Mustard bran in lactating dairy cow diets

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ABSTRACT

Two trials using lactating Holstein cows were conducted to evaluate effects of a diet containing oriental mustard bran on dry matter intake (DMI), milk production, milk components, and organoleptic properties. In experiment 1, 34 lactating cows (24 multiparous and 10 primiparous; days in milk ≥ 50 d) were used in a switchback design to determine the lactational response and organoleptic quality of milk when the diet contained 8% oriental mustard bran (MB) versus a control diet (CON). Mustard bran replaced a portion of soybean meal and all the beet pulp in the CON diet. Milk yields were greater for cows fed the MB diet; however, no differences were found in DMI, 3.5% fat (FCM) or solids-corrected milk. Milk components and components production were not affected by treatment. Milk organoleptic qualities were not affected by diet. In experiment 2, 22 lactating cows (16 multiparous and 6 primiparous; days in milk ≥ 21 d) were assigned randomly within parity to receive MB or CON from wk 4 to 19 postpartum in a randomized complete block design. Cows were fed CON wk 1 to 3 postpartum. The MB diet contained the same ingredients as the CON, except sunflower seed and a portion of soybean meal were replaced with mustard bran. Milk and components data were collected during wk 3 postpartum and used as covariates to adjust treatment means. Intake was greater for cows fed the MB diet; however, daily milk, 3.5% FCM, and solids-corrected milk yields were not different between diets. Milk components and component yields were not affected by treatment. Milk urea concentration was less for cows fed the MB diet. Although cows fed the MB diet had greater DMI, this was not translated into a higher milk 3.5% FCM/DMI production efficiency ratio. During experiment 2, many cows fed MB experienced minor to severe hemolysis with bloody urine. This hemolysis believed to be caused by the S-methyl-cysteine sulfoxide contained in

mustard bran could have affected milk production efficiency. The increased milk yield observed in experiment 1 was not observed in experiment 2. Adding 8% of MB to lactating cow diets had a mixed effect on DMI and milk production. Milk component yields and milk quality were not affected. Feeding this level of MB presents a hemolytic danger to lactating dairy cows.

Key words: oriental mustard bran, milk, organoleptic, lactating cow

INTRODUCTION

Brown, oriental, and yellow are the main mustard species grown in the Midwestern United States and western Canada for food and oil production. Mustard bran is the outer coat derived from the processing of the seed for mustard flour. Oriental mustard bran production is estimated to be 150 to 200 t of bran yearly in the Midwestern United States. The chemical analysis of oriental mustard bran samples we analyzed was highly variable. Crude protein content varied from 15.5 to 21.5%, crude fat from 15.5% to 20.0%, and NDF from 38 to 55% of DM. Composition may also be influenced by processing methods. Processing techniques that leave more whole seed, more gluten in the bran, or both, will result in a more variable chemical composition. Despite variability in its chemical content, the nutrient profile of mustard bran makes it, a priori, an attractive alternative feed for cattle.

Early studies with mustard meal indicated it can be fed to sheep (Peterson, 1970), swine (Peterson and Danielson, 1972), beef cattle (Peterson and Thomas, 1970; Thomas, 1972), and dairy cows (Moss, 1975) with no adverse effects on DMI. Milk and milk component yields were not affected by 7.5 and 15% mustard meal in diets (Moss, 1975). To our knowledge, no studies have been conducted with feeding mustard bran to dairy cattle.

The objective of this study was to evaluate the effect of an isocaloric and isonitrogenous diet containing 8% oriental mustard bran on milk production, milk composition, DMI, and milk organoleptic quality. Because experiment 1 was a short-term study of 28-d experimental

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periods, which may not capture true differences in DMI and production responses, experiment 2, a longer study of 16 wk, was designed to validate results found in experiment 1.

MATERIALS AND METHODS

All procedures were approved by and conducted according to the University of Minnesota Animal Care and Use Committee guidelines.

Experiment 1

Animals and Diets. Thirty-four lactating Holstein cows (24 multiparous and 10 primiparous) were paired within parity by milk production level ($\text{DIM} \geq 50$ d) and assigned randomly to 2 treatment diets in a switchback design of two 28-d periods, with the first 14 d of each period serving as adaptation to treatment and the last 14 d as data collection. The number of cows used was determined by the power test procedures described by McMillan (2004), based on DMI using a standard deviation of 2 kg/d. Cows were fed either the control diet (**CON**) or the oriental mustard bran-containing diet (**MB**) in which mustard bran replaced 26% of CP from soybean meal (**SBM**) and all of the NDF from beet pulp in the CON diet (Table 1). Diets were fed as a TMR and were formulated to contain 16% of CP and 1.74 Mcal of NE_L /kg, which called for 8% mustard bran inclusion rate in the MB diet. Because mustard bran contains 16.5% fat, TMR were also formulated to keep fat concentration below 6%, which is the safe level for ruminants (Palmquist and Jenkins, 1980). Cows were housed in a tie-stall barn and fed individually a TMR once daily for ad libitum intake (5% orts). Amounts of feed fed and orts were recorded daily to determine DMI.

Sample Collection and Analytical Procedures.

Cows were milked twice daily and milk weights were recorded at each milking during the entire experiment. Two 24-h (p.m. and a.m.) milk samples (~500 mL) were collected during the experiment (one each week) and shipped to Stearns County DHIA laboratories (Sauk Center, MN) for analysis for fat, protein, lactose, SNF, TS, MUN, and SCC.

Milk samples (~500 mL, p.m. and a.m.) were also collected daily during the last 7 d of each treatment period for organoleptic (odor and taste) evaluation. Mustard has a special odor and contains allyl isothiocyanate which is responsible for the hot taste and might affect milk quality (Moss, 1975). A panel of 2 experienced dairy products judges and 1 beginner evaluated milk samples from 10 cows/treatment ($\text{SCC} \leq 300 \times 10^3/\text{mL}$). The panel scored milk odor and taste on a scale

of 1 to 10 using evaluation of milk off-flavors guidelines described by Nelson and Trout (1981). A score of 1 indicates strong mustard odor or strong hot taste whereas a score of 10 indicates no defects.

Samples of TMR, corn silage, haylage, wheat straw, concentrates, and mustard bran were obtained weekly during the entire feeding period and frozen at -20°C . Samples were oven-dried at 55°C for 48 h and ground through a Wiley mill (1-mm screen; Arthur H. Thomas Co., Philadelphia, PA). All samples were analyzed for DM, CP, ether extract, ash, acid detergent lignin, Ca, and P (methods 934.01, 2001.11, 945.16, 942.05, 973.18, 968.08, and 965.17, respectively; AOAC, 2010). The NDF (with amylase and without sulfite) and ADF were analyzed with a fiber analysis unit (Ankom Technology, Fairport, NY) using solutions of Van Soest et al. (1991). Neutral detergent insoluble CP and acid detergent insoluble CP were determined by the Kjeldahl procedure (method 2001.11, AOAC, 2010) and used to calculate NE_L ($3\times$; NRC, 2001).

Statistical Analysis

Milk production and composition, and DMI data were subjected to ANOVA for a switchback design using generalized least squares procedures (Proc Mixed of SAS; SAS Inst., Cary, NC). The model for intake, milk production, and efficiency included fixed effects of parity, period, treatment, interactions, and day within period was a repeated variable. Covariance structures were first-order autoregressive, compound symmetry, or unstructured, whichever minimized fit statistics. The model for milk components and component production

Table 1. Ingredient composition of TMR for cows fed the control (CON) or the oriental mustard bran (MB) diets in experiment 1

Item, % of DM	Diet	
	CON	MB
Alfalfa haylage	26.7	25.5
Corn silage	26.8	25.5
Wheat straw	1.0	1.0
High-moisture corn (26% DM)	21.0	20.6
Soybean meal (46% CP)	11.0	9.0
MB	—	7.9
Sunflower seed	3.0	3.0
Dried corn distillers grains	5.7	5.7
Dried beet pulp	3.0	—
Dicalcium phosphate (CaHPO_4)	0.30	0.30
Sodium bicarbonate (NaHCO_3)	0.75	0.75
Trace-mineralized salt ¹	0.60	0.60
Vitamin ADE ²	0.15	0.15

¹Provided per kilogram of diet DM: 36.5 mg of Zn; 26 mg of Mn; 20.5 mg of Fe; 9.1 mg of Cu; 0.60 mg of I; 0.4 mg of Co; and 0.3 mg of Se.

²Provided per kilogram of diet DM: 6,875 IU of supplemental vitamin A; 1,379 IU of supplemental vitamin D; and 23 IU of supplemental vitamin E.

Table 2. Ingredient composition of TMR for cows fed the control (CON) or the oriental mustard bran (MB) diets in experiment 2

Item, % of DM	Diet	
	CON	MB
Alfalfa haylage	24.5	23.5
Corn silage	24.5	23.5
Straw, wheat	3.2	2.8
High-moisture shelled corn	21.5	21.5
Soybean meal	12.2	9.4
MB	—	8.0
Sunflower seed	2.8	—
Dried corn distillers grains	8.7	8.7
Dicalcium phosphate (CaHPO ₄)	0.29	0.29
Sodium bicarbonate (NaHCO ₃)	1.00	1.00
Calcium carbonate (CaCO ₃)	0.50	0.50
Trace-mineralized salt ¹	0.60	0.60
Vitamin ADE ²	0.21	0.21

¹Provided per kilogram of diet DM: 36.5 mg of Zn; 26 mg of Mn; 20.5 mg of Fe; 9.1 mg of Cu; 0.60 mg of I; 0.4 mg of Co; and 0.3 mg of Se.

²Provided per kilogram of diet DM: 6,875 IU of supplemental vitamin A; 1,379 IU of supplemental vitamin D; and 23 IU of supplemental vitamin E.

only included fixed effects of parity, period, treatment, and interactions. The model for organoleptic data comprised the fixed effect of treatment and random effect of juror.

Experiment 2

Animals and Diets. Twenty-two lactating Holstein cows (16 multiparous and 6 primiparous; DIM $\geq$ 21 d) were assigned randomly within parity to receive a diet containing 8% MB or CON, from wk 4 to 19 postpartum in a randomized complete block design. The num-

ber of cows used was determined as in experiment 1. The CON diet was made of corn silage, alfalfa haylage, wheat straw, and the concentrate portion containing rolled high-moisture corn, SBM, sunflower seed, dried distillers grains, minerals, and vitamins. The MB diet contained the same ingredients as the CON, except sunflower seed and a portion of SBM were replaced with mustard bran from the same source as in experiment 1 (Table 2). Diets were formulated as in experiment 1 to be isocaloric, and isonitrogenous, and to contain 8% MB.

Cows were housed in the same tie-stall barn as in experiment 1 and fed individually a TMR once daily for ad libitum intake (5% orts). Cows were fed the CON diet during the pre-treatment period (calving to wk 3 postpartum) then switched to treatment diets after collection of pretreatment milk samples and recording of milk weights. Cows were fed experimental diets from wk 4 through 19 postpartum. Amounts of feed fed and orts were recorded daily to determine DMI.

Sample Collection and Analytical Procedures.

Cows were milked twice daily and milk weights were recorded at each milking during the entire experiment. Two 24-h (p.m. and a.m.) milk samples (~500 mL) were collected from each cow during wk 3 postpartum (before they were fed the experimental diets), and one 24-h (p.m. and a.m.) milk sample was taken weekly throughout the experiment. Milk samples were analyzed for fat, protein, lactose, SNF, MUN, and SCC, as described previously in experiment 1. Frozen samples were also shipped to the Northern Great Plains Research Laboratory USDA (Mandan, ND) for fatty acid analysis via direct transesterification (Whitney et al.,

Table 3. Chemical composition of forages, mustard bran (MB), and control (CON) and oriental MB diets fed in experiment 1

Item	Forage			Diet		
	Alfalfa haylage	Corn silage	Wheat straw	MB	CON	MB
DM, %	43.2	38.3	90.0	94.5	53.1	54.3
Chemical composition, DM basis						
CP, %	19.5	7.3	3.6	17.5	16.4	16.3
NE _L , ¹ Mcal/kg	1.43	1.72	0.97	1.73	1.74	1.74
Ether extract, %	2.4	3.0	1.8	16.5	4.1	5.2
NDF, %	42.3	38.9	85.0	44.5	31.0	32.7
ADF, %	30.8	19.0	54.0	31.3	18.0	18.8
Lignin, %	7.3	2.2	6.5	5.1	3.1	3.5
NFC, ² %	25.8	46.7	1.8	41.5	41.7	39.5
Starch, ³ %	—	—	—	—	28.9	27.8
Ash, %	10.0	4.1	7.8	4.9	7.0	6.6
Ca, %	1.55	0.21	0.18	1.19	0.73	0.69
P, %	0.50	0.27	0.05	0.34	0.43	0.40
Mg, %	0.36	0.24	0.12	0.24	0.29	0.29

¹Calculated using $3 \times$ maintenance NE_L equation from NRC (2001).

²NFC = 100 - (% NDF + % CP + % ether extract + % ash) according to the NRC (2001) model.

³Estimated for TMR only using Spartan Dairy program of Michigan State University (East Lansing).

Table 4. Intake, milk production and composition, and organoleptic quality of milk for cows fed control (CON) and oriental mustard bran (MB) diets in experiment 1

Item	Diet		SEM	P-value
	CON	MB		
Cows, n	34	34		
DMI, kg/d	22.3	22.7	0.56	0.65
Milk production				
Milk, kg/d	38.8	42.0	0.97	0.04
3.5% FCM, ¹ kg/d	40.6	41.0	0.96	0.72
SCM, ² kg/d	35.0	35.5	0.83	0.70
Milk component				
Fat, %	3.45	3.37	0.07	0.44
Protein, %	2.87	2.86	0.04	0.85
Lactose, %	4.89	4.89	0.03	0.90
SNF, %	7.76	7.74	0.05	0.82
TS, %	11.21	11.12	0.11	0.52
Urea, mM	13.31	12.91	0.31	0.35
SCC, $\times 10^3$ /mL	385	315	108	0.65
Milk component production				
Fat, kg/d	1.41	1.41	0.04	0.99
Protein, kg/d	1.17	1.20	0.03	0.47
Lactose, kg/d	2.00	2.05	0.05	0.44
SNF, kg/d	3.17	3.25	0.07	0.42
TS, kg/d	4.58	4.66	0.10	0.58
Milk production efficiency				
3.5% FCM/DMI	1.78	1.80	0.05	0.74
Organoleptic evaluation ³				
Taste	9.57	9.81	0.16	0.07
Off flavor	9.74	9.89	0.08	0.08

¹3.5% FCM = $(0.432 \times \text{kg/d of milk}) + (16.23 \times \text{kg/d of fat})$ (Britt et al., 2003).

²SCM = $(12.3 \times \text{kg/d of fat}) + (6.56 \times \text{kg/d of SNF}) - (0.0752 \times \text{kg/d of milk})$ (Tyrrell and Reid, 1965).

³Scored on a scale of 1 to 10. Score of 1 means strong mustard odor or strong hot taste, and score of 10 = no noticeable defect.

1999) with an alkaline catalyst (KOH), as described by Murrieta et al. (2003). Separation of fatty acid methyl esters was achieved by GLC (Model CP-3800; Varian, Inc., Palo Alto, CA) with a $100 \text{ m} \times 0.25 \text{ mm}$ (i.d.) $\times 0.2 \mu\text{m}$ (film thickness) capillary column (SP-2560, Supelco, Bellefonte, PA) and H_2 gas as the carrier at 1.5 mL/min. The initial oven temperature was maintained at 120°C for 2 min, increased to 210°C at 6°C/min, and then increased to 250°C at 5°C/min. The injector temperature was 260°C and the flame ionization detector temperature was 300°C. Identification of peaks was accomplished using purified fatty acid standards (Sigma-Aldrich, St. Louis, MO; Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA). One milligram of methyl tridecanoic acid (13:0) was used as an internal standard.

Samples of TMR, corn silage, haylage, wheat straw, concentrates, and mustard bran were obtained weekly during the entire feeding period and frozen at -20°C . Samples were oven-dried at 55°C for 48 h and ground through an ultracentrifuge mill (1-mm screen; Brinkmann Instrument Co., Westbury, NY). All samples were analyzed for DM, CP, ether extract, ash, NDF, ADF, acid detergent lignin, Ca, and P content as in

experiment 1. Mustard bran samples were shipped to Olson Station Biochemistry Laboratory (South Dakota State University, Brookings, SD) for AA content determination. Amino acid content was analyzed by methods 988.15 and 994.12 AOAC (2010). Also, samples of MB were sent to the Experiment Station Chemical Laboratory (University of Missouri–Columbia) for fatty acid analysis by the AOAC (2010) method 972.28. In vitro DM digestibility of MB was determined by the modified 2-stage technique of Tilly and Terry (1963) as described by Holden (1999). The NDF digestibility was estimated after a single-stage, 48-h in vitro incubation.

Statistical Analysis

Data of milk production and composition obtained from wk 4 to 19 were adjusted using wk 3 data as covariates if $P \leq 0.15$. All data were subjected to ANOVA for a randomized complete block design using generalized least squares (Proc Mixed of SAS). The model included treatment, parity, and their interaction. Day was a repeated variable for milk production, intake, and milk production efficiency (3.5% FCM/DMI). Week was a repeated variable for milk components and component

Table 5. Chemical composition of forages, mustard bran (MB), TMR control (CON), TMR MB diets fed in experiment 2

Item	Forage			Diet		
	Alfalfa haylage	Corn silage	Wheat straw	MB	CON	MB
DM, %	48.5	39.0	90.0	92.8	56.8	58.1
Chemical composition, DM basis						
CP, %	19.3	7.3	3.6	21.0	17.4	17.2
NE _L , ¹ Mcal/kg	1.34	1.50	0.97	2.36	1.74	1.74
Ether extract, %	2.8	3.5	1.8	20.8	4.9	5.2
NDF, %	39.6	41.5	85.0	36.0	29.1	29.6
ADF, %	30.6	22.9	54.0	23.4	17.4	17.7
Lignin, %	7.31	2.34	10.0	5.1	3.8	3.8
NFC, ² %	31.1	43.5	1.8	18.4	41.9	41.5
Ash, %	7.3	4.2	7.8	4.8	6.7	6.6
Ca, %	1.52	0.17	0.18	0.92	0.87	0.89
P, %	0.21	0.21	0.05	0.45	0.44	0.44
Mg, %	0.35	0.23	0.12	0.26	0.29	0.29

¹Calculated using $3 \times$ maintenance NE_L equation from NRC (2001).²NFC = $100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ ether extract} + \% \text{ ash})$ according to the NRC (2001) model.

production. Covariance structures were first-order autoregressive, first-order ante dependence, compound symmetry, or unstructured, whichever minimized fit statistics.

RESULTS AND DISCUSSION

Experiment 1

Nutrient Content of Diets. The chemical composition of forages, oriental mustard bran, and experimental diets is presented in Table 3. Crude protein, ADF, and calculated NE_L ($3 \times$) content of diets were similar between diets. The NDF percentage was slightly higher in the MB diet because of the high fiber content of the MB but this NDF is highly digestible (see discussion for experiment 2). Ether extract content was higher for the MB diet because of the high fat content of MB.

DMI, Milk Yield, and Composition. Daily DMI (Table 4) for cows fed CON and MB diets were not different ($P = 0.65$). Feeding 7.5% mustard meal did not affect DMI (Moss, 1975). Peterson (1970), as quoted by Moss (1975), observed no palatability problems when lambs or beef heifers were fed protein supplements containing 20% mustard meal. Like the mustard meal studies, our results show that mustard bran did not affect intake or apparent palatability. The allyl isothiocyanate contained in mustard (Moss 1975) did not adversely affect DMI of cows fed the MB diet. Daily milk production of cows fed MB diet was greater ($P = 0.04$) than that of cows fed the CON diet. However, 3.5% FCM and SCM yields were not affected ($P \geq 0.70$) by diets. This is because milk fat production from cows fed the CON diet was not different ($P = 0.99$) but offset the greater milk yield of cows fed the MB

diet. A high milk production from feeding the MB diet could possibly be explained in the short-term by a better energetic utilization of this diet. The concentrations of milk fat, protein, lactose, SNF, TS, urea, SCC, and milk production efficiency (3.5% FCM, kg/kg of DMI) were not different between treatments ($P \geq 0.35$). In addition, daily production of milk fat, protein, lactose, SNF, and TS were not different between treatments ($P \geq 0.42$). Milk yield, milk fat, SNF, and milk protein

Table 6. Amino acid composition of oriental mustard bran fed in experiment 2

Item	Basis		
	DM ¹	CP ²	EAA ³
CP, %	21.4	—	—
AA			
Alanine, %	0.79	3.69	—
Arginine, %	1.06	4.95	12.97
Aspartic acid, %	1.37	6.40	—
Cystine, %	0.56	2.62	—
Glutamic acid, %	2.92	13.64	—
Glycine, %	0.99	4.63	—
Histidine, %	0.45	2.10	5.51
Isoleucine, %	0.78	3.64	9.55
Leucine, %	1.23	5.75	15.06
Lysine, %	1.36	6.36	16.65
Methionine, %	0.31	1.45	3.79
Phenylalanine, %	0.75	3.50	9.18
Proline, %	1.55	7.24	—
Serine, %	0.92	4.30	—
Threonine, %	0.97	4.53	11.87
Tryptophan, %	0.20	0.93	2.45
Valine, %	1.06	4.95	12.97
TEAA ⁴	8.17	38.18	—

¹AA as a percent of DM.²AA as a percent of CP.³Essential AA as a percent of total EAA.⁴Total EAA.

Table 7. Fatty acid composition (% of total fatty acids) of oriental mustard bran (MB), control diet (CON), and MB diet in experiment 2

Fatty acid	MB	TMR	
		CON	MB
14:0	0.09	0.35	0.35
14:1	0.00	0.25	0.20
15:0	0.00	0.07	0.07
16:0	4.28	17.04	15.83
16:1	0.65	0.26	0.47
17:0	0.00	0.10	0.11
18:0	1.72	3.70	3.15
18:1 <i>trans</i> -9	0.19	0.00	0.00
18:1 n-9	19.53	42.31	28.85
18:1 n-7	3.59	1.08	2.22
18:2	23.46	53.27	49.90
18:3	10.34	8.58	10.08
18:4	0.00	0.13	0.12
20:0	1.00	0.74	0.93
20:2	1.13	0.00	0.44
20:1 n-9	11.04	0.87	4.26
20:3 <i>trans</i> -3	0.24	0.00	0.00
22:0	0.64	0.94	0.85
22:1 n-9	23.17	0.94	7.90
22:2 n-6	0.59	0.56	0.64
24:0	0.53	1.27	1.21
24:1 n-9	1.69	0.07	0.63

were not affected when mustard meal was fed to dairy cows (Moss, 1975).

Organoleptic Evaluation. No taste or odor differences ($P \geq 0.07$) of milk from cows fed the CON or the MB diets were observed (Table 4). The taste panel was unable to differentiate milk from cows fed the CON diet or the MB diet. No objectionable taste or offensive odors of any of the milk samples were detected. Similar observations were reported by Moss (1975) when mustard meal was feed to lactating Holstein cows.

Experiment 2

Nutrient Content of Diets. The chemical composition of forages, MB, and TMR is presented in Table 5. The chemical content of experimental diets was similar. The AA composition of MB is presented in Table 6. Concentrations of essential AA as percentages of DM were lower compared with those of SBM; however, as percentages of CP, lysine, and methionine were very close to those of SBM (NRC, 2001). This AA makeup would complement corn protein in a corn-based diet well. The fatty acid composition of MB and TMR is presented in Table 7. The fatty acid content of the MB diet was greater for *cis*-vaccenic (18:1 n-7), linolenic (18:3 n-3), arachidic (20:1 n-9), and erucic (22:1 n-9) acids than those of the CON diet. Brassicas are known to contain high level of erucic acid (DePeters and Bath, 1986). The oleic (18:1 n-9) and linoleic (18:2) content was much greater in the CON than in the MB diet. The

high linoleic acid in the CON diet was due to sunflower seed, which has higher linoleic acid (68.6%) content (Peng et al., 2010) than does MB (23.46%). The MB had an in vitro DM digestibility of 66.8% and an in vitro NDF digestibility of 81.6%.

DMI, Milk Yield, and Composition. During experiment 2, many cows fed the MB diet experienced minor to severe hemolysis with bloody urine. Four first-lactation cows, with severe hemolysis that appeared after wk 4 of the experiment, were removed from the experiment and replaced by new cows of same parity and similar production level. Three older cows and one first-lactation cow with minor hemolysis were kept in the study; however, they appeared not to be cured from the hemolysis over the feeding period. Brassicas contain S-methyl-cysteine sulfoxide (Gustine, 1985; Fales et al. 1987; Lambert et al. 1987), which is converted by rumen microorganisms to the toxic S-methyl cysteine dimethyl disulfide. This disulfide was shown to cause hemolytic anemia in cattle and sheep (Gustine, 1985). The nature of the hemolysis described by Gustine (1985) is very similar to the one observed in experiment 2.

In the short-term switchback study (experiment 1) with 28-d feeding periods, cows did not show any apparent sign of hemolysis. In the long term study (experiment 2), cows did not show hemolysis until after 4 wk in the experiment. The lack of hemolysis in cows used in the short-term study could be because of insufficient exposure and conversion rate of S-methyl cysteine sulfoxide to the toxic dimethyl disulfide. The level of S-methyl-cysteine sulfoxide in the MB and its conversion rate to its toxic form in the rumen is not known. We consulted with several analytical laboratories and were unable to find one that was currently measuring S-methyl-cysteine sulfoxide.

Dry matter intake was greater ($P = 0.03$) for cows fed the MB diet (Table 8); however, intake interacted ($P = 0.004$) with parity, where multiparous cows fed MB ate more feed than did cows fed CON and primiparous cows were not different (Table 9). However, in experiment 1, MB did not affect DMI. No palatability problem was observed with mustard meal diets fed to ruminants (Peterson, 1970), swine (Peterson and Danielson, 1972), beef cattle (Peterson and Thomas, 1970; Thomas, 1972), and dairy cows (Moss, 1975). Mustard is in the family of brassicas (e.g., rapeseed, turnip, cabbage, radish, kale, and cauliflower). Brassicas contain toxic or anti-nutritional factors such as erucic acid (DePeters and Bath, 1986), and glucosinolate (Bell, 1984; Fales et al. 1987; Maesoomi et al. 2006), precursors of thiocyanate goitrogenic compounds. A high DMI intake of the MB diet over the CON diet is an indication that the MB diet was palatable and apparently the anti-nutritional factors of mustard reported in these

Table 8. Intake and milk composition for lactating dairy cows fed control (CON) and oriental mustard bran (MB) diets in experiment 2

Item	Treatment (Trt)		SEM	P-value	
	CON	MB		Trt	Trt × parity
Cows, n	11	11			
DMI, kg/d	20.4	22.0	0.50	0.03	0.004
Milk production					
Milk, ¹ kg/d	41.0	41.8	0.82	0.48	0.007
3.5% FCM, ^{1,2} kg/d	42.8	43.5	1.6	0.76	0.17
SCM, ^{1,3} kg/d	39.4	39.9	1.5	0.81	0.23
Milk component					
Fat, %	3.87	3.85	0.12	0.85	0.49
Protein, ¹ %	2.92	2.87	0.02	0.12	0.55
Lactose, ¹ %	4.86	4.89	0.02	0.26	0.006
SNF, ¹ %	8.79	8.74	0.04	0.40	0.07
TS, ¹ %	12.61	12.55	0.13	0.73	0.22
Urea, ¹ mM	17.42	15.52	0.33	<0.001	0.05
SCC, ¹ × 10 ³ /mL	427	348	76	0.46	0.19
Milk component production					
Fat, ¹ kg/d	1.56	1.58	0.07	0.84	0.25
Protein, ¹ kg/d	1.21	1.20	0.05	0.90	0.27
Lactose, ¹ kg/d	2.06	2.10	0.05	0.51	0.29
SNF, ¹ kg/d	3.73	3.86	0.10	0.33	0.32
TS, ¹ kg/d	5.09	5.16	0.20	0.81	0.25
Milk production efficiency					
3.5% FCM/DMI	2.1	1.98	0.14	0.53	0.86

¹Pretrial measures were used as covariates ($P \leq 0.13$).

²3.5% FCM = (0.432 × kg/d of milk) + (16.23 × kg/d of fat) (Britt et al., 2003).

³SCM = (12.3 × kg/d of fat) + (6.56 × kg/d of SNF) – (0.0752 × kg/d of milk) (Tyrrell and Reid, 1965).

studies did not interfere with DMI. Daily milk production and milk components were not different ($P \geq 0.12$) between diets (Table 8); however, an interaction ($P \leq 0.05$) with parity for milk production, milk lactose concentration, and milk urea concentration was observed (Table 9). Multiparous cows fed MB produced more milk than CON, whereas no difference existed between primiparous heifers. The increase in milk produced by multiparous cows consuming MB appears to be related to their increased intake. However, in experiment 1, cows produced more milk and intake was not different. Concentration of lactose was greatest for primiparous cows among the 4 parity-treatment groups. This change was small and is likely to be of little biological importance. Urea concentration was less for multiparous cows

fed MB than CON and no treatment differences were apparent within primiparous cows. The concentrations of milk fat, protein, TS, and SCC were not affected ($P \geq 0.12$) by diet, which is in agreement with experiment 1 data. The 3.5% FCM production efficiency ratio was not different between treatments (2.01 vs. 1.98 kg of 3.5% FCM/kg of DMI for CON and MB, respectively).

The fatty acid profile of milk fat was altered by MB (Table 10). The proportion of monounsaturated fatty acids was increased ($P = 0.02$) in the milk from cows fed the MB diet due primarily to more C18:1 *cis*-6 (petroselenic), C18:1 *trans*-11 (vaccenic), and C22:1 (erucic). Vaccenic acid has anticarcinogenic effect in human foods, mediated through its conversion to rumenic acid [*cis*-9, *trans*-11 conjugated linoleic acid

Table 9. Intake and milk composition for the interaction between treatment and parity (T × P) for lactating dairy cows fed control (CON) and oriental mustard bran (MB) diets in experiment 2

Item	Heifer		Cow		SEM ¹	P-value		
	CON	MB	CON	MB		Treatment	Parity	T × P
Cows, n	3	3	8	8				
DMI, kg/d	19.0	18.3	21.8	25.8	0.86	0.03	<0.001	0.004
Milk, kg/d	39.8	37.2	42.2	46.3	1.49	0.82	0.001	0.007
Lactose, %	4.85	4.96	4.88	4.82	0.03	0.26	0.08	0.006
SNF, %	8.83	8.89	8.76	8.60	0.08	0.40	0.02	0.07
Urea, mM	16.73	15.83	18.13	15.21	0.56	<0.001	0.42	0.05

¹SEM associated with an n of 3.

Table 10. Effect of mustard bran on fatty acid composition of milk in experiment 2

Fatty acid, g/100 g of fatty acid	Treatment		SEM	P-value
	Control	Mustard bran		
4:0	1.59	1.64	0.04	0.45
8:0	1.10	1.16	0.04	0.32
10:0	2.33	2.36	0.17	0.88
11:0	0.27	0.27	0.01	0.81
12:0	3.08	3.17	0.15	0.67
14:0 ¹	11.09	10.86	0.34	0.63
14:1 ¹	0.79	0.77	0.04	0.83
15:0	1.05	0.99	0.03	0.22
16:0 ¹	24.35	23.17	0.78	0.28
16:1 <i>cis</i> ¹	1.55	1.47	0.06	0.34
16:1 <i>trans</i>	0.32	0.34	0.02	0.53
17:0 ¹	0.55	0.56	0.01	0.50
17:1 ¹	0.16	0.18	0.01	0.24
18:0	12.35	12.01	0.52	0.65
18:1 <i>cis</i> -6	0.31	0.72	0.10	0.01
18:1 n-9 <i>cis</i> ¹	24.37	24.39	0.72	0.98
18:1 <i>cis</i> -11	0.68	0.70	0.02	0.12
18:1 <i>trans</i> -11 ¹	3.01	3.50	0.08	<0.001
18:2 n-6 <i>cis</i> ¹	2.75	2.98	0.05	0.006
18:2 n-6 <i>trans</i>	0.30	0.30	0.01	0.82
18:2 <i>cis</i> -9 <i>trans</i> -11 ¹	0.66	0.65	0.05	0.88
18:2 <i>trans</i> -10, <i>cis</i> -12 ¹	0.002	0.009	0.001	0.001
18:3 n-3	0.015	0.023	0.002	<0.0001
18:3 n-6	0.34	0.51	0.01	0.03
20:0	0.14	0.39	0.01	<0.001
20:4 n-6	0.13	0.13	0.01	0.88
22:0 ¹	0.054	0.110	0.002	<0.001
22:1 n-9 ¹	0.01	0.23	0.01	<0.001
Saturated ¹	58.41	57.66	1.14	0.63
Monounsaturated ¹	35.33	37.46	0.57	0.02
Polyunsaturated ¹	4.65	4.92	0.17	0.27
n-3 ¹	0.42	0.57	0.02	<0.001
n-6 ¹	3.50	3.71	0.10	0.16
Total, mg/g of DM	318	323	13	0.80

¹Pretrial measures were used as covariates ($P \leq 0.15$).

(CLA)] (Lock et al., 2004). Erucic acid is believed not to have a nutritional value (DePeters and Bath, 1986). The proportion of one CLA isomer (C18:2, *trans*-10, *cis*-12) was increased ($P < 0.001$). The increase in milk C18:2 *trans*-10, *cis*-12, a potent inhibitor of milk fat synthesis (McGuire and McGuire, 2000; Bauman and Griinari, 2003), was not great enough to suppress milk fat synthesis in cows fed the MB diet. The rumenic acid, reported to be the only CLA shown unequivocally to inhibit carcinogenesis in experimental animals (Kelly et al., 1998), was not changed by diet. The proportion of α -linolenic acid (C18:3 n-3), and gamma-linolenic acid (C18:3 n-6) was increased ($P \leq 0.03$) by the MB diet. The n-3 fatty acids were also increased ($P < 0.001$) in the milk of cows fed the MB diet, which are believed to have an anticarcinogenic effect in human diets (Gleissman et al., 2010). Diets did not affect ($P \geq 0.37$) the proportion of short-, medium-, or long-chain fatty acids (data not shown). Changes in fatty acids profile did not affect milk fat percentage and yield.

CONCLUSIONS

In experiment 1, adding 8% MB to lactating cow diets increased daily milk production by 3.2 kg, but did not affect DMI, percentages and production of milk fat, protein, lactose, SNF, and TS, or urea N or SCC. Milk organoleptic qualities (odor and taste) were not affected by feeding MB to dairy cattle. In experiment 2, feeding a diet containing 8% of MB to lactating cows increased DMI only in multiparous cows, with corresponding changes in milk production; however, component yields were not affected by feeding MB. But MB presents some danger of hemolysis to lactating dairy cows. Future studies could explore safe levels of MB and S-methyl cysteine sulfoxide in dairy cattle diets, and further investigate nutrient digestibility of MB.

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