Effects of Probiotic-vitacogen and β1-4 Galacto-oligosaccharides Supplementation on Methanogenesis and Energy and Nitrogen Utilization in Dairy Cows

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ABSTRACT : The effects of probiotic-vitacogen and galacto-oligosaccharides (GOS) supplementation on methanogesis, energy and nitrogen utilization in replacement dairy cows were evaluated. A basal diet comprising orchardgrass hay, lucerne hay cube and concentrate (2:2:1, DM basis) were fed with or without supplements to four cows at 80 g DM /kgBW$^{0.75}$ per day in a 4×4 Latin square arrangement. The four treatments were; 1) basal diet, 2) basal diet plus 100 g probiotic-vitacogen, 3) basal diet plus 50 g GOS, 4) basal diet plus 50 g GOS and 100 g probiotic-vitacogen. Nutrient apparent digestibility was not altered by the effect of supplementation. Nitrogen intake was significantly ($p<0.001$) higher for the two vitacogen-supplemented diets compared to control and GOS supplemented diets. However, vitacogen supplemented diets had numerically higher fecal and urinary nitrogen losses, thereby, having lower nitrogen retention compared to control and GOS supplemented diets. Gross energy intake was also significantly ($p<0.05$) higher for vitacogen-supplemented diets compared to control and GOS diets, however, due to higher losses in feces, urine, methane and heat, GOS supplemented diet had numerically higher energy retention. There was an 11% reduction in methane emission (liters/day) in GOS supplemented diet compared to control diet. However, the combination of GOS with vitacogen resulted in an increased methane emission. When expressed per unit of animal production (g/kg live-weight gain), methane production tended to be lower in vitacogen-supplemented diets compared to control and GOS diets. The supplementation of replacement dairy cows with GOS reduced methane emission (liters/day), while, vitacogen supplementation reduced methane emission per unit animal production. The two feed supplements may contribute to the abatement of methane as a greenhouse gas. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 349-354)***

Key Words : Methanogenesis, Probiotics, Galacto-oligosaccharides, Cows

INTRODUCTION

Methane emissions from ruminant animals do not only reduce the energy utilization by animals, but also contributes to the greenhouse gas effect. Methane production by cattle typically accounts for 5.5 to 6.5% of gross energy intake (Johnson and Ward, 1996). Johnson et al. (2001) reported that due to their large population, size and appetite, ruminants produce about 77 Tg of methane enterically and 7 Tg from manure annually. Modifications of rumen fermentation in order to reduce methane production by dietary or chemical (e.g Ionophores) means have been reported (Mathison et al., 1998; Hegarty, 1999). Addition of microbial feed supplements to ruminant diets is known to alter rumen fermentation and increase livestock productivity. However, results have been variable and inconsistent. The reported positive effects include increased feed intake (Phillips and VonTungeln, 1985; Wohlt et al., 1998), weight gain (Fallon and Harte, 1987; Abe et al., 1995) and milk production (Hoyos et al., 1987; Teh et al., 1987).

Galacto-oligosaccharides (GOS) are non-digestible carbohydrates resistant to gastrointestinal enzymes, but are selectively utilized by bifidobacteria. The GOS have been used as a prebiotic food ingredient in Japan and show bifidogenic effects in human studies (Sako et al., 1999). Beneficial effects to humans include increased energy value (Ohtsuka et al., 1990), improvement of intestinal microbial ecology and host physiology (Ishikawa et al., 1995) and elimination of ammonia (Tamai et al., 1992).

Gamo et al. (2002) observed a reduction in methane production in vitro with GOS supplementation.

This study evaluated the effects of a probiotic-vitacogen and β1-4 galacto-oligosaccharides supplementation on methanogenesis, energy and nitrogen utilization in replacement dairy cows fed on orchardgrass hay, lucerne hay cube and concentrate in 2:2:1 proportion on DM basis.

MATERIALS AND METHODS

Animals

Four Holstein dairy cows of 10 months old with initial weight of 273.5±20.66 kg were used in a 4×4 Latin square design. The four treatment diets were; a basal diet comprising orchardgrass hay, lucerne hay cube and concentrate in 2:2:1 proportions on DM basis and a basal diet fed with either 50 g GOS, 100 g vitacogen or a combination of 50 g GOS and 100 g vitacogen. Since GOS

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Table 1. Chemical composition and energy content of orchard grass hay, lucerne hay cube, concentrate, and the mixed ration on dry matter basis

	Orchard grass hay	Lucerne hay cube	Concentrate[1]	Mixed diet[2]
Dry matteer	85.9	88.0	88.9	87.4
Crude protein	12.1	16.0	19.0	15.0
NDF	56.3	40.8	19.0	42.6
ADF	34.5	32.5	6.2	28.0
Hemicellulose	21.8	8.3	12.9	14.6
GE (kcal/g DM)	4.41	4.39	4.40	4.40
Ingredient composition of concentrate				
Cereals	52%	Heat treated corn, maize, soybeans and rye		
Oils	34%	Soybean oil		
Bran type	8%	Corn gluten feed		
Others	6%	Alfalfa meal, molasses, $CaCo_3$ powder, dicalcium phosphate, salt, malt, yeast, lactic acid bacteria, bacillus, streptococcus and minerals and vitamins[3]		

[1] Commercially prepared by Chubu Co. Ltd. Hokkaido, Japan. [2] Mixed diet: 40% orchard grass, 40% Lucerne hay cube and 20% concentrates.
[3] 34,350 IU of vitamin A/kg, 6,870 IU of vitamin D3/kg, 46 mg of vitamin E/kg, 229 mg/kg of Zn, 126 mg of Mn, 69 mg/kg of Fe, 33 mg/kg of Cu, 2.6 mg/kg of I, 0.8 mg/kg of Co, 0.46 mg/kg of Se.

products contain 40% lactose and 19% glucose, the equal amounts were added to a control diet as well as to vitacogen supplemented diets in order to eliminate the effect of lactose and glucose contained in GOS product. Cows were housed in individual metabolism cages with stanchions and equipped with a ventilated respiratory gas collection hood. Feces and urine were separated automatically. The animals were fed a mixed ration at 80 g DM/kgBW$^{0.75}$ per day in two equal meals at 07:00 and 18:00 h. Supplements were top-dressed on the basal rations. Fresh drinking water and mineral blocks containing Fe 1,232; Cu 150; Co 25; Zn 500; I 50; Se 15 and Na 382 mg /kg were always available to the animals.

Supplements

A probiotic, vitacogen (Hi-vitacogen, Seiwa, Co. Ltd., Mie, Japan) is a fermented product containing several species of bacteria and yeasts in a powder form. Wakita et al. (1987) reported a microbial total count to be 10^9/g after a direct microscopic examination count of vitacogen. Twenty-nine non-pathogenic bacterial species belonging to nine genera (*Micrococcus, Staphylococcus, Pediococcus, Leuconostoc, Paracoccus, Streptococcus, Lactobacillus, Gluconobacter and bacillus*) were identified with 26 to 35 species of yeast in vitacogen.

Galacto-oligosaccharides (Yakult, Co. Ltd., Tokyo, Japan) were synthesized enzymatically from lactose by transgalactosidase activity of β-galactosidases derived from *Aspergillus oryzae* or *Streptococcus thermophilus*. In a standardized industrial production process, using β-galactosidase derived from *Bacillus circulans*, more than 55% of lactose is converted to GOS (Ishikawa et al., 1995).

Experimental procedure

Each experimental period consisted of seven days adaptation period succeeded by five days digestion trial and a one-day respiratory trail. All cows were being weighed before and after every experimental period in order to determine the amount of feed to offer, calculate metabolic rates and weight gains.

Digestion trial : During the five-day collection period, feed intake was recorded and a machine device was separating feces and urine output automatically. Urine was collected in dilute sulfuric acid. At the end of each collection period, feces and urine were pooled for each animal and sub samples taken for analysis. Fecal samples were initially dried in a forced air oven at 60°C for 72 h while urine samples were stored in freezers at -20°C. Samples of feed and dried feces were Wiley mill ground (1 mm screen) for dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and energy analyses.

Respiratory trial : Oxygen consumption and carbon dioxide and methane production from each cow were monitored by an open circuit respiratory system using a hood over animal's head (Takahashi et al., 1998). Oxygen and methane concentration in inlet air and exhaust gas from the ventilated hood were analyzed with a paramagnetic oxygen analyzer (model Shimazu MAG-2) and a non-dispersive infrared methane analyzer (model Horiba VIA-300, VIA-500), respectively. Data were pooled into the computer (NEC PC-9801 VM) from the analyzer through an interface at 1 m interval and then standardized automatically at 0°C, 101 kPa and zero water vapour pressure. Heat production was calculated using Brouwer's equation (Brouwer, 1965). Methane volume was converted to mass value using the conversion factor 0.716 g/l (Brouwer, 1965).

Laboratory analyses : The DM, OM and Kjeldahl-N of feed and fecal samples were determined by AOAC (1990) procedures. The NDF and ADF were determined according to Goering and van Soest (1970) as modified by van Soest et al. (1991). Hemicellulose was calculated as differences between NDF and ADF. Energy content in feed, feces and

Table 2. Nutrient digestibility (%) of cows fed basal diet alone or supplemented with GOS, vitacogen or the combination of GOS and vitacogen

	Treatments				SEM	P value
	Control	Vitacogen	GOS	Vitacogen and GOS		
Dry matter	68.2	67.5	68.7	67.3	0.69	0.523
NDF	63.3	62.6	64.4	62.2	0.96	0.454
ADF	57.8	57.1	59.9	57.7	0.99	0.287
Hemicellulose	73.9	73.0	72.9	70.7	1.71	0.630
Nitrogen	72.6	72.4	72.5	71.3	0.70	0.581
Energy	69.6	68.9	69.6	68.6	0.66	0.658

Table 3. Nitrogen utilization (g/kg $BW^{0.75}$ per day) in cows fed basal diet alone or supplemented with GOS, vitacogen or the combination of GOS and vitacogen

	Treatments				SEM	P value
	Control	Vitacogen	GOS	Vitacogen and GOS		
N intake	1.92^{a}	1.96^{b}	1.92^{a}	1.95^{b}	0.001	0.001
Fecal N	0.53	0.54	0.53	0.56	0.01	0.431
Digested N	1.39	1.42	1.39	1.39	0.01	0.550
Urinary N	0.71	0.74	0.71	0.75	0.05	0.884
Retained N	0.68	0.67	0.69	0.64	0.05	0.920

Means within a row with different superscript differ significantly by the P value in that row.

Table 4. Energy utilization (MJ/kg $BW^{0.75}$ per day) in cows fed basal diet alone or supplemented with GOS, vitacogen or the combination of GOS and vitacogen

	Treatments				SEM	P value
	Control	Vitacogen	GOS	Vitacogen and GOS		
Gross energy intake	1.45^{a}	1.50^{b}	1.48^{c}	1.50^{b}	0.001	0.001
Fecal energy	0.45	0.47	0.45	0.47	0.001	0.339
Digestible energy	1.00	1.03	1.03	1.03	0.010	0.974
Urinary energy	0.60	0.61	0.57	0.59	0.002	0.602
Methane energy	0.06	0.06	0.05	0.06	0.004	0.473
Metabolizable energy	0.91	0.91	0.92	0.91	0.012	0.845
Heat production	0.37	0.37	0.37	0.38	0.006	0.422
Retained energy	0.54	0.54	0.56	0.53	0.015	0.700

Means within a row with different superscript differ significantly ($p<0.05$).

freeze-dried urine were determined by adiabatic bomb calorimeter (CA-4P, Shimadzu, Japan).

The nutrient composition of orchardgrass hay, lucerne hay cube and concentrates as well as the ingredient composition of concentrate is presented in Table 1.

Statistical analysis

Data was analyzed in a 4×4 Latin square design using a general linear models procedure of SAS (SAS Inst. Inc., Cary, NC) with the effects of cow, period, and dietary treatment included in the model. In case of significant difference in main effects, contrasts were evaluated and least square means were separated using the least significant differences. The overall means obtained were least square means and were assessed for significant differences at $p<0.05$.

RESULTS AND DISCUSSION

Nutrient digestibility

The apparent nutrient digestibility of DM, NDF, ADF, hemicellulose, nitrogen and energy were not significantly altered by supplementations (Table 2). However, GOS supplemented diets had numerically higher nutrient digestibility compared to the combination of GOS and vitacogen supplementation. There was no additive effect on nutrient digestibility of vitacogen when supplemented with GOS. The lack of responses in nutrient digestibility after the addition of probiotics has been reported (Harrison et al., 1988; Williams and Newbold, 1990; Williams et al., 1991). Probiotics may change bacteria numbers that may influence the rate of fiber digestion but not the digestibility of the diet, which is more related to the physicochemical structure of the feed (Hovell et al., 1986).

Nitrogen utilization

Nitrogen intake was significantly ($p<0.001$) higher in vitacogen-supplemented diets compared to control and GOS supplemented diets (Table 3). The reason for higher nitrogen intake in vitacogen-supplemented diets is the high protein content in the probiotic-vitacogen. However, vitacogen supplemented diets had numerically higher fecal

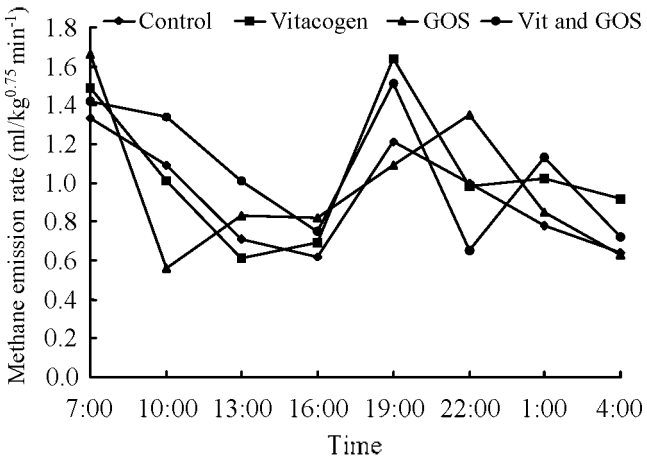

Figure 1. Diurnal changes in methane emission rate by replacement cows fed on orchardgrass hay, lucerne hay cube and concentrates (2:2:1 on DM basis) without supplements (♦), supplemented with vitacogen (■), GOS (•) and vitacogen plus GOS (▲).

and urinary nitrogen losses as compared to control and GOS supplemented diets. Consequently, control and GOS supplemented diets had numerically higher retained nitrogen than vitacogen-supplemented diets.

Energy balance

The gross energy intake was significantly ($p<0.001$) higher for the two vitacogen-supplemented diets compared to the control and GOS supplemented diets (Table 4). However, GOS supplemented diet had significantly ($p<0.05$) higher energy intake compared to control diet. The observed higher energy intakes in supplemented diets were due to higher energy contents in vitacogen and GOS supplements. Energy losses through feces, urine, methane and heat were relatively higher for two vitacogen-supplemented diets compared to control and GOS supplemented diets. Therefore, GOS without vitacogen-supplemented diets had numerically higher energy balance than the control and the two-vitacogen-supplemented diets.

Gaseous exchange

Rates of oxygen consumption and carbon dioxide production were not significantly different between treatments. Methane emissions are plotted against time in Figure 1. Methane emission increased immediately after feeding. There were no supplementary effects of vitacogen, GOS or both on the profile of gaseous exchange.

Methane emission

Synthesis of methane was rapidly stimulated by feeding (Figure 1) and gradually reduced with time. The methane production data is presented in Table 5. Methane emission (liters/day) was reduced by 11% in GOS supplemented diet compared to control diet. However, when GOS was supplemented together with vitacogen methane emission increased by 17.5%. Methane production expressed as methane conversion ratio (MCR %, energy loss as methane per unit of GE intake) by the Intergovernmental Panel on Climate Change (1996) was between 3.6 to 4.2%. The probiotic-vitacogen significantly ($p<0.05$) increased methane production in vitro (Takahashi et al., 2000) and tended to increase methane in sheep (Zhou et al., 2001). The microorganisms in the probiotic-vitacogen may not have suppressed methanogenesis but rather promoted its activity. The GOS, on the other hand, may have enhanced the propionate producers such as *Selenomonas*, *Succinomonas* and *Megasphaera*. Since propionate production is in direct competition with methanogenesis for available hydrogen, the increased molar production of propionate resulted in lower methane emission. In a rumen fermentation trail, Santoso et al. (2003) reported an increase in the proportion of propionate (mol/100 mol of total VFA) in dairy cows fed orchardgrass silage supplemented with GOS. However, methane production when expressed per unit of animal production (i.e. g/kg live-weight gain) was lower ($p>0.05$) for vitacogen-supplemented diets compared to control and GOS supplemented diets due to higher live-weight gain in viatcogen-supplemented diets. Kurihara et al. (1999) observed a curvilinear association between live-weight gain and methane production (g/kg live-weight gain) in Brahman heifers and suggested that methane production can only be reduced for cattle experiencing low live-weight gains. In some in vitro studies (Chaucheyras et al., 1995; Gamo et al., 2002) some strains of lactic acid bacteria and yeast has been shown to reduce methane production. Gamo et al. (2002)

Table 5. Methane emission and live weight gain in cows fed basal diet alone or supplemented with GOS, vitacogen or the combination of GOS and vitacogen

	Treatments				SEM	P value
	Control	Vitacogen	GOS	Vitacogen and GOS		
LWG[1] (kg/day)	0.65	1.24	0.76	1.04	0.301	0.517
Methane production						
L/day	107.0	104.7	95.4	112.1	7.21	0.476
L/DMI[2]	18.75	18.15	16.83	19.55	1.29	0.473
MCR[3]	4.04	3.91	3.61	4.21	0.28	0.520
g/kg live wt gain	179.8	75.7	119.8	111.8	43.05	0.424

[1] Live weight gain. [2] Dry matter intake. [3] Methane conversion ratio (% Methane per gross energy intake).

also reported a significant reduction in methane production with GOS addition in vitro. In contrast Santoso et al. (2002) reported an increase in methane emission in cows fed silage with GOS.

CONCLUSION

Results from the present experiment reveal that methane emission (liters/day) in growing dairy cows could be reduced by the supplementation of the daily ration with galacto-oligosaccharides. Probiotic-vitacogen increases methane emission, but when expressed per unit of animal production (i.e. g/kg live weight gain), methane emission in cows fed vitacogen-supplemented diet was 2.4 fold lower than in control diet. The reason for increased live weight gain in replacement dairy cows fed on vitacogen-supplemented diets could not be explained with the parameters measured in the present study. Therefore, the mode of action of the probiotic-vitacogen need to be further elucidated. Probiotic-vitacogen supplementation to growing cows or fattening steers could be beneficial for increased weight gain as well as abatement of methane emission.

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