Alfalfa Containing the Glyphosate-Tolerant Trait Has No Effect on Feed Intake, Milk Composition, or Milk Production of Dairy Cattle

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ABSTRACT

The objective of this experiment was to assess if feeding glyphosate-tolerant alfalfa affects feed intake, milk composition, or milk production of dairy cows. One alfalfa (*Medicago sativa*), variety expressing the CP4 EPSPS protein and grown in southeastern Washington State was harvested at the late vegetative stage as hay. Three commercial conventional varieties of alfalfa hay of similar nutrient composition and harvested in the same geographic region were fed to cows as controls. The commercial hays were selected to be similar in crude protein [18% of dry matter (DM)] and neutral detergent fiber (40% of DM) to the glyphosate-tolerant hay. Sixteen multiparous Holstein cows were fed diets containing alfalfa hay (39.7% of diet DM) from either the glyphosate-tolerant alfalfa, or 1 of the 3 conventional varieties. Diets contained at least 15.7% crude protein and 29% neutral detergent fiber. Experimental design was a replicated 4 × 4 Latin square. Periods were 28 d and feed intake, milk yield, and milk composition were summarized over the last 14 d of each period. Daily milk yield (38.0 kg) and 4% fat-corrected milk (34.7 kg) were not affected by treatment. Milk fat (3.44%) and milk true protein (2.98%) were also not affected by source of hay. Milk lactose (4.72%) and solids-not-fat (8.5%) did not differ due to treatment. Dry matter intake was similar across treatments (24.4 kg/d). These results are consistent with data from feeding trials with other glyphosate-tolerant crops and previously reported compositional comparisons of glyphosate-tolerant alfalfa with controls. Milk production, milk composition, feed intake, and feed efficiency were not affected by feeding diets that contained nearly 40% glyphosate-tolerant alfalfa hay to lactating dairy cows. **Key words:** glyphosate, Roundup Ready, alfalfa, dairy cow

INTRODUCTION

Glyphosate is a nonselective herbicide that has been used commercially in the United States for over 35 yr. Glyphosate kills annual and perennial plants by disrupting the shikimate pathway (Duke et al., 2003). The enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) is inhibited by glyphosate, which prevents the plant from synthesizing the aromatic amino acids phenylalanine, tyrosine, and tryptophan. A glyphosate-tolerant form of the *epsps* gene, *cp4 epsps*, has been stably incorporated into soybeans, canola, cotton, corn, and sugar beets (Cerdeira and Duke, 2006) and has dramatically changed cropping practices in the United States. Glyphosate is now the most used herbicide in the United States, with >46 million kilograms of active ingredient used in 2002 (Gianessi and Reigner, 2006). This gene (*cp4 epsps*) encoding a glyphosate-tolerant form of EPSPS from *Agrobacterium* spp. is very effective at producing glyphosate tolerance (Padgett et al., 1996a). The Monsanto Company (St. Louis, MO) and Forage Genetics International (West Salem, WI) have developed varieties of alfalfa that are tolerant to glyphosate ("Roundup Ready") using the *cp4 epsps* gene. Glyphosate tolerance in alfalfa was developed using *Agrobacterium*-mediated transformation to incorporate into the alfalfa genome the *cp4 epsps* coding sequence that produces a glyphosate-tolerant form of EPSPS. The production of the CP4 EPSPS protein in plant tissues is the basis of the trait in Roundup Ready Alfalfa events J101 and J163 [Organisation for Economic Co-operation and Development (OECD) unique identifiers: MON-00101-8 and MON-00163-7, respectively]. Planting glyphosate-tolerant alfalfa and subsequent application of a glyphosate agricultural herbicide has the potential to offer significant agronomic, economic, and environmental benefits. Such planting systems would offer alfalfa growers a new opportunity for improved weed control, crop safety, and forage quality by eliminating weeds associated with stands of alfalfa similar to planting systems currently approved for corn, soybeans, canola, cotton, and sugar beets. Feeding trials with other glyphosate-tolerant crops have been con-

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ducted showing no negative effects on the performance of farm animals (Flachowsky et al., 2005). To date, the effects of glyphosate-tolerant alfalfa on feed intake or milk production of dairy cows have not been published in the peer-reviewed literature. The purpose of this experiment was to assess if feeding glyphosate-tolerant alfalfa to dairy cows affects feed intake, milk composition, and milk production compared with feeds composed of conventional varieties of alfalfa that are similar in nutrient composition.

MATERIALS AND METHODS

Alfalfa Production, Harvest, and Chemical Composition

The Roundup Ready alfalfa (test alfalfa) was genetically modified alfalfa line J101 × J163 expressing the CP4 EPSPS protein. The test alfalfa forage field was planted in spring 2004 in southeastern Washington State, and glyphosate was applied to control weeds before first cutting. The test alfalfa was first cut in June followed by a second cutting in July 2004 at the late vegetative stage and harvested without the use of preservatives into large (approximately 680 kg) bales as hay. Three conventional commercial alfalfa varieties were similarly planted and harvested in southeastern Washington. One conventional alfalfa variety (control) was grown next to the test field, weeds were controlled using nonglyphosate postemergence herbicides, and the hay harvested into large bales. Two other conventional varieties (**REF1** and **REF2**) were grown in the same region by commercial hay producers, planted and managed similarly to the control, and harvested, instead, into small (approximately 36 kg) bales. All hays were cut and allowed to dry in the field to approximately 15% moisture before baling with only first-year second-cutting hay used in this study. The 2 reference hay lots were purchased from commercial hay producers and intentionally selected for use because they were nutritionally similar to the test and control hays (i.e., values for CP and NDF composition were similar). Each bale of each of the 4 hays was individually labeled and shipped to the University of Wisconsin Agricultural Experiment Station at Arlington during the fall of 2004. All alfalfa hays were stored at ambient temperature in 1 of 2 secured buildings until the feeding trial. Before feeding, hays were chopped through an AgriVal Agri-Chopper (DC Atlas Company, Loyal, WI) without the grate and placed in wagons, which were stored in a hay shed at the Dairy Cattle Center during the feeding study.

Nutrient Composition of Hays. Before the start of the study, all hays, corn silage, high-moisture corn, and grain mixes were sampled and analyzed for DM, CP,

NDF, P, Ca, K, and Mg at the University of Wisconsin Soil and Forage Analysis Lab (Marshfield, WI). The analytical DM was determined by oven drying at 105°C for 3 h. Samples were analyzed for CP (method 988.05; AOAC, 1990) with the following modifications: 1 g of sample was analyzed; 30 mL of H₂SO₄, 10 g of K₂SO₄, 0.3 g of CuSO₄, and 0.1 g of pumice were added to each tube for digestion, and digestion time was 2 h; 250 mL of water was added to the tubes after digestion, and samples underwent steam distillation into a boric acid solution (4% wt/vol) containing 0.20% methyl red indicator solution and 0.98% bromocresol green solution; and 0.1 N H₂SO₄ was used to titrate the distillate. Neutral detergent fiber was determined according to Van Soest et al. (1991) using α -amylase and sodium sulfite and was not corrected for ash concentration. Analysis of P, Ca, K, and Mg were according to Schulte et al. (1987).

Confirmation of Hay Trait Status. Samples of the 4 hays were sent to Monsanto (St. Louis, MO) to confirm that the test alfalfa contained the CP4 EPSPS protein and that the 3 conventional hays did not. All 4 hays were tested for the CP4 EPSPS protein using the Western blot method (Rogan et al., 1999). Hay samples were ground with dry ice and stored in a -80°C freezer before analysis. Hay samples were homogenized in Tris-borate buffer [100 mM Tris base, 100 mM Na₂B₄O₇·10H₂O, 10 mM MgCl₂, 0.05% (vol/vol) Tween-20, and 0.2% (wt/vol) L-ascorbic acid] at a tissue-to-buffer ratio (wt/vol) of 1:50. Extracts were filtered, diluted with sample loading buffer (Laemmli, 1970), and analyzed by SDS-PAGE using the Criterion system (BioRad, Hercules, CA). Western blot analysis was conducted as described previously (Rogan et al., 1999) with the exception that primary antibody bound to polyvinylidene difluoride membrane was detected with a 1:5,000 dilution of rabbit anti-goat IgG antibodies conjugated to horseradish peroxidase (Sigma, St. Louis, MO). The test and conventional hay samples were also analyzed for glyphosate (Cowell et al., 1986) and aminomethylphosphonic acid (**AMPA**; degradation product of glyphosate) residues (Cowell et al., 1986).

Cows, Diets, and Sampling

The feeding study was conducted at the Emmons Blaine Dairy Center at the University of Wisconsin-Arlington Research Farm. All procedures involving animals were reviewed and approved by the University of Wisconsin-Madison, College and Agricultural and Life Sciences Animal Care and Use Committee. Sixteen multiparous cows were used in a replicated 4 × 4 Latin square design. Cows were blocked into 4 groups based upon DIM and previous 2-wk milk yield. Each block of 4 cows was assigned to a square, and cows within square

were randomly assigned to treatments. Periods were 28 d with first 14 d for treatment adaptation. Feed intake, milk yield, and milk composition measurements were collected over the last 14 d of each period.

Cows were fed diets containing test alfalfa or 1 of the 3 conventional varieties. Chopped hay was incorporated into each TMR as a fixed percentage (39.7% of diet) on a DM basis. Diets were formulated to meet or exceed NRC (2001) requirements for energy, CP, Ca, P, salt, and vitamin A, D, and E requirements for multiparous dairy cows producing 40.9 kg of milk per day. Diets were adjusted weekly to accommodate variation in DM content of feeds. The TMR were offered such that between 2 to 6 kg (as fed) of refusals was recorded daily.

Cows were milked twice daily in a milking parlor, and milk yields were recorded after each milking. Because diet shifts among periods occurred after the morning milking of d 1, daily milk weights were calculated based upon afternoon and subsequent morning milkings. During the last 2 wk of each period, afternoon and morning milk samples from each cow were collected during 4 consecutive milkings and analyzed for fat, true protein, lactose, SNF, and MUN (method 972.16; AOAC, 1990) by Fourier transform infrared spectroscopy (Foss FT 6000, Foss Electric, Hillerød, Denmark) and SCC (method 978.26; AOAC, 1990) by flow cytometry (Foss 400, Foss Electric). Milk samples were analyzed at AgSource (Verona, WI).

On d -2 and -1 (before initiation of the study) and on d 27 and 28 of each period, BW were measured. On d -2 and 27 of each period, cows were scored for BCS using a 5-point system (quarter-point basis; Edmonson et al., 1989). Body condition scoring was done independently by the same 2 people over the course of the study.

Statistical Analysis

All data were analyzed as a replicated 4×4 Latin square using the MIXED procedure in SAS (SAS Institute, 1999). The model for milk yield, 4% FCM yield, milk fat percentage, milk protein percentage, milk lactose percentage, milk SNF percentage, milk SCC, MUN, DMI, hay intake, BCS, BW, BW change, and feed efficiency (kg of 4% FCM/kg of DMI) was: $Y = \mu + S_i + C_{(j)i} + P_k + T_l + e_{ijkl}$; where μ = overall mean, S_i = random effect of square ($i = 1$ to 4), $C_{(j)i}$ = random effect of cow within square ($j = 1$ to 4), P_k = fixed effect of period ($k = 1$ to 4), T_l = fixed effect of treatment ($l = 1$ to 4), e_{ijkl} = random residual error.

Data are reported as least squares means with standard errors. Significant treatment effects were declared when the P -value of the overall treatment effect was <0.05 . When a significant effect due to treatment was declared, differences among treatment means were de-

termined with the "diff" function in the MIXED procedure of SAS.

RESULTS AND DISCUSSION

Feed and Diet Composition

The concentration of glyphosate residue was near the lower limit of detection (0.2 ppm) for the test alfalfa hay (0.25 ppm) and under the detection limit for the 3 conventional hays (<0.2 ppm; i.e., not detected). No AMPA was detected in any of the 4 hays. Presence of the CP4 EPSPS protein in the test alfalfa hay was confirmed by the Western blot analysis and no detectable level of the protein was detected in the REF1 and REF2 alfalfas. Trace levels of CP4 EPSPS were detected in control alfalfa, but at levels that were at 0.33% of what was found in the test hay, suggesting slight mechanical cross contamination of the control hay from sampling or dust.

The chemical composition of the alfalfa hays was similar (Table 1). The CP composition of the hays ranged from 16.9 to 20.7%, and NDF ranged from 38.7 to 43.4% of DM. Mineral contents of the 4 hays were similar except for potassium, which ranged from 2.34 to 4.58% of DM. In a previous study, McCann et al. (2006) reported that there were no statistical differences in CP, fiber values, or mineral composition of test and conventional alfalfa varieties grown over 3 yr across several field study sites consistent with the finding that test and conventional alfalfas are compositionally equivalent. Glyphosate-tolerant corn (Ridley et al., 2002) and soybeans (Padgett et al., 1996b) were also not found to differ in nutrient content or composition when compared with conventional grains. Nutrient profiles of the diets are summarized in Table 2. Differences in CP and NDF composition between the alfalfa hays had minimal effect on the final nutrient composition of the test diets. The diet containing the test alfalfa inadvertently contained approximately 1% less CP (15.7% of DM) than the diets formulated with the conventional alfalfa hays, but was still adequate to support 40 kg of potential milk production for the cows in this study (NRC, 2001). The diet containing REF1 was approximately 1.5% higher in NDF than the other 3 diets. The greater level of potassium in the test alfalfa than conventional hays resulted in a slightly greater dietary concentration of potassium for the diet containing test alfalfa hay.

Milk Yield, Milk Composition, and Feed Intake

All animals except one remained on the study until the end of the fourth 28-d period. One cow was removed during the fourth period because it became apparent that the on-hand supply of test hay was not adequate

Table 1. Chemical composition of feeds (DM basis)

Feed	DM % as fed	CP	NDF	P	Ca	K	Mg
	% of DM						
Corn silage	39.3 ± 1.9 ¹	7.8 ± 0.3	39.2 ± 2.0	0.22 ± 0.07	0.16 ± 0.10	1.05 ± 0.10	0.19 ± 0.02
High-moisture shelled corn	69.23 ± 1.6	8.8 ± 0.6	14.6 ± 5.0	0.34 ± 0.02	0.01 ± 0.00	0.49 ± 0.03	0.20 ± 0.12
Protein/mineral supplement ²	90.0 ± 1.6	46.5 ± 3.1	6.1 ± 1.3	1.31 ± 0.05	4.73 ± 1.28	1.30 ± 0.16	0.46 ± 0.06
Alfalfa hay, test	87.8 ± 2.5	16.9 ± 2.4	38.7 ± 1.9	0.27 ± 0.04	1.58 ± 0.61	4.58 ± 1.54	0.46 ± 0.15
Alfalfa hay, control	86.1 ± 2.8	19.8 ± 0.8	40.1 ± 4.0	0.34 ± 0.03	1.59 ± 0.79	2.77 ± 1.08	0.41 ± 0.19
Alfalfa hay, reference 1	85.5 ± 3.0	19.6 ± 1.4	43.4 ± 1.6	0.37 ± 0.15	1.07 ± 0.06	3.28 ± 0.27	0.31 ± 0.02
Alfalfa hay, reference 2	85.1 ± 3.0	20.7 ± 2.0	38.5 ± 1.7	0.30 ± 0.04	1.27 ± 0.07	2.34 ± 0.10	0.46 ± 0.15

¹Mean ± standard deviation of samples by period, n = 4.

²Supplement composed of soybean meal (48% CP), 44.9%; corn gluten meal (60% CP), 37.2%; salt, 6.4%; calcium carbonate, 4.3%; dicalcium phosphate, 2.7%, magnesium oxide 0.8% and vitamin premix (682 IU vitamin A/kg, 227 IU vitamin D/kg, and 2,273 IU vitamin E/kg), 3.5%.

to allow all animals to finish the 28-d final period. One cow assigned to the test hay treatment was chosen at random and dropped from the study. All data from this cow for the final period were omitted before statistical analyses.

Milk yield and composition data are presented in Table 3. Yields of milk (38.0 ± 2.0 kg/d) and 4% FCM (34.7 ± 1.9 kg/d) were not affected by treatment. Milk fat percentage ($3.44 \pm 0.10\%$) and milk true protein percentage ($2.98 \pm 0.07\%$) were also not affected by source of hay. Milk lactose and SNF did not differ due to treatment. These results are consistent with data from feeding trials with other glyphosate-tolerant crops. Feed intake and milk production were similar in dairy cows fed glyphosate-tolerant corn silage and glyphosate-tolerant corn grain when compared with cows fed diets that were formulated with conventional corn silage and corn grain (Donkin et al., 2003; Ipharra-

guerre et al., 2003). Grant et al. (2003) reported that milk yield and 4% FCM production was reduced when cows were fed diets containing glyphosate-tolerant corn silage and corn grain, compared with conventional controls. The reduction in milk production reported by Grant et al. (2003) appeared to be due to differences in the DM content of the corn silages, which was attributed to faster-than-normal drying conditions. In their study, feed intake was depressed but milk production per unit of feed intake was similar when the glyphosate-tolerant corn diet was compared with conventional controls. There is no evidence to suggest that the gene modification in alfalfa would be toxic or produce any products that would impair digestion and metabolism in dairy cattle. To the contrary, a direct benefit of improved weed control in alfalfa fields will be to remove weed species that are known to be toxic to livestock, reduce feed palatability, impart off-flavors to milk, or

Table 2. Formulation and nutrient composition of diets

Item	Treatment ¹			
	Test	Control	Reference 1	Reference 2
Alfalfa hay, test	39.7	—	—	—
Alfalfa hay, control	—	39.7	—	—
Alfalfa hay, reference 1	—	—	39.7	—
Alfalfa hay, reference 2	—	—	—	39.7
Corn silage	22.2	22.2	22.2	22.2
High-moisture shelled corn	27.7	27.7	27.7	27.7
Protein/mineral supplement	10.4	10.4	10.4	10.4
DM, % as fed	65.7 ± 0.2	65.1 ± 0.2	66.3 ± 0.4	62.3 ± 0.3
	% of DM			
CP	15.7 ± 1.4 ²	16.8 ± 0.5	16.7 ± 1.1	17.2 ± 1.3
NDF	28.9 ± 1.2	29.4 ± 2.3	30.8 ± 1.2	28.8 ± 0.9
Ca	1.16 ± 0.19	1.16 ± 0.27	0.95 ± 0.11	1.03 ± 0.14
P	0.39 ± 0.03	0.41 ± 0.02	0.43 ± 0.05	0.40 ± 0.03
K	2.32 ± 0.61	1.60 ± 0.42	1.80 ± 0.10	1.43 ± 0.05
Mg	0.28 ± 0.09	0.31 ± 0.11	0.26 ± 0.04	0.33 ± 0.10

¹Test treatment was a TMR containing glyphosate-tolerant alfalfa hay. Control, reference 1, and reference 2 were TMR containing commercial, conventional hays.

²Mean ± standard deviation, based on analysis of feed components across periods.

Table 3. Least squares mean milk production and composition of cows fed diets with or without glyphosate-tolerant alfalfa hay¹

Item	Treatment ²				SE	P-value
	Test	Control	Reference 1	Reference 2		
Milk, kg/d	38.4	37.8	38.2	37.6	2.0	0.69
4% FCM, kg/d	35.0	34.4	35.1	34.3	1.9	0.5
4% FCM/intake	1.39	1.39	1.47	1.43	0.05	0.18
Milk composition						
Fat, %	3.41	3.43	3.49	3.42	0.10	0.56
True protein, %	2.96	3.02	2.96	2.98	0.07	0.44
Lactose, %	4.59	4.74	4.78	4.78	0.07	0.14
SNF, %	8.16	8.65	8.64	8.65	0.20	0.20
SCC, × 1,000	159 ^a	371 ^b	219 ^a	213 ^{ab}	97	<0.05
MUN, mg/dL	13.3 ^a	14.9 ^{ab}	17.6 ^c	15.6 ^{bc}	0.9	<0.01

^{a-c}Means in rows with different superscripts are different ($P < 0.05$).

¹Intake and milk data were summarized over the last 14 d of each experimental period.

²Test treatment was a TMR containing glyphosate-tolerant alfalfa hay. Control, reference 1, and reference 2 were TMR containing commercial, conventional hays.

be of low feed value. Harrison et al. (1996) reported that the CP4 EPSPS enzyme introduced into Roundup Ready soybeans was not toxic to mice and that insertion of this gene into soybeans did not result in production of any compounds that were antinutritive or allergenic to mice. Hartnell et al. (2005) reported no differences in apparent digestibility in sheep of OM, CP, NDF, or digestible energy of beets or beet pulp in which the CP4 EPSPS enzyme had been incorporated to produce glyphosate tolerance.

Milk SCC was similar for cows consuming diets containing the test, REF1, and REF2 alfalfas. The elevated mean SCC for cows consuming diet with control alfalfa was due primarily to 1 animal that had an elevated SCC during one period. Milk urea nitrogen concentrations differed among treatments but were similar when cows consumed diets containing control and test alfalfa. Differences in MUN were relatively small across treatments. The slightly lower MUN for cows fed test alfalfa were likely due to the lower dietary CP level, not factors

that could be attributed uniquely to the test hay. Milk fat, protein, SCC, and MUN concentrations also did not differ when cows were fed diets containing glyphosate-tolerant corn grain and corn silage compared with conventional controls (Donkin et al., 2003; Grant et al., 2003; Ipharraguerre et al., 2003).

Feed intake data are summarized in Table 4. Dry matter intake did not differ across treatments (24.4 ± 1.4 kg/d). Intake of hay was also similar across treatments. Feed efficiency (4% FCM produced per unit of feed intake) also did not differ due to treatment. Our results are similar to those reported by Donkin et al. (2003) and Ipharraguerre et al. (2003).

BW and BCS

Body weights and BCS data are summarized in Table 4. Mean BW of cows and BW change differed by treatment, but were not consistently due to factors associated with feeding test hay. Cows fed diets with control

Table 4. Least squares mean feed intake, BW, and BCS of cows fed diets with or without glyphosate-tolerant alfalfa hay¹

Item	Treatment ²				SE	P-value
	Test	Control	Reference 1	Reference 2		
DMI, kg/d	25.1	26.0	24.0	24.0	1.4	0.49
Hay intake, kg of DM/d	10.0	10.3	9.5	9.5	0.6	0.48
Mean BW, kg	669 ^c	664 ^{bc}	656 ^a	657 ^{ab}	16	<0.01
BW change, kg	15.5 ^c	13.4 ^c	-8.3 ^a	1.6 ^b	3	<0.01
Mean BCS	2.7	2.6	2.6	2.6	0.1	0.59
BCS change	0.06	-0.03	-0.02	0.01	0.06	0.74

^{a-c}Means in rows with different superscripts are different ($P < 0.05$).

¹Intake measurements averaged over the last 14 d of each period; BW and BCS are summarized over the entire 28-d period.

²Test treatment was a TMR containing glyphosate-tolerant alfalfa hay. Control, reference 1, and reference 2 were TMR containing commercial, conventional hays.

and test hay had similar mean BW and changes in BW by period. Body condition scores of cows averaged 2.6 ± 0.1 and did not differ due to treatment; BCS did not change significantly over the duration of the trial. These data suggest that cows on all treatments consumed adequate energy to support milk production during each period and over the duration of the trial.

CONCLUSIONS

No significant differences were observed in lactating dairy cow performance when fed glyphosate-tolerant alfalfa compared with conventional alfalfa when formulated to a similar TMR nutrient basis. Results of this experiment indicate that milk production, milk composition, feed intake, and feed efficiency were not affected by feeding diets that contained nearly 40% glyphosate-tolerant alfalfa hay to dairy cows.

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