Influence of *Mentha×piperita* L. (Peppermint) Supplementation on Nutrient Digestibility and Energy Metabolism in Lactating Dairy Cows

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ABSTRACT : The characteristic smell of cow milk was suppressed when herbs were consumed by lactating dairy cows. But it is unclear whether or not peppermint ingestion affects the nutritional and milk production parameters in lactating dairy cows. The objective of this study was to examine the effect of peppermint feeding to lactating dairy cows on nutrient digestibility, energy metabolism, ruminal fermentation and milk production. Eight Holstein cows were given a diet supplemented with or without 5% of dried peppermint per diet on a dry matter basis. The digestion of nutrients from cows fed the diet with peppermint was significantly lower than that of the control group. Energy loss as methane and methane released from cows receiving the peppermint treatment was significantly lower than that in the control cows. Peppermint feeding to cows resulted in the promotion of thermogenesis. However, ruminal fermentation and milk production were not affected by peppermint feeding. In conclusion, peppermint ingestion by lactating dairy cows reduces the nutrient digestibility and methanogenesis, and changes energy metabolism. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 12 : 1721-1726)***

Key Words : Dairy Cows, Peppermint, Digestibility, Methane, Energy Metabolism, Milk Production

INTRODUCTION

Herbs have been used to remove the smell from meat or fish and to flavor food while cooking. Ando et al. (2001) demonstrated that when dried herbs were consumed by lactating dairy cows, the components peculiar to such herbs were transferred to the cows' milk and the characteristic smell of cow milk was suppressed. People who dislike milk may tolerate it when the cow has ingested herbs, because more than half of people who dislike milk object to its smell and flavour in Japan (The National Dairy Promotion and Research Association, 2000).

We previously examined which herb is acceptable by cattle and effective to suppress the characteristic smell of cow milk in panel test with various herbs offered to dairy cattle. As a result of these studies, *Mentha×piperita* L. (peppermint) proved to be one of the suitable herbs for dairy cattle supplement among common herbs (unpublished). However, to develop a technique for controlling or suppressing the milk flavor by feeding peppermint requires knowledge of how digestion, metabolism, and milk production will be affected in lactating dairy cows. Peppermint has been reported to have antimicrobial activities *in vitro* (Pattnaik et al., 1996; Montes-Belmont and Carvajal, 1998; Imai et al., 2001) and pharmacological activities on digestive organs in monogastric animals (Leicester and Hunt, 1982; May et al., 2000; Kline et al., 2001).

Recently, Ando et al. (2003) reported that peppermint feeding to steers changed the concentration of ruminal ammonia-nitrogen and the number of protozoa but did not affect nutrient digestibility. However, little information on the effect of peppermint feeding to lactating cows is available. Therefore, the influence of peppermint as a feed supplement in lactating dairy cows on nutrient digestibility, energy metabolism, ruminal fermentation, and milk production was investigated.

MATERIALS AND METHODS

The animals were cared according to the Guide for the Care and Use of Experimental Animals (Animal Care Committee, National Institute of Livestock and Grassland Science) based on Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (Curtis and Nimz, 1988).

Animals and their management

Eight multiparous, late-lactation Holstein cows [body weight, 593.1±16.1 kg; milk yield, 29.1±4.1 kg; days in milk, 231.1±12.5; parity, 3.1±0.8 (mean±SD)] were tethered in stalls and had free access to water. Eight cows were randomly assigned to two dietary treatments: 1) diet only (control), and 2) diet supplemented with 5% of peppermint per diet (peppermint treatment) on dry matter basis. The diet consisted (dry matter basis) of first-cut Italian ryegrass hay (40%) and concentrate (60%) containing concentrate

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Table 1. Ingredient and nutrient composition of the diet and peppermint

Diet	%
Ingredient composition (DM)[1]	
Italian ryegrass hay	40.0
Concentrate mix pellet [2]	22.4
Flaked maize	19.0
Soybean meal	16.5
Mineral mix [3]	1.5
Vitamin mix [4]	0.1
Salt	0.5
Nutrient composition (DM)	
Organic matter	93.0
Crude protein	16.5
Neutral-detergent fiber	34.7
Acid-detergent fiber	19.1
Ether extracts	2.4
Peppermint	
Dry matter	90.4
Nutrient composition (DM)	
Organic matter	87.3
Crude protein	23.7
Neutral-detergent fiber	32.9
Acid-detergent fiber	21.0
Ether extracts	3.8

[1] DM: Dry matter.
[2] Used the same lot during the experiment. Contained (per kg) 500 g of grain (milo, maize, and barley), 200 g of bran (wheat and rice), 80 g of soybean meal.
[3] Contained (per kg) 110 g of P, 220 g of Ca, 100 g of Mg.
[4] Contained (per kg) 1,200 mg of vitamin A, 10 mg of vitamin D, and 20,000 mg of DL-α-tocopherol acetate.

mix pellets, flaked maize, soybean meal, mineral mix, vitamin mix, and salt (Table 1). Cows received a diet to meet the lactating cow requirements of the Japanese Feeding Standard for Dairy Cattle (Agriculture, Forestry and Fisheries Research Council Secretariat, 1999) twice daily at 09:30 and 18:00 h in equal amounts. The concentrate and forage were offered separately. Peppermint (Table 1, sun-dried, imported from Egypt, purchased from Kaneka Sun Spice co., Ltd., Osaka, Japan) was mixed with the concentrate and given to the animals.

The cows were milked twice daily at 09:00 and 17:30 h, and individual milk yields were recorded. Milk samples were collected at each milking and analyzed for fat, protein, lactose, and solids-not-fat by Milko-Scan 133B (N. Foss Electric, Denmark). Each milk sample was individually mixed in proportion to the milk yields, and mixed samples were used for a determination of gross energy (GE).

Balance trial and sample collection

The experimental period was 22 days, with the first 14 days for adaptation and the last 8 days as the test period. The balance trial was conducted to investigate whole tract digestibility by the collection of total feces and urine using a feces-urine separating machine an 8-day test period. The feces and urine were recovered from the machine and the outputs were recorded every morning. The samples (100 g/kg) were collected and stored at -20°C until use. Each fecal and urinary sample was individually mixed, and used for chemical analysis. For 2 days of the test period (except the calibration time for analyzers), a head hood-type respiration chamber was used to measure methane and carbon dioxide production and oxygen consumption. Air collected from the respiration chamber was analyzed every 10 seconds to determine the concentrations of methane, carbon dioxide, and oxygen with a methane infrared gas analyzer (ZRF1EGY1, Fuji Electric Co., Ltd., Tokyo, Japan), a carbon dioxide infrared gas analyzer (ZRF1DKY1, Fuji Electric Co., Ltd.), and a paramagnetic oxygen analyser (Model No.490, P. K. Morgan Ltd., Kent, UK), respectively. Gas analyzers were calibrated using span gases as reference every morning. Air flow (L/min) was measured using flow meter (TBX100F, Aichi tokei denki co., Ltd, Okazaki, Japan) and converted to the standard condition at 0°C (temperature) and 760 mmHg (pressure).

Ruminal fluid was collected via the mouth using a rumen catheter (SANSHIN INDUSTRIAL Co., Ltd., Yokohama, Japan) just before and 3 hour after the morning meal on last day of the test period. The pH of the ruminal fluids was measured with a pH meter (D-24, HORIBA, Ltd., Tokyo, Japan). Ruminal fluids were separated from the feed particles through four layers of gauze, and centrifuged at 1,200×g for 15 min. The supernatants were added with a perchlorate solution to deproteinize and then stored at -20°C until the assay.

Chemical analyses and calculations

Dry matter (DM) of feed was examined by drying the sample at 100°C for 18 h, and that of feces was examined by drying at 135°C for 2 h after at 60°C for 24 h. Wet feces were used for analyzing nitrogen. Feed and feces samples were dried at 60°C and then ground (1 mm) prior to analysis. Crude protein (CP), ether extract (EE), crude ash (AOAC INTERNATIONAL, 2000), neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) (Van Soest et al., 1991) were determined. Organic matter (OM) was calculated as the weight loss upon ashing. The GE of sample was determined using an auto-calculating bomb calorimeter (CA-4PJ, Shimadzu, Kyoto, Japan). Urine and milk samples for GE analysis were lyophilized before use.

Deproteinized ruminal fluid samples were neutralized with potassium hydroxide solution and centrifuged at 400×g for 10 min. The supernatants were applied to ammonia (Weatherburn, 1967) and volatile fatty acid (VFA) analyses. Volatile fatty acid analysis was performed using the high performance liquid chromatograph (HPLC) organic acid analysis system (Shimadzu). The supernatants were shaken with cation exchange resin (Amberlite, IR 120B H AG,

Table 2. Apparent digestibility of nutrient composition in cattle fed a diet with or without peppermint (%)

	Treatment		SEM	Significance
	Control	Peppermint		
Dry matter	70.3	66.7	0.7	*
Organic matter	72.6	69.2	0.7	*
Crude protein	69.1	59.8	2.3	*
Neutral-detergent fiber	55.8	51.7	1.0	*
Acid-detergent fiber	55.8	51.6	1.1	*
Ether extracts	73.9	70.5	1.8	
Gross energy	70.3	66.5	0.7	*

Statistical significance * $p<0.05$. SEM: Standard error of mean.

Table 3. Energy intake, partition and utilization in cattle fed a diet with or without peppermint

	Treatment		SEM	Significance
	Control	Peppermint		
Energy intake (kJ/kg metabolic body size/day)				
Gross energy (GE)	2,836.2	3,010.6	119.8	
Digestible energy	1,993.3	1,999.4	72.2	
Metabolizable energy (ME)	1,736.6	1,755.7	70.1	
Partition of GE (%)				
Feces	29.7	33.5	0.7	*
Urine	2.1	2.0	0.1	
Methane	6.9	6.2	0.2	*
Milk	25.5	24.6	0.8	
Heat production	34.1	36.7	0.8	($p = 0.06$)
Retention	1.6	-3.0	1.1	*
Partition of ME (%)				
Milk	41.7	42.2	1.4	
Heat production	55.8	62.9	1.4	*
Retention	2.5	-5.1	1.9	*
Metabolizability (ME/GE)	0.612	0.584	0.006	*

Statistical significance * $p<0.05$. SEM: Standard error of mean.

ORGANO CORPORATION, Tokyo, Japan) and centrifuged at 6,500×g for 5 min. The supernatants were passed through a 0.45 μm filter under pressure, and filtrates were then injected into an HPLC system. The analytical conditions were as follows: column, SCR-101H (7.9 mm×30 cm) attached to a guard column SCR(H) (4.0 mm×5 cm) (Shimadzu); oven temperature, 40°C; mobile phase, 4 mM *p*-toluenesulfonic acid aqueous solution; reaction phase, 16 mM Bis-Tris aqueous solution containing 4 mM *p*-toluenesulfonic acid and 100 μM ethylenediaminetetraacetic acid; flow rate of the mobile and reaction phase, 0.8 ml/min; detector, conductivity detector (CDD-6A, Shimadzu).

Methane energy was calculated as 39.45 kJ/L (Brouwer, 1965). Heat production (kJ/day) was calculated using Brouwer's formula: $16.18\times O_2$ (L/day)+$5.02\times CO_2$ (L/day)-$2.17\times CH_4$ (L/day)-$5.99\times N$ (g/day) (Brouwer, 1965). Energy retention (MJ/day) was calculated using the following formula: metabolizable energy (ME)-energy loss as milk-heat production.

Statistical analysis

Statistical analysis of data was performed by ANOVA with dietary treatment as a factor (SAS Institute, 1988). Data was shown as means and standard error of mean.

RESULTS

Nutrient digestibility

Table 2 shows the digestibility of nutrients in lactating dairy cows fed a diet with or without peppermint supplementation. The digestibilities of all nutrients, i.e., DM, OM, CP, NDF, ADF and GE, in the peppermint treatment group were significantly lower ($p<0.05$) than those in the control group, except for that of EE.

Energy utilization

There were no significant differences in GE, digestible energy, and ME intake between the control and peppermint treatment groups (Table 3). The fecal energy loss was higher ($p<0.05$) in the peppermint treatment group than that in the control. The energy partitions from GE intake to urine or milk were unaffected by peppermint feeding. Energy loss as methane in cows fed peppermint was significantly lower ($p<0.05$) than that in the control cows. The percentages of heat production to GE and ME in peppermint treatment group were higher ($p = 0.06$ and $p<0.05$) than in the control.

Table 4. Methane release by cattle fed a diet with or without peppermint

	Treatment		SEM	Significance
	Control	Peppermint		
Methane release				
(L/day)	598.9	560.5	18.1	
(L/kg DMI)[1]	32.6	29.0	0.8	*
(L/kg DOMI)[2]	48.2	45.0	1.0	(p = 0.08)

Statistical significance * $p<0.05$. SEM: Standard error of mean.
[1] DMI: Dry matter intake.
[2] DOMI: Digestible organic matter intake.

Peppermint supplementation to the dairy cow feed resulted in negative energy retention, which was significantly lower ($p<0.05$) than that of the control. The metabolizability (ME/GE) of cows receiving the peppermint treatment was lower ($p<0.05$) than that in the control.

Methane production

There was no significant difference in methane release (L/day) between the control and peppermint treatment groups. However, methane releases per dry matter intake and digestible organic matter intake from cows fed peppermint were lower ($p<0.05$ and $p = 0.08$) than those from the control group (Table 4).

Ruminal fermentation

Ruminal pH and ammonia concentration were not affected by peppermint feeding (Table 5). The concentration of total VFA and the molar rate of acetate, propionate, and butyrate in peppermint treatment group were not different form those in the control group. There was no difference in the acetate to propionate ratio of ruminal fluid between the control and peppermint treatment groups.

Milk production

The yield of average milk and 4% fat-corrected milk in cows received peppermint treatment were not different from those in the control cows (Table 6). The compositions of the milk, i.e., fat, protein, lactose, and solids-not-fat, were unaffected by peppermint supplementation to the feed.

Table 6. Average milk yield and composition in cattle fed a diet with or without peppermint

	Treatment		SEM	Significance
	Control	Peppermint		
Milk yield (kg/day)	28.6	29.5	2.2	
FCM yield (kg/day)[1]	28.1	28.6	2.0	
Composition (g/kg)				
Fat	38.8	38.1	1.5	
Protein	32.4	32.6	1.1	
Lactose	43.1	44.6	0.7	
Solids-not-fat	85.6	87.1	1.1	

SEM: Standard error of mean. [1] FCM: 4% fat-corrected milk.

DISCUSSION

Although feeding herbs to dairy cows resulted in improvements in the flavor of milk (Ando et al., 2001), experiments had not been conducted to determine the effect of feeding peppermint to lactating dairy cows on nutritional and milk production parameters. Therefore, in the present study, the use of dried peppermint as a feed supplement on lactating dairy cows and its effects on nutrient digestibility, energy metabolism, ruminal fermentation, and milk production were investigated.

The digestibilities of DM, OM, CP, NDF, ADF, and GE in cows fed the diet additionally supplemented with peppermint were lowered in comparison with that of cows fed no peppermint in the present study (Table 2). These results may be explained by potent antimicrobial activity of peppermint (Pattnaik et al., 1996; Montes-Belmont and Carvajal, 1998; Imai et al., 2001), which may have decreased ruminal microbial activity involved in nutrient

Table 5. Ruminal fermentation in cattle fed a diet with or without peppermint before and 3h after morning feeding

	Time[1]	Treatment		SEM	Significance
		Control	Peppermint		
Ruminal pH	0 h	6.8	6.8	0.1	
	3 h	6.4	6.4	0.1	
Ammonia (mg/100 ml)	0 h	10.6	10.1	1.4	
	3 h	10.8	8.1	1.1	
Total volatile fatty acids (VFA) (mmol/L)	0 h	74.6	74.6	8.0	
	3 h	95.9	88.9	5.8	
Molar rate of VFA (%)					
Acetate	0 h	67.5	67.6	0.4	
	3 h	65.2	67.6	1.0	
Propionate	0 h	18.0	19.0	0.5	
	3 h	19.5	19.6	0.4	
Butyrate	0 h	10.6	9.8	0.7	
	3 h	12.4	10.3	1.2	
Acetate to propionate ratio	0 h	3.8	3.6	0.1	
	3 h	3.3	3.5	0.1	

SEM: Standard error of mean. [1] Time after morning meal.

digestibilities. However, Ando et al. (2003) reported that feeding peppermint had no effect on nutrient digestion, which does not agree with our findings. In this study, we fed cattle peppermint at a concentration of 5% per diet, and Ando et al. (2003) used peppermint at about 2.9% per diet (6 kg timothy hay, 1 kg concentrate, and 0.2 kg peppermint per day). This difference in feeding rate of peppermint may contribute to these different results. Moreover, the present study suggests that the allowable upper limit of peppermint in feed, which would cause no negative effects in nutrient digestibility, was exceeded.

For lactating dairy cows fed a diet with peppermint, the ratio of heat production to ME intake was increased, although ME intakes in two treatments were not different (Table 3). This finding demonstrates that peppermint has an activity that promotes heat production in dairy cows. Peppermint has been used as a traditional folk remedy for indigestion, nausea, sore throat, diarrhea, colds, headaches, toothaches and cramps (Leung and Foster, 1996), and is also believed to promote perspiration in human, which offers support for the results in this experiment. In addition, this finding also indicates that the substance(s) in peppermint that produces pharmacological activity, such as heat-generating activity, was absorbed from the digestive tract, escaping from decomposition or metabolism, and exerted an influence in the body of the ruminant animal.

The percentage of energy loss as methane to GE intake and the methane production (L/kg DM intake) in cows receiving the peppermint treatment were significantly lowered in comparison with those in control cows (Tables 3 and 4), showing that peppermint fed to dairy cows suppresses methanogenesis. Whitelaw et al. (1984) observed that the absence of ruminal ciliate protozoa led to a reduction of methane release from cattle, which supports our findings because peppermint fed to cattle decreases the number of protozoa in rumen (Ando et al., 2003). Whereas, the decrease in methane production from ruminant animals has been reported to be accompanied with an increase in the molar proportion of propionate per total VFA (Thivend and Jouany, 1983; Whitelaw et al., 1984; Kung et al., 2003) because hydrogen is required for methane synthesis in rumen. In the present study, the molar ratio of propionate and acetate to propionate ratio in cows fed the diet with peppermint did not increase despite the fact that daily production of methane was significantly inhibited. Therefore, the regulatory mechanism of peppermint feeding for the reduction of methane production in ruminant animals remains unclear.

Ingestion of peppermint had no effect on milk yields and compositions (Table 6), indicating that the absorbed component(s) of peppermint that has a pharmacological activity does not affect the syntheses of milk and its components. However, energy retention for cows fed peppermint was negative; therefore, long-term feeding of peppermint at allowance level in the present study may result in a decrease in the milk yield and composition.

In conclusion, the results in the present study are the first to show that peppermint added to feed at 5% per diet for lactating dairy cows decreases nutrient digestibility and methane production. Peppermint has a component(s) containing an activity that promotes heat generation. This component(s) is absorbed and then acts internally in dairy cows, suggesting that peppermint ingestion alters energy metabolism and partition in ruminants. However, at this point, not enough is known to establish a technique to control the flavor of milk by feeding herb(s) to cows. More research will be necessary to fully understand the effects of feeding peppermint to lactating cows on their digestion, metabolism, and milk production.

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