Effects of Feeding Rations with Genetically Modified Whole Cottonseed to Lactating Holstein Cows

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ABSTRACT

Two experiments were conducted to evaluate dry matter intake (DMI), milk yield, and milk composition from feeding rations that contained different sources of genetically modified whole cottonseed to Argentinean Holstein dairy cows. Twenty-four lactating multiparous Argentinean Holstein dairy cows were used in 2 experiments with a replicated 4 × 4 Latin square design, with cows averaging 565 kg body weight and 53 d in milk at the beginning of the experiments. Treatments in Experiment 1 were: Bollgard cotton containing the *cry1Ac* gene, Bollgard II cotton containing *cry1Ac* and *cry2Ab* genes, Roundup Ready cotton containing the *cp4 epsps* gene, and a control nongenetically modified but genetically similar cottonseed. In Experiment 2, two commercial sources, a parental control line, and the transgenic cotton containing both *cry1Ac* and *cp4 epsps* genes were used as treatments. All cows received the same total mixed ration but with different whole cottonseed sources. Cottonseed was included to provide 2.50 kg per cow daily (dry matter [DM] basis) or about 10% of the total diet DM. The ingredient composition of the total mixed ration was 32% alfalfa hay, 28% corn silage, 22% corn grain, 17% soybean meal, and 2% minerals and vitamins. In addition, genomic DNA was extracted from a subset of milk samples and analyzed by polymerase chain reaction followed by Southern blot hybridization for small fragments of the *cry1Ac* transgene and an endogenous cotton gene, *acp1*. No sample was positive for transgenic or plant DNA fragments at the limits of detection for the assays following detailed data evaluation criteria. The DMI, milk yield, milk composition, body weight, and body condition score did not differ among treatments. Cottonseed from genetically modified varieties

used in these studies yielded similar performance in lactating dairy cows when compared to non-transgenic control and reference cottonseed.

(**Key words:** dairy cow, cottonseed, genetically modified, performance)

Abbreviation key: GM = genetically modified, THI = temperature-humidity index, WCS = whole cottonseed.

INTRODUCTION

Classical plant breeding has led to improved agricultural crops used either for consumption by humans or as animal feed for the production of meat, milk, and eggs. This global effort has made major progress, especially over the last century. In the last 15 yr, the ability to insert target genes to confer specific traits has been achieved, and this provides opportunities for more rapid developments in animal and plant genetic improvements (Beever and Phipps, 2001). Currently, most genetically enhanced plants in the marketplace provide insect protection or herbicide tolerance, and are being used as feed for livestock (Clark and Ipharraguerre, 2001). Utilization of these crops offers producers an alternative strategy for managing weed pressure and insect pests. Cotton has been modified by the introduction of specific DNA sequences containing either the *cry1Ac* gene (Bollgard cotton) or both the *cry1Ac* and *cry2Ab* genes to produce Bollgard II cotton, which provides protection against insect pests. This insect protection is conferred to the plant by the Cry1Ac and Cry2Ab proteins derived from *Bacillus thuringiensis*. The Cry1Ac protein binds to specific receptors in the midgut of susceptible insects (i.e., a specific group of lepidopteran insects, such as cotton bollworm) and forms ion-selective channels in the membrane, but does not affect mammals or insects that lack those receptors (Betz et al., 2000; English and Slatin, 1992). The cells swell due to an influx of water, which leads to cell lysis and death (Knowles and Ellar, 1987). Cotton has also been modified to gain tolerance to the Roundup family of agricultural herbicide prod-

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ucts. Roundup herbicide tolerance is conferred to the plant by inclusion of the *cp4 epsps* gene (Roundup Ready). The production of the CP4 EPSPS protein by the plant provides tolerance to glyphosate, the active ingredient in Roundup (Harrison et al., 1996).

Ginned cottonseed (linted or commonly called "whole") is utilized extensively in dairy cattle rations as an energy, fiber, and protein source. The objective of this research was to evaluate DMI, milk yield, milk composition, and BCS of dairy cows fed different genetically modified (GM) whole cottonseeds (WCS) compared with the control, nontransgenic cottonseed.

MATERIALS AND METHODS

Cows, Diets, and Experimental Design

Two experiments were conducted concurrently at the Instituto Nacional de Tecnología Agropecuaria experimental research station located in Rafaela, Santa Fe, Argentina (31°11' south lat) from September 25, 2000 to January 15, 2001, encompassing the late-spring and early-summer seasons. Twenty-four multiparous Argentinean Holstein cows (initial means [$\pm$ SD] of 565.6 $\pm$ 52 kg BW, 2.88 $\pm$ 0.61 BCS, 53 $\pm$ 9.6 DIM, and 32.9 $\pm$ 4.2 kg of milk production) were randomly assigned into three groups (squares) of four cows in each experiment. A 4 $\times$ 4 Latin square design was used with treatments (4), cows (12), squares (3) and periods (4) as sources of variation in each experiment. The experimental periods consisted of wk 1 to 3 for diet adaptation, and wk 4 for feed sampling, intake, BW, BCS, milk yield, and milk composition measurements. All cows were fed the same blended mixture of corn grain, soybean meal, alfalfa hay, corn silage, minerals, and vitamins referred to as TMR. This mixture was weighed into individual cow feeders. Then, WCS of different origins was added by hand to the feeder based on the treatment assignment for the individual cow, blended in, and fed. At each morning and evening feeding, 1.25 kg of WCS was added, providing a total of 2.50 kg/d of WCS DM per cow. The diets were formulated to meet or exceed animal requirements according to the Cornell Net Carbohydrate and Protein System (CNCPS), version 4.0 (Fox et al., 2000) and NRC (1989).

Treatments in Experiment 1 were a Bollgard cotton (DP50B) containing the *cry1Ac* gene, Bollgard II cotton (DP50BII) containing the *cry1Ac* and *cry2Ab* genes, Roundup Ready cotton (DP50RR) containing the *cp4 epsps* gene, and a control non-GM but genetically similar control cottonseed (DP50). Cotton was sown in 60 900-m rows separated by 0.96 m in Chaco, Argentina. An 11.52-m buffer was planted in sorghum to separate the varieties. In Experiment 2, the transgenic cotton

(Chaco520BGRR) containing both *cry1Ac* and *cp4 epsps* genes, two commercial sources (Guazuncho II INTA and Porá INTA; National Institute of Agricultural Technology, Genética Mandiyú SRL, Avia Teray, Chaco, Argentina), and a parental control line (Chaco520 INTA; National Institute of Agricultural Technology, Genética Mandiyú SRL) were used as treatments. Cotton was sown in 48 1160-m rows separated by 0.96 m in Chaco, Argentina. An 11.52-m buffer was planted in sorghum to separate the varieties. All cotton was ginned and the fuzzy seed sent to the trial site. Only the Roundup Ready cotton varieties were sprayed with glyphosate during the growing of the cotton. During both trials, test and control WCS were stored at ambient temperature in labeled paper bags on dry concrete flooring in a completely enclosed shed.

Cows were housed in individual pens in a roofed shed, and had free access to water. All cows were removed daily from pens to a drylot and natural shade. The total time for staying outside in the drylot or in the milking parlor was 6 h/d, typically from 0530 to 0900 h and from 1430 to 1700 h. The TMR was offered twice daily at approximately 0900 and 1500 h and was fed for approximately 10% refusal from wk 1 through 3 and for approximately 5% refusal during wk 4 based on the mean consumption during wk 3. Feed refusal for each cow was collected and recorded once daily at approximately 0800 h. At the beginning of the study, and on d 28 of each experimental period, BW was measured and BCS scored using the 5-point system (Mullvany, 1977) to the nearest one-quarter unit. The same two people did the body condition scoring independently over the course of the study.

Sample Preparation and Analysis

The same procedures were used in both experiments for sampling and analyses. Prior to the study initiation, one representative WCS sample of each variety was taken for different analytical purposes. The WCS samples (approximately 200 g) were characterized at Monsanto (St. Louis, MO) using event-specific PCR to confirm the identity of the test and control varieties. Each variety (approximately 200 g) of WCS was analyzed for mycotoxins (aflatoxin B1, B2, G1, and G2; ochratoxin A; T-2 Toxin; HT-2 toxin; Neosolaniol; Fusarenon X; deoxynivalenol; 15 acetyl-DON; 3 acetyl-DON; nivalenol; zearalenone; and Fumonisin B1, B2, and B3) by a combination of HPLC and TLC (Romer Labs, Inc., Union, MO)], for pesticide (organophosphates, organonitrogens, organochlorinates, and *n*-methylcarbamates; Griffitt and Craun, 1974) and gossypol analysis (AOCS, 1998) by Covance Labs (Madison, WI). An additional ~400-g sample was taken to

the Forage Analysis Laboratory, Rafaela Experimental Station (Rafaela, Argentina), where ~200 g was used for nutrient analysis and ~200 g was stored frozen for any other potential need.

All dietary ingredients were analyzed (one pooled sample per period) for DM at 60°C for 48 h, and then ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA). The ADF, NDF, and acid detergent lignin were analyzed according to Van Soest et al, (1991), using heat-stable amylase and sodium sulfite for NDF analysis. The CP, fat (ether extract, **EE**), calcium, phosphorus, magnesium, potassium, sodium, and ash were assayed according to AOAC (1990). These analyses were carried out at the Forage Analysis Laboratory, Rafaela Experimental Station and the Faculty of Chemistry. Nonfibrous carbohydrates were estimated by difference (NFC, % = 100 - [CP, % + NDF, % + fat, % + ash, %]), as was indicated by the NRC (2001). The TMR and varieties of WCS were sampled daily during a 7-d sample collection period, composited, and analyzed as noted above.

Cows were milked twice daily, and individual milk yields were recorded at each milking using the AFIM-ILK (AFIKIM S.A.E., Kibbutz Afikim, Israel) data system. Milk samples (~50 mL per cow, daily) were collected at 2 consecutive (1630 h and 0630 h) milkings through the 7-d sample collection period and analyzed for fat, protein, lactose, MUN, and SNF by infrared methods (Milkoskan Model 4000, Foss Electric, DK-3400, Hillørd, Denmark). Concentrations and yields of fat, protein, lactose, MUN, and SNF were computed as the weighted means for evening and morning milk yields on each test day.

Analysis of Milk for Transgenic and Endogenous Cotton DNA

A second set of evening and morning milk samples (~30 mL/cow respectively) was stored frozen for transgenic and endogenous cotton DNA analysis in milk. After thawing, the analyses were carried out according to the method described by Jennings et al. (2003a). A total of 24 milk samples were collected from 10 dairy cows for *cry1Ac* analysis. Twelve of the samples were collected from animals fed either DP50B or DP50BII WCS (both events contain the *cry1Ac* transgene), and 12 of the samples were collected from animals fed either DP50RR or conventional WCS (neither contains the *cry1Ac* transgene). A total of 47 milk samples were used from 12 cows for *acp1* analysis. *Acp1* encodes the endogenous cotton gene for acyl carrier protein. The samples for *acp1* analysis were taken from animals fed DP50, DP50B, DP50BII, or DP50RR. Milk samples were collected using procedures to mini-

mize environmental contamination, and they were shipped to the laboratory on dry ice. All samples were analyzed by PCR coupled to Southern blot hybridization.

Statistical Analysis

In both experiments, data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). The statistical model accounted for variation due to cow, square, and period, along with comparisons among the treatments. Only treatment effects were considered fixed. Square, period, cow(square), and period $\times$ square interaction were considered random effects. The interaction of treatment $\times$ square was considered part of the error term. Treatment means were reported as least squares means with the associated standard error. Observations that were at least two standard deviations outside the treatment means were investigated. Observations deemed to be outliers were eliminated from the analysis. An adjusted Tukey test was used to compare treatments least squares means, and differences were considered to be significant at $P < 0.05$.

RESULTS AND DISCUSSION

The effect of cow nested within square was significant ($P < 0.05$) for all variables analyzed. No interaction between period $\times$ square was detected ($P > 0.05$). These results do not affect the conclusions respect to treatments.

Chemical Composition of Feeds

The nutrient content of individual ingredients is shown in Table 1. The high fiber content in ground corn could be related to some harvesting residues in the original corn. The average ingredient composition (% $\pm$ SD) of TMR was 32 $\pm$ 2.3 for alfalfa hay, 28 $\pm$ 3.4 for corn silage, 22 $\pm$ 8.1 for corn grain, 17 $\pm$ 2.8 for soybean meal, and 2 $\pm$ 0.1 for minerals and vitamins. The nutrient composition of WCS varieties used in Experiments 1 and 2 are shown in Table 2. Within experiments, there were minor differences in nutrient composition among varieties of WCS. Because WCS contributed 11% of the DMI, the small differences in nutrient composition observed among varieties had minimal effects on the nutrient composition of the total diet consumed. All cottonseeds used in this study were grown in Argentina under similar conditions, thereby eliminating potential confounding factors of this aspect in interpreting results.

Mycotoxins and pesticide residues were not detected in any WCS samples, except for marginal zearalenone

Table 1. Nutrient composition (DM basis, %) of feedstuffs used in TMR.

Feedstuffs	DM (n = 4)	CP (n = 4)	NDF (n = 4)	ADF (n = 4)	ADL ¹ (n = 4)	EE ² (n = 4)	Ash (n = 4)	ADIN ³ (n = 4)
Corn silage	27.8 ± 1.9 ⁴	8.6 ± 0.5	48.3 ± 0.9	27.4 ± 1.2	2.6 ± 0.1	ND ⁵	8.3 ± 0.5	12.1 ± 3.1
Alfalfa hay	84.2 ± 6.7	19.6 ± 1.8	40.8 ± 4.3	31.0 ± 4.0	8.3 ± 1.5	2.9 ± 1.0	11.5 ± 1.0	ND
Ground corn	89.1 ± 1.1	9.7 ± 0.8	18.6 ± 1.5	5.6 ± 1.3	1.4 ± 0.5	5.0 ± 0.9	3.3 ± 0.2	ND
Dehulled soybean meal	90.1 ± 1.3	38.9 ± 9.8	18.6 ± 6.2	11.0 ± 3.6	1.4 ± 1.2	4.6 ± 0.1	6.7 ± 0.6	ND

¹ADL = Acid detergent lignin.²EE = Ether extract.³ADIN = Acid detergent insoluble nitrogen.⁴Mean ± SD.⁵ND = Not determined.

toxin levels corrected for hull contents in two treatments in each experiment: namely, DP50 and DP50BII in Experiment 1 and Chaco520 and Chaco520BGRR in Experiment 2. Due to separation of the hull from the hull contents during grinding, the hulls and hull contents were weighed. This proportion was used in calculating the overall toxin concentrations in the WCS. The samples could not be analyzed for citrinin and diacetoxyscirpenol due to the complex sample matrix. According to Coppock et al. (1987) and Arieli (1998), free gossypol for all the WCS samples was at a normal level, with no more than 0.5% found, except for Guazuncho and Pora in Experiment 2, which had 0.54 and 0.55%, respectively.

Test substance characterization confirmed the expected transgenic content in both experiments. The exception was DP50BII (Bollgard II) in Experiment 1. The PCR analysis of DP50BII indicated the sample was contaminated with Roundup Ready cottonseed. The PCR analysis produced a faint band, lower in intensity than the control, and this was likely due to cross pollination by insects because 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 this study because there were no differences in cow performance observed among any of the treatments, including cows fed Roundup Ready cottonseed. Additionally, the nontransgenic control was confirmed to be free of the GM components. Thus, the appropriate comparisons could still be made without confounding the assessment of potential effects of feeding transgenic WCS to cows. In Experiment 2, Chaco520 cottonseed presented a faint positive band suggestive of a very low level of Roundup Ready trait contamination. The two commercial cottonseed products (Guazuncho and Pora) included in this study were negative for both transgenic traits.

Dry Matter Intake, Body Weight, and Body Condition Score

Treatment effects on DMI, BW, and BCS during the experiments are presented in Table 3. The WCS was fed at a fixed amount of 2.50 kg of DM per cow daily for all treatments. The GM WCS did not affect the total DMI of the animals. These results are consistent with summaries of Beever and Phipps (2001) and Clark and Ipharraguerre (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 BCS were not affected by treatments (Table 3). However, significant differences ($P < 0.01$) in BW and BCS were observed among experimental periods unrelated to treatment. Cows lost BW from periods 1 to 4, with a significant variation in BCS. This loss in BW is considered to be due to the effects of hot weather on the cattle that occurred during the study. The thermal-neutral zone for Holstein dairy cattle is 5 to 20°C (NRC, 1981), but can vary among animals. Temperatures below or above the thermal-neutral range alter intake and metabolic activity, and thus the maintenance requirements of the animal (NRC, 2001). The temperature-humidity index (THI), obtained by equation from the relative humidity and the air temperature (Bianca, 1962), was used to evaluate the body comfort of the animals. A THI value above 72 may decrease DMI and, as a consequence, lactational performance. In our study, the minimum temperatures were between 10 to 20°C, providing a relatively good environment for a thermal-neutral zone during the nights. An average THI of 70 was registered during the day, with values above 72 occurring in almost all experimental periods.

The eating behavior of cows at night, achieving higher DMI than might be expected, probably indi-

Table 2. Chemical composition of TMR and whole cottonseed treatments in each experiment.

Components	TMR		Experiment 1								Experiment 2							
			DP50		DP50B		DP50BII		DP50RR		Chaco520		Chaco520BGRR		Guazuncho		Pora	
	Mean ⁴	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, %	53.8	2.7	91.4	1.5	91.6	1.2	91.2	1.4	91.2	0.9	92.6	0.9	91.8	1.0	92.0	1.2	92.2	1.1
CP, %	18.1	0.7	17.6	4.4	18.1	2.7	16.7	1.4	19.6	1.7	21.4	1.8	21.5	2.1	22.3	1.2	21.6	2.1
NDF, %	36.7	1.4	52.6	1.8	52.7	1.2	52.8	1.6	48.8	2.2	46.1	1.9	47.1	2.0	46.4	0.9	47.9	1.1
ADF, %	23.0	2.3	37.3	0.7	37.5	1.1	39.4	1.8	34.9	2.3	31.1	1.1	32.9	1.9	32.5	1.4	33.0	3.1
ADL ¹ , %	4.2	0.4	8.0	0.8	8.5	0.9	7.7	0.9	8.3	1.1	9.3	0.7	9.8	0.8	9.1	0.8	9.3	0.5
EE ² , %	4.1	1.0	15.9	1.0	15.4	0.5	14.2	1.0	17.3	0.6	21.0	1.8	19.0	2.0	20.3	1.8	18.9	0.8
Ash, %	9.4	0.4	4.3	0.2	4.2	0.3	3.9	0.4	4.3	0.4	4.3	0.2	4.7	0.4	4.3	0.2	4.4	0.1
NFC, % ³	31.7		9.9		9.6		12.4		10.0		7.2		7.7		6.7		7.2	
Ca, %	0.96	0.25	0.14	0.01	0.15	0.01	0.15	0.01	0.14	0.01	0.13	0.02	0.14	0.01	0.14	0.01	0.15	0.01
P, %	0.49	0.03	0.64	0.05	0.61	0.09	0.58	0.05	0.61	0.05	0.67	0.03	0.69	0.02	0.68	0.02	0.66	0.03
Mg, %	0.26	0.03	0.38	0.04	0.34	0.02	0.36	0.02	0.36	0.02	0.33	0.03	0.32	0.04	0.35	0.02	0.35	0.01
K, %	2.5	0.4	1.4	0.2	1.6	0.1	1.4	0.1	1.4	0.1	1.4	0.1	1.6	0.2	1.4	0.2	1.4	0.2
Na, %	1.3	0.2	0.02	0.007	0.02	0.005	0.03	0.004	0.02	0.003	0.02	0.005	0.02	0.004	0.02	0.004	0.02	0.006
Fe, mg/kg	399	19	37.6	2.1	37.1	2.4	37.3	3.0	38.3	3.7	38.1	2.5	36.5	2.5	38.6	3.6	36.8	4.1
Zn, mg/kg	56.0	14.3	34.0	7.1	37.5	5.6	36.1	7.8	35.0	7.6	37.6	8.4	37.9	6.8	37.4	5.7	37.6	6.9
Cu, mg/kg	10.4	1.0	4.6	0.3	4.5	0.5	4.5	0.6	4.7	0.4	5.0	0.5	4.8	0.4	4.9	0.4	4.9	0.4
Mn, mg/kg	85.0	9.1	13.5	0.8	13.0	0.5	13.1	1.1	13.8	0.7	14.3	0.7	14.1	1.0	13.9	0.6	14.3	1.0

¹ADL = Acid detergent lignin.

²EE = Ether extract.

³NFC = 100 – (CP + NDF + EE + ash).

⁴(n = 8).

Table 3. Total mixed ration, DMI, BW, and BCS of cows fed whole cottonseed (WCS) from varieties with and without expression of the Cry1Ac, Cry2Ab, or CP4 EPSPS proteins.

Item	WCS treatments				SE	<i>P</i> <	Periods				SE	<i>P</i> <
	DP50	DP50B	DP50BII	DP50RR			1	2	3	4		
Experiment 1												
Intake, kg/(cow·d)												
TMR DMI	23.4	23.8	23.9	23.7	1.1	0.67	24.9 ^a	25.2 ^a	23.7 ^b	21.1 ^c	0.6	0.01
BW, kg	567.4	567.3	566.8	568.7	25.0	0.97	573.2 ^a	572.5 ^a	565.5 ^b	558.9 ^b	24.8	0.01
BCS ¹	2.30	2.30	2.30	2.34	0.08	0.62	2.27 ^{bc}	2.41 ^a	2.25 ^c	2.33 ^b	0.07	0.01
Item	WCS treatments				SE	<i>P</i> <	Periods				SE	<i>P</i> <
	Chaco520	Chaco520BGRR	Guazuncho	Pora			1	2	3	4		
Experiment 2												
Intake, kg/(cow·d)												
TMR DMI	23.0	22.4	22.9	23.0	0.9	0.41	24.0 ^a	24.2 ^a	22.3 ^b	20.8 ^c	0.4	0.01
BW, kg	548.3	539.3	548.1	539.9	15.8	0.36	558.6 ^a	547.7 ^{ab}	537.0 ^{bc}	533.5 ^c	15.0	0.01
BCS	2.34	2.25	2.31	2.36	0.08	0.27	2.19 ^b	2.44 ^a	2.24 ^b	2.39 ^a	0.06	0.01

^{a,b,c}Means in the same row with unlike superscripts differ $P < 0.01$.

¹BCS scale ranged from 1 (thin cow) to 5 (fat cow).

cates 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; Davison et al., 1996). The effects of environmental temperature on the cows in this study did not affect the interpretation of data based on the experimental design employed in this study.

Table 4. Effect of whole cottonseed (WCS) from varieties with and without expression of the Cry1Ac, Cry2Ab, or CP4 EPSPS proteins on milk yield and milk composition.

	WCS treatments					
Item	DP50	DP50B	DP50BII	DP50RR	SE	<i>P</i> <
Experiment 1						
Milk yield, kg/cow/d	26.9	26.7	27.6	27.4	2.9	0.69
Milk composition, %						
Fat	3.59	3.60	3.52	3.59	0.19	0.78
Protein	3.15	3.14	3.14	3.13	0.07	0.95
Lactose	4.97	5.01	5.04	5.00	0.06	0.28
SNF	8.84	8.91	8.96	8.90	0.10	0.39
MUN, mg/100 mL	18.77	19.49	20.66	20.01	1.96	0.27
Yield, kg/cow/d						
Fat	0.95	0.95	0.95	0.97	0.07	0.86
Protein	0.84	0.84	0.86	0.85	0.08	0.81
Lactose	1.34	1.34	1.39	1.37	0.15	0.46
SNF	2.37	2.38	2.47	2.44	0.25	0.49
MUN, g/d	5.17	5.19	5.75	5.54	0.78	0.18
	Chaco520	Chaco520BGRR	Guazuncho	Pora	SE	<i>P</i> <
Experiment 2						
Milk yield, kg/cow/d	27.5	26.5	26.8	27.4	2.2	0.56
Milk composition, %						
Fat	3.32	3.35	3.36	3.24	0.13	0.69
Protein	3.16	3.20	3.15	3.11	0.09	0.60
Lactose	4.83	4.83	4.86	4.86	0.09	0.82
SNF	8.72	8.77	8.73	8.71	0.12	0.69
MUN, g/100 mL	17.10	14.71	15.65	16.63	1.70	0.10
Yield, kg/cow/d						
Fat	0.90	0.88	0.89	0.89	0.07	0.99
Protein	0.86	0.85	0.84	0.85	0.06	0.91
Lactose	1.33	1.28	1.30	1.33	0.12	0.59
SNF	2.39	2.33	2.33	2.38	0.19	0.73
MUN, g/d	4.75	4.03	4.35	4.58	0.59	0.15

Milk Yield and Milk Composition

Milk production, milk composition, and milk component yields were not affected by the different GM WCS when compared with control nontransgenic WCS (Table 4). These results are in accordance with two recent reviews (Clark and Ipharraguerre, 2001; Beever and Phipps, 2001). Milk yield averaged more than 26 kg per cow daily over all treatment groups and experiments; milk contained, on average, 3.58, 3.14, 5.0, and 8.9% (Experiment 1) and 3.32, 3.16, 4.85, and 8.73% (Experiment 2) fat, protein, lactose, and SNF, respectively.

In Experiment 1, the concentration of MUN ranged from 18.8 (DP50) to 20.7 mg/100 mL of milk (DP50BII). In Experiment 2, the range was from 14.7 (Chaco520BGRR) to 17.1 mg/100 mL of milk (Chaco520). These values, and lack of significant differences, indicate that cows were fed with similar and relatively well balanced diets (Gustaffson, 1993; Linn and Olson, 1995; Broderick and Clayton, 1997).

Analysis of Milk for Transgenic and Endogenous Cotton DNA

Milk samples were analyzed for transgenic and endogenous cotton DNA. A total of 24 milk samples were analyzed for a 215-bp fragment of the *cry1Ac* transgene, and 47 samples were analyzed for a 400-bp fragment of the endogenous cotton gene, *acp1*. Both assays used PCR coupled to Southern blot analysis to achieve an extremely high level of sensitivity approaching the theoretical limits of detection for PCR (as little as a single cotton cell). No sample was positive for transgenic or cotton DNA fragments at the limits of detection for the assays following detailed data evaluation criteria (Jennings et al., 2003a). The lack of detection of transgenic DNA from cottonseed is supported by the lack of transgenic DNA detected in milk or meat from livestock that have consumed GM corn or soybean meal (Phipps and Beever, 2001; Phipps et al., 2002; Jennings et al., 2003b).

CONCLUSIONS

Lactating dairy cattle fed WCS derived from cottonseed expressing proteins conferring insect protection and/or tolerance to glyphosate in plants supported similar performance as the non transgenic commercial varieties as indicated by DMI, milk yield, milk composition, BW, and BCS under controlled feeding conditions. Therefore the nutritional value of whole cottonseeds from genetically modified cotton was equivalent to cottonseed from nontransgenic cotton varieties.

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