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ORIGINAL ARTICLE

Effects of long-term feeding of genetically modified corn (event MON810) on the performance of lactating dairy cows

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Summary

A long-term study over 25 months was conducted to evaluate the effects of genetically modified corn on performance of lactating dairy cows. Thirty-six dairy cows were assigned to two feeding groups and fed with diets based on whole-crop silage, kernels and whole-crop cobs from Bt-corn (Bt-MON810) or its isogenic not genetically modified counterpart (CON) as main components. The study included two consecutive lactations. There were no differences in the chemical composition and estimated net energy content of Bt-MON810 and CON corn components and diets. CON feed samples were negative for the presence of Cry1Ab protein, while in Bt-MON810 feed samples the Cry1Ab protein was detected. Cows fed Bt-MON810 corn had a daily Cry1Ab protein intake of 6.0 mg in the first lactation and 6.1 mg in the second lactation of the trial. Dry matter intake (DMI) was 18.8 and 20.7 kg/cow per day in the first and the second lactation of the trial, with no treatment differences. Similarly, milk yield (23.8 and 29.0 kg/cow per day in the first and the second lactation of the trial) was not affected by dietary treatment. There were no consistent effects of feeding MON810 or its isogenic CON on milk composition or body condition. Thus, the present long-term study demonstrated the compositional and nutritional equivalence of Bt-MON810 and its isogenic CON.

Introduction

Since the first commercial cultivation of genetically modified plants (GMP) in 1996, the worldwide agricultural area on which GMP is grown increased from 1.7 million hectares to 125 million hectares in the year 2008 (James, 2008). Most of the genetically modified corn plants are tolerant to herbicides (glyphosate or glufosinate) or resistant against insects such as the European corn borer or corn rootworm. One of numerous insect-resistant corn hybrids is Bt-Corn event MON810, which contains the gene for Cry1Ab protein originating from naturally occurring soil-born bacterium *Bacillus thuringiensis* (*Bt*). The crystal protein (Cry protein) is an endotoxin that is specific for certain Lepidoptera such as the European corn borer. Due to the rising global cultivation of GMP, the importance of this plant cultivar and its use in animal nutrition grows in almost the same manner. To answer the question of the nutritional assessment of feeds from GMP and their influence on the performance and health of animals and quality of the food of animal origin, more than 100 feeding studies were published worldwide. In all experiments, feeds from GMP were compared with their conventional counterparts

(CONs) or commercial conventional hybrids. The numerous studies have determined the effects of feeding GMP (e.g. soybeans, corn, canola, sugar beet and cotton) to various animal species (e.g. lactating dairy cows, beef cattle, sheep, swine and poultry). The results of these studies were summarized and discussed in various review articles (Hammond et al., 1996; Aulrich et al., 2001, 2002; Clark and Ipharraguerre, 2001; Faust, 2002; Aumaitre, 2004; Flachowsky and Chesson, 2004; Flachowsky et al., 2007). Depending on the aim of those studies, the duration of these trials shows a high variability. While digestibility trials were carried out over a period of approximately 10–30 days, fattening trials usually lasted over a longer period of 250 days for beef cattle or approximately 100 days for growing–finishing pigs. In contrast, in dairy cows only short-term studies were conducted over 21–91 days. This lack of data resulted in the question of impact assessment of long-term feeding of Bt-corn (MON810) on performance variables of dairy cows. For this reason, the current study aimed to evaluate the effect of Bt-corn on feed intake, milk production, milk composition and body condition in lactating dairy cows compared with the isogenic CON over a period of 25 months.

Materials and methods

Crop and processing of corn products

Genetically modified corn (Bt-MON810) and its isogenic not genetically modified CON were cultivated and harvested under the same agronomic conditions (cultivation, sowing, fertilizing, plant protection and harvesting) in 2004, 2005 and 2006 at the Bavarian State Research Center for Agriculture (Freising, Germany). Degradation characteristics of the Cry1Ab protein and *cry1Ab* DNA depend on the processing of the forage (Aulrich et al., 2004; Lutz, 2005; Lutz et al., 2006). Therefore, the crop was processed directly after harvest into different corn products (silage, whole-crop cobs and kernels) to maximize the intake of Cry1Ab protein and *cry1Ab* DNA. Thereby, whole-crop cobs were prepared from chopped whole corn plants with special respect to careful heat treatment (approximately 7 min, approximately 580 and 93 °C at product entry and exit respectively) and low-pressing temperature (83–85 °C directly after pelleting, approximately 90 bar pressure, continuous product flow). This product, which is produced from the whole corn plant, is defined as whole-crop cobs in the present paper. Nutrient and energy contents of the different corn components are shown in Table 1.

Animals, experimental design and diets

Thirty-six Simmental dairy cows were selected from the Bavarian State Research Center for Agriculture and stratified by performance variables and lactation number and assigned to two groups. Eighteen dairy cows (nine primiparous, nine multiparous) were fed genetically modified corn (Bt-MON810), and the other 18 cows (nine primiparous, nine multiparous) were fed its isogenic CON over a period of 25 months. The cows had *ad libitum* access to their basic ration. During the experimental period, nine cows per group were exchanged because of illness or infertility and replaced by first-lactation cows. Thus, the study includes data from animals with complete lactations, as well as data from animals with partial

Table 1 Nutrient and energy contents (mean ± SD) of CON and Bt-MON810 corn components (g/kg DM unless stated)

	Whole-crop silage		Grain		Whole-crop cobs	
Component	CON	Bt-MON810	CON	Bt-MON810	CON	Bt-MON810
N	27	27	26	26	26	26
Dry matter (g/kg)	361 ± 30	351 ± 26	885 ± 11	895 ± 11	905 ± 11	907 ± 11
Crude ash	28 ± 2.3	29 ± 4.3	15 ± 2.1	15 ± 1.3	33 ± 4.6	32 ± 4.7
Crude protein	80 ± 8.6	84 ± 4.9	102 ± 8.1	98 ± 5.5	82 ± 7.5	83 ± 3.9
Crude fat	31 ± 5.4	28 ± 3.3	41 ± 8.7	40 ± 4.2	28 ± 5.8	30 ± 4.7
Crude fibre	176 ± 11	190 ± 15	23 ± 3	21 ± 2	170 ± 10	164 ± 9
ADFom*	188 ± 9	229 ± 26	27 ± 5	24 ± 2.2	193 ± 11	195 ± 7
NDFom*	370 ± 17	402 ± 37	105 ± 17	94 ± 9.5	434 ± 54	393 ± 22
NEL (MJ/kg DM)	6.74 ± 0.34	6.67 ± 0.34	8.75 ± 0.08	8.81 ± 0.19	6.99 ± 0.06	7.01 ± 0.04

CON, isogenic control corn; Bt-MON810, corn resistant to European cornborer; ADFom, acid detergent fibre expressed exclusive of residual ash; NDFom, neutral detergent fibre expressed exclusive of residual ash; NEL, net energy for lactation, calculated using digestibility coefficients determined according to standard methods with wethers (GfE, 1991).

*n = 7 and 10 for CON and Bt-MON810 silage, n = 4 for CON and Bt-MON810 grain, n = 5 for CON and Bt-MON810 whole-crop cobs.

lactations. The study covered two consecutive lactations from 2 weeks ante partum (a.p.) to 45 weeks post partum (p.p.) in the first lactation and to 29 weeks p.p. in the second lactation of the trial. The animals were housed in a tie-stall barn equipped with individual feeding places and were fed twice daily. Feed refusals were removed once a day, weighed and taken into account for determination of feed intake. Each group had a separate feed bunk to avoid a contamination of the CON diet with Cry1Ab protein or *cry1Ab* DNA from the Bt-MON810 diet. During the experiment, three diets for each feeding group were used according to the different energy and nutrient requirement of cows in early or late lactation and dry period. One was a partial mixed ration (PMR) designed for cows producing 22 kg milk per day (PMR 1), the second one was a ration for cows with less than 18 kg milk per day (PMR 2) and the third ration was designed for dry cows (PMR 3). PMR 2 and PMR 3 were based on PMR 1, which was mixed up with increasing amounts of straw (24.7% or 35.7% of DM, respectively, in PMR 2 and PMR 3). The diets were designed in such a way that the corn components (silage, whole-crop cobs and kernels) were from the same hybrid for each diet to maximize any possible effect of the Cry1Ab protein and *cry1Ab* DNA on animal performance. Overall, the PMR contained a proportion of 71% corn components. The ingredient composition of the experimental diets is shown in Table 2. For daily milk production higher than 22 kg/day, additional concentrates (41.2% corn kernels (either transgenic or isogenic), 34.4% rapeseed meal, 19.9% dried molassed beet pulp, 3.2% mineral mixture and 2.2% rape seed oil on dry matter basis) were fed individually on the top of the PMR according to actual milk yield.

Data collection, sampling and analysis

Individual feed intake was recorded daily. For chemical analysis, samples of corn components (silage, whole-crop cobs and kernels), straw, concentrates and diets were collected weekly. After determination of DM, these silage, straw and diet samples were pooled monthly for the analysis of chemical composition. The corn grains, whole-crop cobs and concentrates samples were also pooled and analyzed monthly. The other diet and concentrates components (molasses, dried molassed beet pulp and rapeseed meal) were collected and analyzed per batch. Feed samples were analyzed for crude nutrients

Table 2 Ingredient (% of DM) and chemical composition (g/kg DM unless stated) of diets (mean ± SD) derived from CON and Bt-MON810 corn components

	PMR1 (designed for 22 kg milk/day)		PMR 2 (for cows < 18 kg milk/day)		PMR 3 (diet for dry cows)	
Ingredient	CON	Bt-MON 810	CON	Bt-MON810	CON	Bt-MON810
Ingredient, % of DM						
Whole-crop silage	41.9	41.9	33.5	33.5	28.6	28.6
Whole-crop cobs	21.2	21.2	17.0	17.0	14.5	14.5
Grass silage	11.0	11.0	8.8	8.8	7.5	7.5
Straw	5.9	5.9	24.7	24.7	35.7	35.7
Molasses	1.4	1.4	1.1	1.1	1.0	1.0
Concentrates*	18.6	18.6	14.9	14.9	12.7	12.7
Chemical composition (g/kg DM)						
N	28	28	28	21	28	21
Dry matter (g/kg)	494 ± 28	490 ± 31	544 ± 25	548 ± 26	573 ± 29	582 ± 29
Crude ash	50 ± 4.2	50 ± 5.7	48 ± 3.3	47 ± 3.4	47 ± 4.0	44 ± 3.0
Crude protein	131 ± 7.6	132 ± 9.7	111 ± 5.7	111 ± 7.6	100 ± 7.2	97 ± 8.3
Crude fat	29 ± 3.4	28 ± 2.9	26 ± 2.6	24 ± 2.0	24 ± 2.4	22 ± 1.9
ADFom†	212 ± 12	213 ± 19	240 ± 16	248 ± 17	277 ± 18	291 ± 32
NDFom†	397 ± 40	395 ± 31	452 ± 26	456 ± 31	502 ± 36	514 ± 52
NEL (MJ/kg DM)	6.67 ± 0.15	6.68 ± 0.14	6.10 ± 0.11	6.09 ± 0.11	5.79 ± 0.10	5.80 ± 0.07

PMR, partial mixed ration; CON, isogenic control corn; Bt-MON810, corn resistant to European cornborer; ADFom, acid detergent fibre expressed exclusive of residual ash; NDFom, neutral detergent fibre expressed exclusive of residual ash; NEL, net energy for lactation, calculated using digestibility coefficients determined according to standard methods with wethers (GfE, 1991).

*Dry matter composition (%): 41.2% corn grain, 51.1% rapeseed meal, 5.3% mineral mixture, 2.4% urea.

†n = 10 and 13 for CON and Bt-MON810 PMR1, n = 9 for CON and Bt-MON810 PMR2, n = 11 and 10 for CON and Bt-MON810 PMR3.

according to Weende analysis (Naumann and Bassler, 2007). For the determination of the ADF and NDF content, samples were pooled for 4-month periods depending on the harvest of the corn products and analyzed according to Naumann and Bassler (2007) with ash correction (ADFom and NDFom). The net energy contents of corn components (silage, whole-crop cobs and kernels), concentrates and diets were calculated according to GfE (2001). The analyzed crude nutrients and the digestibility of the feeds were used for the calculation. The crude nutrient digestibility coefficients of the corn whole-crop cobs, silage and kernels were determined in digestibility trials with wethers (data not shown) according to standard methods (GfE, 1991). For the remaining feeds, table values (Universität Hohenheim—Dokumentationsstelle, 1997) for nutrient digestibility coefficients were used.

Cows were milked twice daily, and the milk yield was recorded (Lactocorder®; WMB AG, Balgach, Switzerland) twice a week from consecutive evening and morning milkings (two complete days of milking: Monday p.m., Tuesday a.m., Thursday p.m. and Friday a.m.) during the experiment. Milk samples were obtained at the same time and analyzed for the content of fat, protein, lactose and urea via an infrared analyzer (MilcoScan; Foss Analytical A/S, Hilleroed, Denmark) and the somatic cell count was measured with a Fossomatic (Foss Analytical A/S). Body condition (body weights, body condition score and backfat thickness) was recorded monthly. Body weights were taken on electronic scales after milking. The body condition was scored according to Edmonson et al. (1989). Determination of backfat thickness was carried out according to Staufenbiel (1992) using an ultrasonic device (Tringa Linear; Esaote Biomedica Deutschland GmbH, Germany).

For the analysis of Cry1Ab protein, weekly samples of corn components (silage, whole-crop cobs and kernels) and diets were collected. The analysis of the samples was carried out by the Institute of Physiology of the Technical University of Munich in Weihenstephan. A highly specific and sensitive ELISA was applied to detect the Cry1Ab protein in feed. One-hundred milligrams of ground feed samples, mixed with ice-cold extraction buffer (8 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl, 1.5 mM potassium phosphate containing 0.1% Tween 20 and protease inhibitors, pH 7.4), were homogenized using the FastPrep technique. The clear sample extract was collected after centrifugation at 15 000 *g* and 4 °C for 15 min. Transgenic silage extracts were directly used in the ELISA, whereas transgenic corn grain and corn cob extracts were diluted 1:5 with extraction buffer (P. Guertler, V. Paul, K. Steinke, S. Wiedemann, W. Preißinger, C. Albrecht, H. Spiekers, F. J. Schwarz, H. H. D. Meyer, unpublished observations). All samples were analyzed as previously described (Paul et al., 2008). The Cry1Ab protein intake of cows was calculated based on the results of the Cry1Ab protein content in feed samples and DMI of the cows.

Statistical analysis

Before analysis, data for DMI, milk production and milk composition were pooled to weekly means for each cow. Subsequently lactation curves were estimated based on these weekly means for the first and the second lactation of the trial. The data were analysed using the MIXED procedure of SAS (version 9.1, SAS Institute, Cary, NC, USA) with dietary treatment, lactation week and the interaction as fixed effects. The following model was used for the analysis of all performance variables:

$$Y_{ijk} = G_i + LW_j + (G^* LW)_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} is the observed value of dietary treatment i, lactation week j, of cow k; G_i the effect of dietary treatment i (i = 1, 2); LW_j is the effect of lactation week j (j = 1,..., maximum 45 [depending on the length of the lactation, maximum 45 lactation week]); $(G * LW)_{ij}$ is the interaction of dietary treatment and lactation week; and ε_{ijk} residual error of dietary treatment i, lactation week j, of cow k.

A normal distribution of the data was assumed for the evaluation. The correlations of errors within the observations of the same cow were taken into account for distribution of residual errors. Significant differences among the dietary treatments were declared at $p < 0.05$.

Results and discussion

Nutrient composition of corn components, concentrates and diets

The nutrient composition and energy content (Table 1) of corn components (silage, whole-crop cobs and kernels), from Bt-MON810 and its isogenic CON were within the range given in current feed tables for ruminants (Universität Hohenheim—Dokumentationsstelle, 1997). Moreover, nutrient and energy concentrations of corn components, concentrates and diets from Bt-MON810 and its isogenic CON were similar throughout the experimental period (Tables 1–3). Thus, compositional equivalence in

Table 3 Nutrient and energy contents (mean ± SD) of CON and Bt-MON810 concentrates (g/kg DM unless stated)

	PMR concentrate		Additional concentrate	
Constituent	CON	Bt-MON810	CON	Bt-MON810
n	28	28	28	28
Dry matter (g/kg)	891 ± 4	895 ± 8	891 ± 4	896 ± 6
Crude ash	86 ± 6.6	85 ± 6.4	71 ± 3.7	68 ± 4.1
Crude protein	297 ± 9.9	296 ± 13.8	206 ± 8.8	199 ± 13.3
Crude fat	39 ± 3.7	39 ± 5.2	35 ± 4.7	34 ± 4.0
ADFom*	110 ± 5	111 ± 7	124 ± 11	120 ± 12
NDFom*	167 ± 12	166 ± 8	198 ± 23	196 ± 19
NEL (MJ/kg DM)	7.30 ± 0.04	7.34 ± 0.04	8.02 ± 0.04	8.01 ± 0.03

CON, isogenic control corn; Bt-MON810, corn resistant to European cornborer; ADFom, acid detergent fibre expressed exclusive of residual ash; NDFom, neutral detergent fibre expressed exclusive of residual ash; NEL = net energy for lactation calculated using digestibility coefficients determined according to standard methods with wethers (GfE, 1991).
*n = 9 for CON and Bt-MON810 PMR concentrate and additional concentrate.

respect to nutrient concentration of genetically modified and unmodified corn, which appears to be a prerequisite in safety and nutritional assessment of GMP (Report of the EFSA GMO Panel Working Group on Animal Feeding Trials, 2008) was demonstrated in the present study. Similarly, Aeschbacher et al. (2005) compared dry matter concentration, chemical composition (crude ash, crude protein, ADF, NDF and crude fat) and energy content of Bt-corn and nonmodified corn and observed no major differences between both corn types. Similar results have been observed in other studies on Bt-corn (Donkin et al., 2003; Rossi et al., 2005) and glyphosate-tolerant corn (Sidhu et al., 2000; Ridley et al., 2002; Ipharraguerre et al., 2003).

Cry1Ab protein analysis of feed samples

The following results were compiled within the same project (P. Guertler, V. Paul, K. Steinke, S. Wiedemann, W. Preißinger, C. Albrecht, H. Spiekers, F. J. Schwarz, H. H. D. Meyer, unpublished observations). The analysis of Bt-MON810 feed samples by ELISA resulted in concentrations of Cry1Ab protein ranging from 91 to 390 ng/g DM (mean ± SD: 195 ± 89 ng/g DM) for corn silage, from 155 to 379 ng/g DM (mean ± SD: 237 ± 55 ng/g DM) for corn kernels, from 226 to 1021 ng/g DM (mean ± SD: 617 ± 222) for corn whole-crop cobs and from 210 to 452 ng/g DM (mean ± SD: 332 ± 59) for PMR. Except for two samples, no immunoreactive fragments of the Cry1Ab protein were detected in CON feed samples above the cut off limit (CCα) and below the detection capability (CCβ). The reason that 2 out of 26 samples of CON corn kernels were positive for Cry1Ab protein (concentrations above the CCβ) is most likely sampling or post-sampling contamination. Thus, as expected, the analysis of feed samples by ELISA not only clearly demonstrated the presence of Cry1Ab protein in Bt-MON810 corn components (silage, whole-crop cobs and kernels) and PMR, respectively, but also demonstrated the absence of Cry1Ab protein in the isogenic CON samples. Therefore, the results confirmed the correct provenance of the Bt-MON810 and CON samples.

In comparison of the different dietary corn components, the lowest concentration of Cry1Ab protein was detected in corn silage, and the highest in corn whole-crop cobs. Given that corn whole-crop cobs and corn silage are both produced from the whole corn plant as the basic material, the differences in Cry1Ab concentration may reflect a different influence of the two conservation methods on degradation of the Cry1Ab protein. We did not measure Cry1Ab concentration of the fresh corn plant, but Lutz et al. (2006) reported an extensive degradation of the Cry1Ab protein during ensiling process of Bt-corn (event Bt176). The complete Cry1Ab protein was only detected in the first 8 days of ensiling, and 61 days after ensiling, only 23.6% of the original Cry1Ab protein amount was detected with ELISA. Similar results were reported by Folmer et al. (2002), who studied the effect of ensiling on the Cry1Ab protein content in early and late maturing Bt-corn hybrids (event Bt11). Data of Lutz (2005) indicate that thermal treatment of corn plant due to whole-crop cobs production reduced Cry1Ab concentration to a lesser extent. In the study of Lutz (2005), 31% of the amount of Cry1Ab protein detected in the fresh plant (expressed on a DM basis) were analyzed in corn whole-crop cobs. Even if the Cry1Ab concentrations of whole-crop cobs and silage

Table 4 Effect of long-term feeding of genetically modified corn (Bt-MON810) and its isogenic counterpart (CON) on feed intake, milk production, milk composition and body condition (mean ± SD)

Lactation of experiment	First			Second		
Group	CON	Bt-MON810	p-value	CON	Bt-MON810	p-value
DMI, kg/day	18.7 ± 0.48	18.9 ± 0.47	0.532	21.0 ± 0.67	20.4 ± 0.68	0.080
Milk yield, kg/day	23.9 ± 0.84	23.7 ± 0.82	0.566	29.2 ± 1.10	28.8 ± 1.12	0.419
ECM, kg/day	23.8 ± 0.8	24.1 ± 0.8	0.438	28.7 ± 1.2	28.5 ± 1.2	0.797
Milk fat, %	3.95 ± 0.09	4.03 ± 0.08	0.015	3.75 ± 0.12	3.86 ± 0.12	0.055
Milk protein, %	3.62 ± 0.04	3.71 ± 0.04	<0.001	3.59 ± 0.06	3.56 ± 0.06	0.299
Milk lactose, %	4.83 ± 0.03	4.82 ± 0.03	0.155	4.75 ± 0.04	4.80 ± 0.04	0.006
MU, mg/l	164 ± 9	181 ± 9	<0.001	179 ± 13	175 ± 14	0.523
SCC ($\times 10^3$ cells/ml)	157 ± 72	205 ± 70	0.073	241 ± 148	220 ± 150	0.754
BW, kg	688 ± 14	690 ± 14	0.977	720 ± 20	691 ± 20	0.014
BCS	3.57 ± 0.1	3.53 ± 0.1	0.395	3.74 ± 0.13	3.42 ± 0.13	<0.001
Back fat thickness, mm	17.6 ± 1.6	18.5 ± 1.6	0.277	20.3 ± 2.0	14.4 ± 2.1	<0.001

CON, isogenic control corn; Bt-MON810, corn resistant to European cornborer; DMI, dry matter intake (kg/day); ECM, energy corrected milk (kg/day); MU, milk urea concentration (mg/l); SCC, somatic cell count ($\times 10^3$ cells/ml); BW, body weight (kg); BCS, body condition score.

measured in the present study suggest that differences in response to variable conservation methods are greater than literature data using transgenic corn of the event Bt176 would suggest, literature as well as our data indicate a lower degradation of Cry1Ab protein degradation during whole-crop cobs production compared to silage preparation.

In the present study, Cry1Ab concentration of corn whole-crop cobs was considerably higher than Cry1Ab concentration in corn kernels or whole-crop silage. Expression of Cry1Ab protein in leaves and in stalks is much higher than expression of Cry1Ab protein in kernels (Nguyen and Jehle, 2007). Whole-crop cobs fed to cows in the present study were prepared from chopped whole corn plants. Thus, the higher concentration of Cry1Ab protein in corn whole-crop cobs compared to kernels can be explained by their high proportions of green plant tissue (leave and stalk) with high Cry1Ab protein concentrations.

Performance variables

The effect of long-term feeding of genetically modified corn (Bt-MON810) and its isogenic CON on performance variables for the first lactation and second lactation of the trial are presented in Table 4. The DMI of cows fed CON and Bt-MON810 diets during

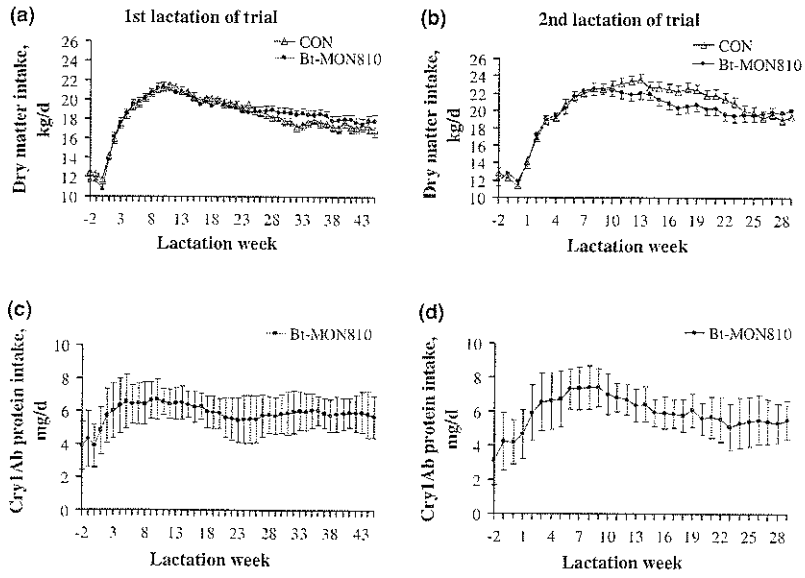

Fig. 1 Dry matter intakes of the two feeding groups in the first (a) and second (b) lactation; Cry1Ab protein intakes of the two feeding groups in the first (c) and second (d) lactation. CON, isogenic control corn; Bt-MON810, corn resistant to European cornborer.

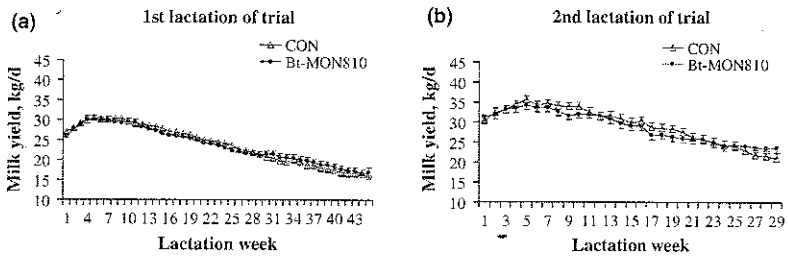

Fig. 2 Milk yield of the two feeding groups in the first (a) and second (b) lactation. CON, Isogenic control corn; Bt-MON810, corn resistant to European cornborer.

the first and second lactation are shown in Fig. 1a,b. There were no differences in DMI between the feeding groups in the first lactation (18.7 vs. 18.9 kg/day) and second lactation (21.0 vs. 20.4 kg/day). These results are comparable to literature data (Folmer et al., 2002; Donkin et al., 2003; Faust et al., 2003; Grant et al., 2003), which also indicate no influence of feeding genetically modified or non-transgenic corn on feed intake in dairy cows.

The Cry1Ab protein intake of cows fed Bt-MON810 corn during both lactations as calculated from feed intake and analyzed Cry1Ab concentrations from feeds is shown in Fig. 1c,d. As a mean of the first lactation, the cows fed with Bt-MON810 corn had an average daily Cry1Ab protein intake of 6.0 mg and in the second lactation of 6.1 mg. Thereby, in the first as well as in the second lactation of the trial, Cry1Ab protein intake appears to be relatively constant over time. Moreover, Cry1Ab intake was the same for each lactation week when first and second lactation of the trial are compared. To our knowledge, there are no data on Cry1Ab intake in dairy cow feeding trials published in literature. However, the PMR 1 used in the present study contained a proportion of 71% corn products, and the additional concentrates contained approximately 41% corn kernels. Thus, taking into consideration current nutrient supply recommendations for dairy cows (GfE, 2001), we included the maximum possible amount of corn products in the diets of the present experiment, and followed therefore the recommendations given in the Report of the EFSA GMO Panel Working Group on Animal Feeding Trials (2008) to create balanced diets with high proportions of GMP in the diets when conducting comparative feeding studies involving GMP of the first generation. Moreover, because we used corn whole-crop cobs with high Cry1Ab concentrations as a dietary component derived from corn plants, we made an additional attempt to maximize the overall Cry1Ab intake in the respective feeding group. In summary, because of these experimental protocols, the possible consequences from Cry1Ab intake should have been visible at each time point throughout the experiment.

Similar to DMI, there were no significant differences in milk yield between the two experimental groups during the two lactations of the trial

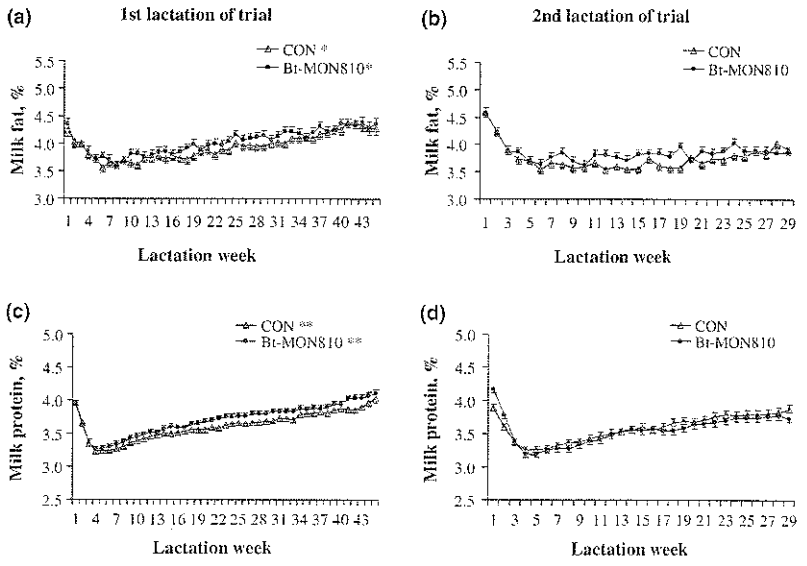

Fig. 3 Milk fat content of the two feeding groups in the first (a) and second (b) lactation; milk protein content of the two feeding groups in the first (c) and second (d) lactation. CON, Isogenic control corn; Bt-MON810, corn resistant to European cornborer. *Differences between the two groups ($p < 0.05$); **Differences between the two groups ($p < 0.01$).

(Fig. 2a,b). Moreover, there were no effects on energy corrected milk yield (Table 4). Similar findings regarding influence of feeding Bt-corn on milk yield were also observed by Barrière et al. (2001), Folmer et al. (2002), Faust et al. (2003), Calsamiglia et al. (2007) and Donkin et al. (2003). Considering that feed intake, dietary nutrient and energy concentrations and, consequently, daily nutrient and energy intakes were not different between the two feeding groups in the present experiment, a lack of influence on daily milk yield seems to be plausible.

As shown in Table 4 and Fig. 3, there were some differences in milk composition and body condition between feeding groups. For example, cows fed Bt-MON810 had higher ($p < 0.05$) milk fat, milk protein and milk urea concentrations (4.03%, 3.71% and 181 mg/l) than cows fed CON diets (3.95%, 3.62% and 164 mg/l) as a mean of the first lactation of the experiment. In the second lactation of the trial, cows fed CON diets had lower ($p < 0.05$) milk lactose concentrations (4.75%) than cows fed Bt-MON810 diets (4.80%), but body weight, BCS and backfat thickness were higher ($p < 0.05$). However, the absolute differences between feeding groups for these variables appear to be small and to fall within the biological variation. Both milk fat and milk protein differed by slightly less than 0.1 percentage units, yet daily ECM yield as an indicator of milk energy output was the same for cows on both diets. In addition, milk urea concentrations of cows on both diets are indicative of very well-balanced diets with regard to protein supply to the cows and nitrogen supply to rumen microorganisms (Lebzien et al., 2006). In summary, no consistent major effects of feeding Bt-MON810 or CON diets on performance variables were observed. Thus, data of the current study demonstrate the compositional equivalence of Bt-MON810 and its isogenic CON, and, as a consequence, its equivalence in nutritional terms.

Conclusions

The nutrient composition and energy content of Bt-MON810 corn components and diets in the present study were not different from the isogenic CON corn components and diets. Feeding of Bt-corn (MON810) over a period of 25 months had no obvious influence on the performance variables of lactating dairy cows in this study. The few differences observed are most likely a result of factors not associated with dietary treatment. Thus, the present long-term study confirms the results of previous short-term studies on the feeding of Bt-corn in dairy cow nutrition.

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