, control and reference plants are expected to be representative of those plants when grown in commercial practice. Appropriate and effective measures were taken to maintain variety identity and to ensure the integrity of all tissues sampled.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the diligent work of Guillermo Videla and Jorge Bosch.

FIGURE 1: FIELD TEST SITE

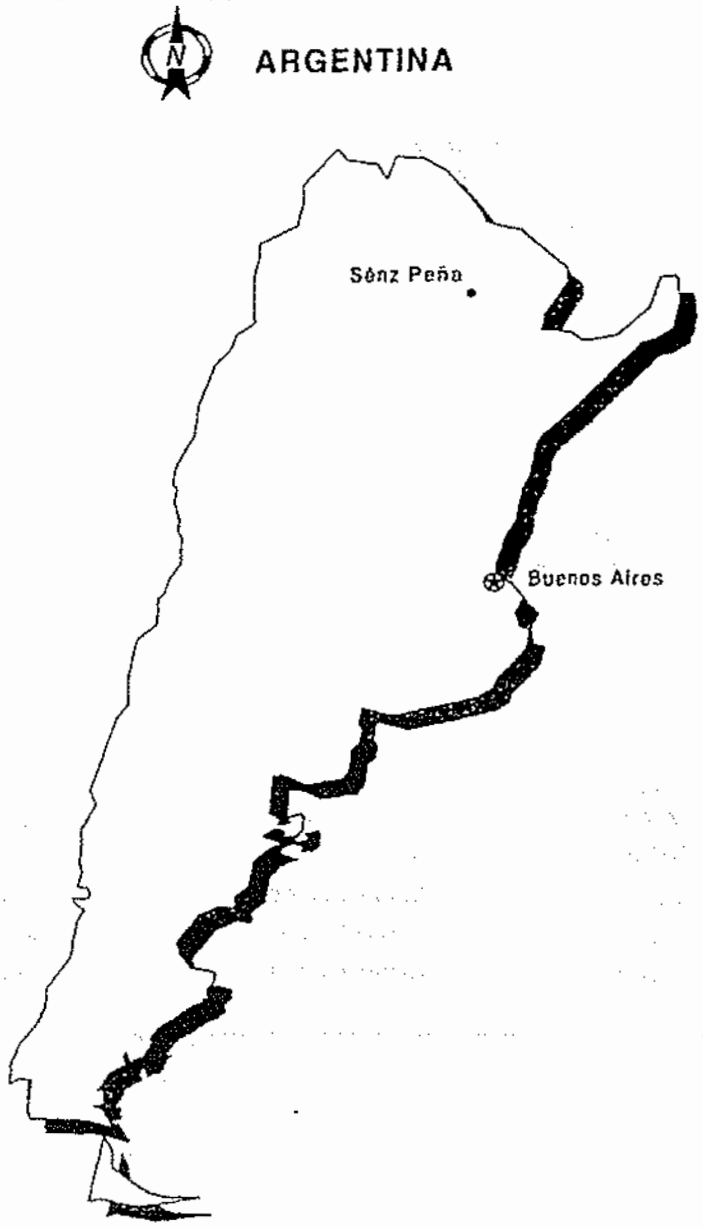


Table 1. Field Site and Field Cooperator

Field Site*	Field Cooperator
Saenz Pena-Chaco, Argentina	Jorge Bosch Monsanto LAS 1 de Marso 1803 3700 Saenz Pena-Chaco Argentina Phone: 54-3732-621456

* Nearest town to field site

Table 2. Soil Information

Soil Series	Texture	OM (%)
N/A	Sandy Loam	2.0

OM = Organic Matter
N/A = Not Available

Table 3. Field History (1997-1998)

Year	Crop	Pesticides Applied	Active Ingredient(s)	Rate (l/ha)
1998	Cotton	Treflan	trifluralin 48%	3 liters
		Cottonex	fluometuron 50%	1 liters
1997	Cotton	Treflan	trifluralin 48%	3 liters
		Cottonex	fluometuron 50%	1 liters

Table 4. Starting Seed Handling

Date Shipped[1]	Date Received[2]	Date Planted	Seed Variety ID	Lot Number	Amount Shipped (kg)	Disposition Unplanted Seed	Phenotype	Seed Storage Temp[3] (°C)
Test Variety								
NA	NA	12/28-29/99	DP50BX	98SEBSA90012SA	113.35	Returned to storage	IPC	15-30
Control Variety								
NA	NA	12/28-29/99	DP50B	50B-H-7055-2MS	113.35	Returned to storage	IPC	15-30
Reference								
NA	NA	12/28-29/99	DP50RR	436R-E-8706-2AZ	113.35	Returned to storage	HT	15-30
NA	NA	12/28-29/99	DP50	50-E-8009-2AZ	113.35	Returned to storage	NT	15-30

[1] Seed shipped directly from Delta and Pine Land.
[2] Seed received by Field Cooperator.
[3] Stored at ambient temperatures (°C) at Genetica Mandiyu Seed Storage Unit.
NT = Non-Transgenic
IPC = Insect Protected Cotton
HT = Herbicide Tolerant

Table 5. Seed Bed Preparation

Pesticides			
Product	**Active Ingredient(s)**	**Rate (kg ai/ha)**	**Date Applied**
None	-	-	-

Cultivation	
Implement	**Date (mm/dd/yy)**
Shredder	07/15/99
Double Action Disc	09/20/99
Double Action Disc	10/10/99
Field Cultivator	12/27/99

Fertilizers			
Composition Amount (% NPK)	**Rate (lb/A)**	**Actual Amount N-P-K (lb/A)**	**Date Applied**
None	-	-	-

Table 6. Planting Information

Date Planted (mm/dd/yy)	**Row Spacing (cm)**	**Planting Depth (cm)**	**Seeding Rate (Seeds/m)**	**Planting Equipment**
12/28-29/99	96	3-4	15-18	Giorgi 12-row Planter

Table 7. Site Maintenance Practices

Pesticides				
Product[1]	**Active Ingredient(s)**	**Crop Growth Stage**	**Rate (l/ha)**	**Date Applied**
Roundup SL	glyphosate 48%	Pre-emergence	9.0	12/31/99
Roundup SL	glyphosate 48%	4th leaf [2]	3.5	01/19/00
Endosulfan EC	endosulfan 35%	11 nodes	1.0	02/10/00
Rogor L + Endosulfan EC	dimetoato 50% + endosulfan 35%	13 nodes	0.7 + 1.6	02/20/00
Pix CS	mepiquat chloride 5%	13 nodes	0.3	02/20/00
Clorpirifos E + Nomolt SC + Cycocel SL	chlorpyrifos 48% teflubenzuron 15% + chloromequat 75%	18 nodes [3]	1.0 + 0.1 + 0.05	03/11/00
Fenom C + Furia EC + Cycocel SL	cypermetrin 40% + prophenophos 2.5 + zetamethrin 18% + chloromequat 75%	21 nodes	1.0 + 0.15 + 0.07	03/19/00
Roundup SL + Diuron SC + MSMA	glyphosate 48%+ diuron 50% + MSMA 96%	21 nodes [4]	2.5 + 1.5+ 1.0	03/20/00
Cycocel SL	chloromequat 75%	cut-out	0.08	04/26/00
Roundup SL	glyphosate 48%	pre-harvest[2]	3.5	6/26/00

Note1: Practices listed are those which occurred after planting and throughout the field season
[1] Commercial name
[2] Only for the RR genotype
[3] Tobacco Budworm, *Heliothis virescens* and *Helicoverpa gelotopoeon*
[4] Only to non-RR genotype,glyphosate applied between rows

Table 7. Site Maintenance Practices (Continued)

Cultivation		
Type	**Implement**	**Date (mm/dd/yy)**
Cultivation	Cultivator	02/01/00

Irrigation		
	Amount (Cm)	**Date (mm/dd/yy)**
None	-	-

Fertilizers			
Composition Amount (% NPK)	**Rate (kg/A)**	**Actual Amount N-P-K (kg/A)**	**Date Applied (mm/dd/yy)**
None	-	-	-

Defoliant(s)			
Product Formulation	**Active Ingredients**	**Rate (l/ha)**	**Date Applied (mm/dd/yy)**
Finish SC	ciclanilide 6% + ethephon 48%	2.85	05/28/00

Table 8. Summary of Crop Growth and Development Observations

Variety ID	Date Planted	Emergence[1] Date	Stand Count 7DAP	Stand Count 14DAP
Test Variety				
DP50BX	12/28-29/99	01/03/00 [5-6]	107.8	65.2
Control Variety				
DP50B	12/28-29/99	01/03/00 [5-6]	49.4	29.8
Reference Varieties				
DP50RR	12/28-29/99	01/03/00 [5-6]	107.6	90.6
DP50	12/28-29/99	01/03/00 [5-6]	75.8	58.6

[1] Date at which 50% of plants emerged [#] = Days after planting
Note: To estimate stand count, 5 sampling stations per plot (in zig-zag fashion) were determined. In each station, plants per 10 meters were counted.

Table 9. Summary of Insect Pest and Disease Observations

Observation Date	Observation Results
01/15/00 [17-18]	Drought reduced the stand of plants from 7DAP to 14DAP
01/25/00 [27-28]	Presence of Aphids with no symptoms of damage. No eggs nor larvae of *Heliothis* observed.
02/11/00 [44-45]	High infestations of Aphids, above economic threshold
02/18/00 [51-52]	Presence of eggs of *Alabams* and *Heliothis*. Presence of Aphids with damage symptoms in certain parts of the plots. More Aphids underneath the leaves. Large population of beneficial insects mainly Coleopterans. Presence of *Alabams* in non-Bt plants (approximately 3-5 per meter in instars L_1, L_2, and L_3).
03/01/00 [62-63]	4-7 larvae of *Alabams* per meter om non-Bt plots, with less than 5% defoliation. Large number of beneficial insects mainly *Coccinellidae*.. *Heliothine* eggs in all varieties.
4/06/00 [98-99]	Presence of Aphids most of them parasitised.

[#] = Days after planting

Table 10. Sprayer Calibration Information

Crop Growth Stage	Date of Calibration	Sprayer Type	Nozzles			Nozzle Spacing (cm)	Sprayer Speed (k/h)	Pressure (PSI)	Target Output (l/ha)
			Type	Size	#				
Preemergence	12/29/99	Yacto Columbia Cross	Teejet	8003	39	48	8	20	155
Early Post Emergence - 4th leaf	12/29/99	Yacto Columbia Cross	Teejet	8003	39	48	8	20	155
Lay-by Post-directed at Base of Plant-15-17 nodes	N/A	Redball 12-row Sprayer	Teejet	110015 (top) 80020 (sides)	3 per row	N/A	7-8	20	95-100 (top) 95-100 (sides)
Pre harvest	12/29/99	Yacto Columbia Cross	Teejet	8003	39	48	8	20	155

N/A = not available

Table 11. Environmental and Crop Conditions at Roundup Applications

Crop Growth Stage	Application Date	Air Temperature (°C)	Wind Speed (km/hr)	Wind Direction
Preemergence	12/31/99	30	< 5	NE
4th Leaf	01/19/00	34	< 5	NE
15-17 Nodes	03/20/00	N/A	N/A	N/A
Pre harvest	06/26/00	15	< 5	S

Table 12. Seed Sampling and Shipping Information

T/C/R Materials	Seed Harvest Date	Seed Cotton Harvest Weight (kg)	Date of Ginning	Ginned Fuzzy Seed Weight (kg)	Seed Shipping Date[1]	Seed Receipt Date
DP50BX	07/5-13/00 [189-197]	5.840	07/06-14/00	3.040	07/29/00	10/06/00
DP50B	07/5-13/00 [189-197]	4.780	07/06-14/00	2.860	07/29/00	10/06/00
DP50	07/5-13/00 [189-197]	5.040	07/06-14/00	2.785	07/29/00	10/06/00
DP50RR	07/5-13/00 [189-197]	3.720	07/06-14/00	2.160	07/29/00	10/06/00

[#] = Days after planting

[1] 1200 grams of each variety were shipped to Monsanto St.Louis.

Remaining seed was shipped to INTA, Santa Fe, Argentina for study 00-04-60-02 on July 18th, 2000. 60 kg of each line was subsequently shipped from INTA to Monsanto St Louis on November 6th, 2000.

Table 13. Crop Destruction

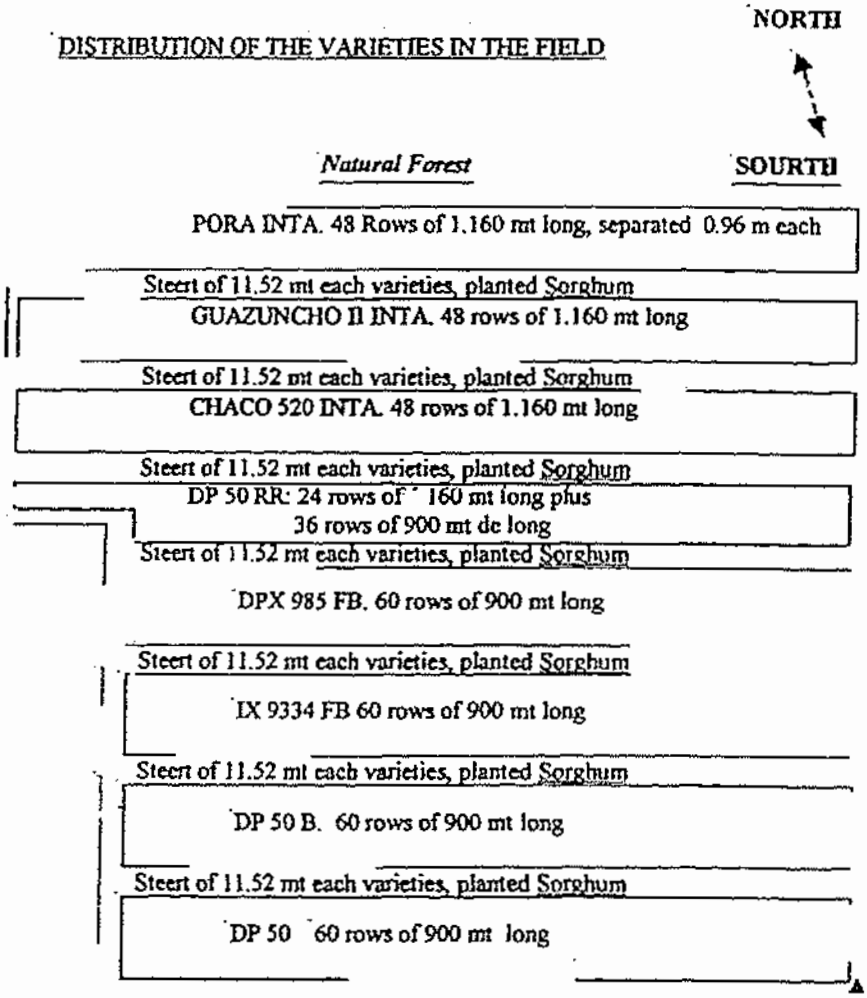
Date of Destruction	Method of Destruction
07/18/00	Crop residues in the plots were destroyed by shredding and plowing down with double action plow.

Table 14. Weather Data, Current Year vs. Normal Conditions

1999/2000 Month	Precipitation (cm)		Air Temperature (°C) Maximum		Minimum	
	Normal	1999/2000	Normal	1999/2000	Normal	1999/2000
December	128.0	63.8	34.2	32.9	19.3	18.4
January	135.4	145.5	33.3	34.3	20.2	19.9
February	124.9	132.8	31.9	31.5	19.5	19.2
March	158.4	35.8	30.7	30.1	18.4	17.9
April	175.7	145.4	26.8	27.5	15.6	16.8
May	58.0	21.1	24.3		12.5	
June	23.7		21.3		9.6	

Note: Total 1999 rainfall and air temperatures are from the date of planting to harvest date. Normal precipitation and air temperatures are the average from a 10 year (1989 - 1998) data collection, which covers same period of time (planting to harvest) and for the climatic division in which the test was located.

APPENDIX 1: PLOT MAP



Summary of Quality Control Review

This report was reviewed to ensure that it accurately reflects the raw data of the study. The raw data was audited for compliance to the Monsanto Company Guidelines for Keeping Research Records (GRR 10/1/99), and where applicable, to Monsanto SOP's.

Reviewed by:

Barbara A. Foy Date: Sept. 24, 2001

Barbara A. Foy
Quality Assurance Specialist
Monsanto Regulatory
Monsanto Company

ANNEXURE 7.2.5(a)

Insect protection protein in cottonseed meal: a dietary toxicity study with the northern bobwhite.

INSECT PROTECTION PROTEIN 2 IN COTTONSEED MEAL: A DIETARY TOXICITY STUDY WITH THE NORTHERN BOBWHITE

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 139-449

MONSANTO STUDY NO.: WL-99-065

MONSANTO REPORT NO.: MSL 16178

FIFRA SUBDIVISION E, SECTION 71-2

AUTHORS: Sean P. Gallagher
Jennie Grimes
Joann B. Beavers

STUDY INITIATION: May 20, 1999

STUDY COMPLETION: September 29, 1999

AMENDED REPORT DATE: February 11, 2000

SPONSOR

Monsanto Company
700 Chesterfield Parkway, North
Chesterfield, Missouri 63198

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601

AMENDED

STATEMENT OF NO DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(1)(A), (B), or (C).

"We submitted this material to the United States Environmental Protection Agency specifically under provisions contained in FIFRA as amended, and thereby consent to use and disclosure of this material by EPA according to FIFRA. In submitting this material to the EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection of rights involving this material that would have been claimed by the company if this material had not been submitted to the EPA."

COMPANY: MONSANTO COMPANY

COMPANY AGENT: _____
Sponsor/Submitter Date

AMENDED

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR part 160, 17 August 1989; OECD Principles of Good Laboratory Practice, (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984. The dates of all audits and inspections and the dates any findings were reported to the Study Director and Laboratory Management were as follows:

ACTIVITY	DATE CONDUCTED	DATE REPORTED TO: STUDY DIRECTOR	MANAGEMENT
Protocol	May 21, 1999	May 21, 1999	May 21, 1999
Test Substance Preparation	May 20, 1999	May 20, 1999	May 20, 1999
Data and Draft Report	July 16, 19, 1999	July 19, 1999	July 22, 1999
Final Report	September 29, 1999	September 29, 1999	September 29, 1999
Amended Final Report	February 10, 2000	February 11, 2000	February 11, 2000

Kimberly A. Hoxter DATE 2-11-00

Kimberly A. Hoxter, B.S.
Quality Assurance Representative

AMENDED

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: Monsanto Company

TITLE: Insect Protection Protein 2 in Cottonseed Meal: A Dietary Toxicity Study with the Northern Bobwhite

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 139-449

STUDY COMPLETION: September 29, 1999

AMENDED REPORT DATE: February 11, 2000

This study was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 160, 17 August 1989; OECD Principles of Good Laboratory Practice, (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984, with the following exception:

The test and control substance characterization and test substance verification portions of the study were conducted by the Sponsor in accordance with the Good Laboratory Practices with the following exceptions:

Characterization of the Cry2Ab2 protein concentration in the test, control and reference substances was not completed prior to initiation of the study.

Homogeneity and stability of the test, control and reference substances in the diet were not determined.

Documentation for the characterization of the Cry2Ab2 protein standard (Study 98-01-39-16) which was used in the diet analysis, was not completed prior to the initiation of this study. Preliminary pre-study data from study 98-01-39-16 confirmed the identity.

STUDY DIRECTOR:

Sean P. Gallagher _____ DATE 2/11/00
Sean P. Gallagher
Senior Biologist

SPONSOR'S APPROVAL:

_____ DATE 2/17/00
Sponsor's Technical Representative

_____ DATE _____
Applicant/Submitter

AMENDED

REPORT SIGNATURE PAGE

This report accurately represents the data developed during the study.

PREPARED BY:

Sean P. Gallagher, B.S.
Study Director

2/11/00
Study Completion Date

APPROVED BY:

Joann B. Beavers, B.S.
Director, Avian Toxicology

2/11/00
Study Completion Date

Susan L. Coleman
Susan L. Coleman, B.A.
Senior Quality Assurance Representative

2-11-00
Date

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-continued-

TABLES AND APPENDICES

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SUMMARY

SPONSOR: Monsanto Company

TEST SUBSTANCES: Cottonseed meal from two genetically modified cotton lines containing Cry2Ab2 Protein (15985 and 15813). Cry2Ab2 is also known as Insect Protection Protein 2.

CONTROL SUBSTANCES: Cottonseed meal from two genetically related cotton lines (DP50 and DP50B)

REFERENCE SUBSTANCES: Cottonseed meal from two non-genetically modified commercial cotton lines

WILDLIFE INTERNATIONAL LTD. PROJECT NO.: 139-449

STUDY: Insect Protection Protein 2 in Cottonseed Meal: A Dietary Toxicity Study with the Northern Bobwhite

RESULTS: The dietary LC50 value for northern bobwhite exposed to cottonseed meal for the two genetically modified cotton lines, 15985 and 15813 tested was estimated to be greater than 100,000 ppm (10% of diet). The no mortality concentrations and the no-observed-effect concentrations were 100,000 ppm for both of the cotton lines.

TEST DATES: Hatch – May 10, 1999
Acclimation – May 10, 1999 to May 20, 1999
Experimental Start – May 20, 1999
Experimental Termination – May 28, 1999

NOMINAL TEST
CONCENTRATIONS: 100,000 ppm (10% cottonseed in diet)

TEST ANIMALS: Northern Bobwhite (*Colinus virginianus*)

AGE TEST ANIMALS: 10 days of age at test initiation

SOURCE TEST ANIMALS: Wildlife International Ltd. Production Flock
8598 Commerce Drive
Easton, Maryland 21601

STUDY COMPLETION: September 29, 1999

AMENDED REPORT DATE: February 11, 2000

AMENDED

ABSTRACT

The objective of this study was to evaluate the effects of cottonseed meal from two genetically modified cotton lines when administered in the diet of juvenile northern bobwhite quail for five days. The test substances were ground cottonseed from the cotton lines 15813 and 15985 genetically modified to contain the Cry2Ab2 protein, also known as Insect Protection Protein 2. The control substances were cotton lines DP50 and DP50B which did not contain the Cry2Ab2 protein and had genetics similar to the test substance lines. Two commercial cotton hybrids, ST474 and DP1266 that had not been genetically modified were tested as reference substances. The test, control, and reference substances were included at 10% of the total diet by incorporating the ground cottonseed into standard game bird diet.

Each of the cotton lines was mixed into the diets and fed to 20 birds evenly distributed in 4 pens. Each group was fed the test, control, or reference substance diet for 5 days and then switched to basal diets for the last 3 days of the study. Food and water were provided *ad libitum*. Food consumption was measured by pen for each day of the exposure period and for the post-exposure observations period (Days 6-8). Individual body weights were recorded at study initiation, Day 5, and study termination. The average temperature in the brooding compartment of the pens was 37.6°C ± 1.6°C. Average ambient room temperature was 28.2°C ± 1.7°C. The photoperiod was 16 hours light/day during acclimation and throughout the test. Birds were observed at least twice daily during the study for mortality or signs of toxicity.

There was no mortality or overt signs of toxicity in birds from any of the treatment groups. No effects on body weight or feed consumption were observed in the test substance groups, 15813 and 15985, compared to the control substance or the commercial cotton line reference groups. These data indicate that quail consuming diets containing 10% cottonseed genetically modified to contain the Cry2Ab2 protein are at minimal risk of adverse effects.

AMENDED

INTRODUCTION

This study was conducted by Wildlife International Ltd. for Monsanto Company at the Wildlife International Ltd. toxicology facility in Easton, Maryland. The in-life portion of the test was conducted from May 20, 1999 to May 28, 1999. Raw data generated at Wildlife International Ltd. and a copy of the final report are filed under Project Number 139-449 in archives located on the Wildlife International Ltd. site. The original final report, analytical summary and raw data generated at Monsanto Company are archived at Monsanto Company.

OBJECTIVE

The objective of this study was to evaluate the effects of cottonseed meal from two different genetically modified cotton lines containing the Cry2Ab2 Protein, when administered to juvenile northern bobwhite in the diet for five days. The Cry2Ab2 protein is also known as Insect Protection Protein 2.

MATERIALS AND METHODS

The methods used in conducting this study were based upon procedures specified in Section 71-2 of the Environmental Protection Agency's Registration Guidelines, *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms* (1); and upon ASTM Standard E857-87, "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species" (2).

Test, Control and Reference Substances

The test substances were ground cottonseed from two cotton lines genetically modified to contain the Cry2Ab2 protein which provides protection against certain insect pests. The two test substances, 15985 and 15813, were received from Monsanto Company on May 5, 1999 and contained 43.23 and 37.13 µg Cry2Ab2 protein/g seed, respectively. 15813 was assigned Wildlife International Ltd. Identification Number 4916 and was identified as: Processed cotton meal 15813; 15985 was assigned Wildlife International Ltd. Identification Number 4917 and was identified as: Processed cotton meal 15985. Processed cottonseed meal in this study refers to ground whole cottonseed.

The control substances were ground cottonseed meal from two cotton lines, DP50 and DP50B with no detectable Cry2Ab2 protein. DP50B (known commercially as Bollgard®) is the

genetically modified parental line for the two Cry2Ab2 lines. Bollgard is an approved genetically modified product in the United States containing an insecticidal protein from a different class of protein. DP50 is not genetically modified. Both DP50 and DP50B provide background genetics representative of the test substance. DP50 and DP50B were received from Monsanto Company on May 5, 1999 and were assigned Wildlife International Ltd. Identification numbers 4918 and 4919, respectively. Control substances were identified as: Processed cotton meal DP50 and processed cotton meal DP50B.

Ground cottonseed meal from two commercial cotton hybrids served as reference substances. The reference substances were received from Monsanto Company on May 5, 1999. One was assigned Wildlife International Ltd. identification number 4920 and was identified as: Processed Cotton Meal DP1266. The second reference standard was assigned Wildlife International Ltd. identification number 4921 and was identified as: Processed Cotton Meal ST474.

All test, control and reference substances were stored frozen at approximately -20°C.

Treatment Groups

Six treatment groups were included in the study: two genetically modified cotton lines (test substances); two genetically related cotton lines (control substances); and two non-genetically modified commercial lines (reference substances). Each treatment group included four replicates of five birds each. Bobwhite chicks were fed diet containing 10% of the appropriate test, control or reference substance and 90% basal ration for 5 days. Consequently the test, control and reference substance concentrations were 100,000 mg/kg diet. Following the five day exposure period all groups were given untreated basal diet for three days.

Duration of the Test

The primary phases of this test and their durations were:

1. Acclimation - 10 days.
2. Exposure - 5 days.
3. Post-exposure observation - 3 days.

Test Birds

All northern bobwhite (*Colinus virginianus*) were 10 days of age and appeared to be in good health at initiation of the test. The birds were obtained from Wildlife International Ltd. Production Flock, Easton, MD and were hatched on May 10, 1999. Birds ranged in weight from 17 to 27 grams at test initiation. All birds were from the same hatch, pen-reared and phenotypically indistinguishable from wild birds. All birds were acclimated to the caging and facilities from the day of hatch until initiation of the test.

Birds were assigned to six experimental groups with 4 replicate pens. Birds were housed in brooding pens containing five birds each. The birds used in this study were immature and could not be differentiated by sex.

Animal Diet

All test birds were fed a game bird ration formulated to Wildlife International Ltd.'s specifications (Appendix I) throughout acclimation and testing. The chicks were given a vitamin supplement in their water from the day they were hatched until the initiation of the test. Water, from the town of Easton public water supply, and feed were provided ad libitum during acclimation and during the test. The birds received no form of antibiotic medication during acclimation or the test.

Diet Preparation

Test diets were prepared by incorporating the appropriate test, control and reference substances directly into basal ration using a Hobart (Model Number AS200T) mixer (Appendix II). An amount of diet sufficient to last the five-day exposure period was prepared on the day of the test initiation and stored frozen. The birds were presented a portion of the diet daily during the exposure period.

Diet Sampling

Samples of the test diets were collected to confirm the presence or absence of the Cry2Ab2 protein in the test, control and reference substance diets. One sample was collected from each diet at preparation on Day 0 and another at the end of the exposure period (Day 5). The Day 5 samples were collected from feed composited by treatment group remaining in the feeders. Samples were stored frozen (-20°C) until transferred to Monsanto Company.

Analysis of Diet

The diet samples were analyzed for the presence of Cry2Ab2 Protein to confirm the identity of the test substance (Appendix III).

Housing and Environmental Conditions

During acclimation and testing, all birds were housed indoors in batteries of thermostatically controlled brooding pens manufactured by Beacon Steel Products Co. (Model No. B735Q). Each pen had floor space that measured approximately 72 X 90 cm. Ceiling height was approximately 23 cm. External walls, ceilings and floors were constructed of galvanized steel wire and sheeting.

Birds were assigned to pens by indiscriminate draw. Each group of birds was identified by pen number and test concentration. Individual birds were identified by leg bands. Four pens that contained five chicks each were assigned to each cottonseed line.

During the test the average temperature in the brooding compartment of the pens was 37.6°C ± 1.6°C (SD). Average ambient room temperature for this study was 28.2°C ± 1.7°C (SD) with an average relative humidity of 34% ± 10% (SD). The photoperiod (maintained by a time clock) was sixteen hours of light per day during acclimation and throughout the test. The light source was fluorescent lights which closely approximate noon-day sunlight. The birds were exposed to an average of approximately 281 lux of illumination.

Housing and husbandry practices were based on guidelines established by the National Research Council (3).

Observations

All birds were observed daily during acclimation. Birds exhibiting abnormal behavior or physical injury were not used. All birds were observed at least twice daily following test initiation and continuing until termination. A record was maintained of all mortality, signs of toxicity and abnormal behavior.

Animal Body Weights/Feed Consumption

Individual body weights were measured at the initiation of the test, on Day 5, and at termination of the test on Day 8. Average feed consumption values were determined by pen for each day of the exposure period (Days 1, 2, 3, 4, 5) and for the post-exposure observation period (Days 6-8). Feed consumption was determined by measuring the change in the weight of the feed presented to the birds over a given period of time. The accuracy of feed consumption values may have been affected by the unavoidable wastage of feed by the birds.

Statistical Analyses

The LC50 value could not be statistically defined because only one concentration was tested. However, based upon the biological response obtained in the treatment groups, the LC50 value was estimated to be greater than the 100,000 ppm concentration. The no-observed-effect concentration was determined by evaluation of the mortality, clinical signs, body weight and feed consumption data.

RESULTS

Diet Analysis

Quail diets prepared using meal from 15813 and 15985 cotton lines were verified to contain Cry2Ab2 protein. Diets from the cotton lines DP50 and DP50B, and commercial lines DP1266 and ST474 did not contain detectable levels of Cry2Ab2 protein (Appendix III). These data serve to confirm the phenotype of the test, control and reference substances as evidenced by the presence of the protein of interest.

Mortalities and Clinical Observations

There were no mortalities in the two genetically related cotton line control groups. One bird in the DP50 control group and one bird in the DP50B control group were noted with foot/leg injuries during the course of the test as a result of penmate aggression. Otherwise, all birds in both control groups were normal in appearance and behavior throughout the test. A single mortality occurred in the DP1266 reference substance group on Day 8 of the test. Since the death occurred after the exposure period and no signs were noted prior to the mortality, the cause of death was considered incidental. In addition, one bird in the ST474 reference substance group was noted with foot injuries on Days 6 and 7 of the test. All other birds in both of the commercial cotton reference substance groups were normal in appearance and behavior for the duration of the test (Table 1, Appendix IV).

There were no mortalities or overt signs of toxicity in any of the birds exposed to the two genetically modified cotton line test subtance groups. One bird in the 15985 treatment group was noted to be slightly lethargic and with a possible eye injury on Day 1 of the test. This same bird was noted with foot lesions as a result of penmate aggression on Day 8 of the test. All other birds in the 15985 and 15813 treatment groups were normal in appearance and behavior for the duration of the test.

Body Weight and Feed Consumption

There were no apparent treatment related effects on body weight or feed consumption in birds fed the reference substances or the test substances when compared to birds fed the control substances (Tables 2 and 3, Appendices V and VI).

CONCLUSION

The dietary LC50 value for northern bobwhite exposed to Cry2Ab2 Protein in cottonseed meal for the two genetically modified cotton lines 15985 and 15813 tested, was estimated to be greater than 100,000 ppm. The no mortality concentrations and the no-observed-effect concentrations were 100,000 ppm for both of the test substance cotton lines.