Methane Emission, Nutrient Digestibility, Energy Metabolism and Blood Metabolites in Dairy Cows Fed Silages with and without Galacto-oligosaccharides Supplementation

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ABSTRACT : This study was conducted to investigate the effect of supplementation of galacto-oligosaccharides (GOS) on methane emission, nutrient digestibility, energy utilization and blood metabolites by Holstein cows fed silages. In two sequential digestion and respiratory trials, two non-lactating Holstein cows were arranged to a balanced incomplete block design. Experimental diets consisted of two silage types: orchardgrass (*Dactylis glomerata* L.) based silage (OS), mixed silage (orchardgrass based silage and alfalfa (*Medicago sativa* L.) silage) (MS), while two GOS levels were without supplementation (0) and 2% of dry matter intake supplementation (2). Four combination diets were OS-0, OS-2, MS-0 and MS-2. Significant effects of silage types and GOS supplementation levels were not observed for DM and OM intake. Whereas the digestibility of OM, NDF and ADF was significantly ($p<0.05$) higher in cows fed OS with and without GOS compared cows fed MS diets. As percentage of GE intake, fecal energy loss for OS diets was significantly ($p<0.05$) declined than for MS diets. In contrast, cows fed MS diets had lower ($p<0.05$) urine energy loss as a proportion of GE intake compared to OS diets. Energy loss as CH_4 and heat production was numerically increased when cows fed both OS and MS with GOS supplementation. Compared to OS, CH_4 emission in cows fed MS was numerically decreased by 10.8 %. Methane conversion ratio (energy loss as CH_4 per unit of GE intake) for OS-0, OS-2, MS-0 and MS-2 were 7.1, 7.2, 6.8 and 7.0, respectively. Plasma of glucose and urea-N concentration were significantly ($p<0.05$) elevated from 1 h to 6 h after feeding, otherwise total protein in plasma was declined ($p<0.01$) at 6 after feeding. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 4 : 534-540)***

Key Words : Galacto-oligosaccharides, Nutrient Digestibility, Energy Utilization, Dairy Cows

INTRODUCTION

Recently, methane (CH_4) production by ruminants has received attention not only due to its effect on substantial loss in efficiency of animal production, but also contributes significantly to greenhouse gases (Mathison et al., 1998). CH_4 is produced as a result of digestible structural carbohydrates fermentation in the rumen by methanogens and is released into the environment by eructation. Furthermore, CH_4 on combustion yield 892.6 kJ (25°C, 1,013 hPa) per mole, and loss of the feed energy is estimated as 7-10% (Takahashi et al., 1997; Takahashi, 2001).

Alfalfa (*Medicago sativa* L.) has been used in many dairy cow rations because it contains high concentration of crude protein (CP) and relatively low concentration of fiber. On the other hand, orchardgrass (*Dactylis glomerata* L.) usually is not considered high quality forage because of high fiber and low CP contents when managed incorrectly. Hence, combination of orchardgrass and alfalfa may be an alternative to improve energy utilization of dairy cow through reducing CH_4 production.

Galacto-oligosaccharides (GOS) are mixture of galactose and glucose that synthesized enzymatically from lactose by the action of β-D-galactosidase derived from *Bacillus circulans* or *Cryptococcus laurentii* (Tanaka and Matsumoto, 1998). GOS is known to promote the growth of bifidobacteria *in vivo* (Bouhnik et al., 1997), and it can be used more readily and selectively by bifidobacteria *in vitro* than other oligosaccharides such as lactulose and raffinose (Sako et al., 1999). Moreover, Gopal et al. (2001) observed *Bifidobacterium lactis* DR10 utilizes tri- and tetra-saccharides of GOS whereas *Lactobacillus rhamnosus* DR20 prefers sugar with a lower degree of polymerization, i.e. disaccharides and monosaccharides.

In the rumen, *Bifidobacterium* and *Lactobacillus* species utilize fructose, galactose, glucose and starch as substrates to produce lactate and acetate (Ogimoto and Imai, 1981). The molar ratio of lactate to acetate in fermentation of glucose by *Bifidobacterium ruminantium* and *Bifidobacterium merycicum* that are isolated from rumen of cattle ranged from 1:2 to 1:5 (Biavati and Mattarelli, 1991). Lactate is intermediate compound of acrylate pathway during propionate production in the rumen. Meanwhile, propionate production is indirect competition with methanogenesis for available hydrogen. By assuming that *Bifidobacterium* and *Lactobacillus* species in the rumen can utilize GOS and produce more lactate, it is expected that

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CH_4 production will reduce. However, the effect of GOS supplementation on CH_4 production by dairy cows has not been well clarified.

The objective of the present study was to determine the effect of GOS supplementation on methane emission, nutrient digestibility, energy utilization and blood metabolites by Holstein cows fed two types of silage.

MATERIALS AND METHODS

Animals and diets

In two sequential digestion and respiratory trials, two non-lactating Holstein cows with an initial body weight 685 and 679 kg were allocated to a balanced incomplete block design. Cows were fed in two equal meal at 08:00 and 16:00 h to meet maintenance level of protein and energy according to Agricultural, Forestry and Fisheries Research Council (1994). Experimental diet was arranged in two-way factorial with two types of silage and with or without GOS supplementation. Type of silage consisted of orchardgrass based silage (OS) and mixed silage (orhardgrass based silage and alfalfa silage) (MS), while GOS treatments were without supplementation (0) and 2% of dry matter (DM) intake (2), based on the previous result of sheep experiment (unpublished). Four combination diets were OS-0, OS-2, MS-0 and MS-2. Ratio of orchardgrass based silage and alfalfa silage was planed 50:50, but due to changes in forage DM, actual mixed silage ratio was 47.1:52.9. The GOS powder used consist of a mixture of saccharides whose formula was Galactose-$(Galactose)_n$-Glucose. $1 \leq n \leq 4$ (monosaccharides 18.3%, disaccharides 38.8%, trisaccharides 23.5%, tetrasaccharides 11.4% and pentasaccharides and higher 4.51%. This powder contained 55% β 1-4 bonds (4`-GOS) (Yakult Central Institute for Microbiological Research, Tokyo, Japan). Chemical composition of silages and the GOS used are presented in Table 1.

Digestion and energy metabolism trials

The trial for each diet was conducted for 14 days with a 9 days adjustment period followed by a 5 days collection period, which included 3 consecutive days for measurement of gaseous exchange. After adjustment period, cows were shifted to respiration chamber. Fresh water and mineral block were freely available at all times. Animals were weighed before and after each collection period.

Table 1. Chemical composition (g/kg DM) of silages and GOS

	OS	MS	GOS
DM (g/kg)	282	288	96
OM	930	911	999
CP	131	136	3
NDF	608	560	ND
ADF	392	413	ND
Hemicellulose	216	147	ND
GE (MJ/kg DM)	20	19	16

ND: Not determined.

Representative sample of silages were taken for three days for each collection period and then dried in the oven 60°C for 48 h. The total amount of feces and urine was separately collected daily and then sampled 10% and 300 ml, respectively. Dried sample of feed and feces were ground to pass through a 1 mm screen for further analyses. The DM content of feed and feces were determined by oven drying at 100±5°C overnight, while organic matter (OM) was determined by using a muffle furnace for 3 h at 550°C. The nitrogen (N) contents of feed, feces and urine were analyzed by using a Kjeltec auto 1035 analyzer (Tecator, Sweden). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were determined using Fibertec 1010 (Tecator, Sweden). Hemicellulose was calculated as difference between NDF and ADF. Bomb calorimeter (Shimadzu CA-4PJ) was used to measure gross energy (GE) content.

Gaseous exchange measurement

The total oxygen (O_2) uptake, and CH_4 and carbon dioxide (CO_2) production were measured by using an open circuit respiration chamber. The respiration chamber was maintained at 18°C and 60% relative humidity throughout the measurement period. During each measurement, air was continuously sampled from the chamber and analyzed for O_2 concentration of with a paramagnetic oxygen analyzer (Horiba, 755A), and CH_4 and CO_2 concentrations with infrared analyzers (Horiba, VIA-510)

Blood and rumen liquor analyses

On the last day of each period, blood samples were collected into heparinized vacuum tubes from jugular vein 1 h and 6 h after feeding. Samples were immediately placed on ice and then centrifuged at 3,000×g for 10 min. Plasma was decanted and stored at -20°C until analysis of total protein, urea N, glucose and non-esterified fatty acids (NEFA) according to technique described by Toharmat et al. (1998).

Samples of rumen liquor were collected 1 h and 6 h after feeding on the last day of each period by using a flexible stomach tube inserted into rumen via the oesophagus. The samples were strained through four layers of cheesecloth and stored at -20°C for further analysis. Concentration of GOS was analyzed by HPLC using a detector (Shodex RI SE-61) and column (8.0×300 mm, Shodex KS 802) maintained at 80°C. The mobile phase was H_2O at a flow rate of 0.5 ml/min.

Calculation and Statistical analysis

Methane gas volume was converted to energy using the conversion factor 39.54 kJ/l (Brouwer, 1965). Heat production was calculated according to Brouwer's equation.

Data were analyzed by the analysis of variance according to balanced incomplete block design (Chakravarti et al., 1967), which period was used as a block and each block contained two cows. Sampling time and interaction of sampling time and diet for blood metabolite parameters were determined using the repeated measures procedure. All statistical analyses described were performed using the general linear model procedure of the SAS (Statistical Analysis Systems Institute Inc., 1990). In the fourth period, gaseous data from one animal was missing due to temperature and humidity fluctuation in the chamber.

RESULTS AND DISCUSSION

Nutrient intake and digestibility

Data on nutrient intake and digestibility by dairy cows are summarized in Table 2. GOS were well accepted by the animals and consumed within 15-20 min after administration. There were no refusals during the experiment because diets offered at maintenance level. Significant effects of silage types and GOS supplementation level were not observed for DM and OM intake. However, their intakes were slightly elevated in OS-2 and MS-2 compared with OS-0 and MS-0.

Digestibility of OM was significantly ($p<0.05$) higher for OS diets compared with MS diets. Similar trend also observed in GOS degradation in the rumen that GOS degraded in OS-2 and MS-2 was 96.9 vs 88.4% for 1 h after feeding and 100 vs 99.3% for 6 h after feeding, respectively. A reason for a higher OM digestibility in cows fed OS diets might have been related to lower rate of passage, hence retention time in the digestive tract for OS diets was longer than for MS diets. Although DM and OM digestibility were not significantly affected by GOS supplementation, cows fed OS-2 and MS-2 tended to be higher than those fed OS-0 and MS-0. It may be due to increased activities of rumen microbes particularly *Bifidobacterium* as contributed from supplementation of GOS, which is composed by galactose and glucose. Lee et al. (1980) reported *Bifidobacterium* species constitute galactokinase, hexose 1-phosphate uridylyltransferase and UDP-galactose-4-epimerase, which are enzymes in conventional pathway of galactose metabolism. Moreover, sugars consisting of galactose, glucose and fructose were fully utilized by bifidobacteria (Minami et al., 1985).

The greater ($p<0.05$) hemicellulose intake in cows fed OS diets than those fed MS diets (1.89 vs 1.20 kg/d) was probably corresponding to relatively higher hemicellulose digestibility in OS than MS diets. Moreover, higher hemicellulose digestibility in OS could be due to highly potential digestibility of hemicellulose in this diet. Cows fed on OS diets had significantly higher NDF digestibility ($p<0.05$) and ADF digestibility ($p<0.01$) compared to cows fed on MS diets. It may be due to fiber fractions for OS diets more digestible than for MS diets, as reported by Nadeau et al. (1996) digestible NDF in orchardgrass and alfalfa silages were 378 and 197 g/kg of DM, respectively. In addition, Weiss and Shockey (1991) demonstrated intake of undigested NDF was higher by cows fed alfalfa silage than by those fed orchardgrass silage.

Energy utilization

The respiratory O_2 and CO_2 are shown in Table 4. The O_2 uptake for cows fed OS diets tended to be higher than MS diets (averaging 3,124.4 vs 2,937.9 l/d). The O_2 uptake by dairy cows tended to increase when GOS was supplemented in either OS or MS. These results shows

Table 2. Nutrient intake and nutrient digestibility of dairy cows fed silages with and without GOS supplementation

	Diets				S.E.[1]	Significance		
	OS-0	OS-2	MS-0	MS-2		Silage	GOS	Int.[2]
Intake (kg/d)								
DM	8.53	8.94	8.23	8.32	0.33	ns[3]	ns	ns
OM	7.96	8.30	7.50	7.57	0.32	ns	ns	ns
CP	1.06	1.20	1.17	1.07	0.04	ns	ns	ns
NDF	5.34	5.24	4.49	4.63	0.30	ns	ns	ns
ADF	3.45	3.36	3.31	3.42	0.13	ns	ns	ns
Hemicellulose	1.89	1.89	1.18	1.21	0.18	*	ns	ns
Digestibility (%)								
DM	61.9	63.4	58.7	60.1	1.2	ns	ns	ns
OM	64.0	65.2	59.7	61.0	1.2	*	ns	ns
CP	60.0	64.4	60.3	58.1	1.5	ns	ns	ns
NDF	65.0	63.4	52.8	54.6	2.4	*	ns	ns
ADF	62.0	61.4	50.3	50.9	2.2	**	ns	ns
Hemicellulose	70.7	67.2	60.8	64.8	2.9	ns	ns	ns

[1] SE: Standard error of least-square means.
[2] Interaction of silage and GOS.
[3] ns: Not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$.

oxygen uptake by dairy cows attributed to DM intake. As reported by Takahashi et al. (1997) that oxygen uptake is generally affected by amount of feed intake, which results in an increased heat increment due to enhanced muscular movements to ingest more feed. Profile of the rate of oxygen uptake in all experimental diets was relatively constant during 8 h observation, ranged from 1.8 to 2.6 l/min (Figure 2). Figure 3 shows supplementation of GOS in either OS or MS had tendency to increase CO_2 production. The peak CO_2 production in all experimental diets was attained 1 h after feeding.

In sequence, energy balance and energy partition by cows fed silages at two levels of GOS supplementation are summarized in Table 3. There were no significant difference in GE intake, fecal energy and digestible energy (DE) between silages and level of GOS supplementation. As percentage of GE intake, fecal energy loss for OS diets was significantly ($p<0.05$) declined than for MS diets. The lower energy loss as feces in OS diets was due to higher energy digestibility, as reported by Agricultural Research Council (1980) increased energy loss in feces and declined apparent energy digestibility proportional as increasing the feeding level of ruminants. In contrast, cows fed MS-0 and MS-2 had lower ($p<0.05$) urine energy loss as a proportion of GE intake compared to OS-0 and OS-2. This proportion in all diets ranging 4.1 to 4.7%, agreed with previous finding (Blaxter and Wainman, 1964) that urine energy loss was not more than 5% of GE intake in sheep and cattle. Energy digestibility (DE/GE) was significantly ($p<0.05$) changed by type of silage, 62.8 and 58.2 MJ respectively for OS and MS diets. However supplementation of GOS in both OS and MS was slightly increased percentage of DE as GE intake, whereas fecal energy and urine energy losses as percentage GE intake were slightly decreased. Energy losses as CH_4 and heat production were numerically increased when cows fed both OS and MS with GOS supplementation. Methane conversion ratio (energy loss as CH_4 per unit of GE intake) for OS-0, OS-2, MS-0 and MS-2 were 7.1, 7.2, 6.8 and 7.0, respectively. Compared to OS, CH_4 production in cows fed MS was markedly decreased by 10.8%, suggesting that combination between orchardgrass based silage and alfalfa silage may be an alternative to reduce energy loss as CH_4 in dairy cows.

Methane emission

The CH_4 emission in dairy cows is summarized in Table 4, while its diurnal change after afternoon feeding is shown in Figure 1. Data of CH_4 for MS-2 were mean of three days measured for one cow. Cows fed both silages with GOS supplementation had higher CH_4 production than those fed silage without GOS supplementation. However, this result led us to reject our hypothesis before experiment that supplementation of GOS in silages diet may reduce CH_4 emission. The higher CH_4 emission in OS-2 and MS-2 probably due to the amount of GOS supplemented was not enough to promote the growth of *Bifidobacterium* species in the rumen. Thus, lactate production by *Bifidobacterium* was not increased as expected in the present study. Trovatelli and Matteuzzi (1976) indicated that bifidobacteria proliferate in the rumen, especially when calves are fed starch-rich diet. Additionally, *Bifidobacterium globosum* and *Bifidobacterium ruminale* were absent or present in very low numbers in calves fed high roughage, whereas with concentrate ration their number was high, usually in order of 10^8 to 10^9/ml of rumen fluid.

Table 3. Energy balance and energy partition of dairy cows fed silages with and without GOS supplementation

	Diets				S.E.[1]	Significance		
	OS-0	OS-2	MS-0	MS-2		Silage	GOS	Int.[2]
Gross energy intake (MJ)	168.5	175.9	157.0	155.2	6.9	ns[3]	ns	ns
Fecal energy (MJ)	63.9	63.8	66.1	64.3	2.1	ns	ns	ns
FE/GE (%)	38.0	36.5	42.2	41.4	1.0	*	ns	ns
Digestible energy (MJ)	104.6	112.1	90.9	90.8	5.8	ns	ns	ns
DE/GE (%)	62.0	63.5	57.8	58.6	1.0	*	ns	ns
Urinary energy (MJ)	7.8	8.1	7.0	6.4	0.2	**	ns	ns
UE/GE (%)	4.7	4.6	4.5	4.1	0.1	*	ns	ns
Methane energy (MJ)	12.0	12.6	10.7	11.7[4]	-	-	-	-
Metabolizable energy (MJ)	84.8	91.5	73.1	79.1	-	-	-	-
Heat production (MJ)	65.4	73.2	61.8	65.4	-	-	-	-
Retained energy (MJ)	19.4	18.3	11.1	13.7	-	-	-	-

[1] SE: Standard error of least-square means.
[2] Interaction of silage and GOS.
[3] ns: Not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$.
[4] Means of three days observation for one cow.

Table 4. Methane emission and respiratory gaseous exchange of dairy cows fed silages with and without GOS supplementation

	Diets			
	OS-0	OS-2	MS-0	MS-2
CH_4 emission (l/d)	303.5	318.9	271.6	295.6[1]
O_2 uptake (l/d)	3,000.2	3,248.6	2,862.7	3,013.2[1]
CO_2 production (l/d)	3,586.7	4,368.4	3,362.1	3,564.7[1]

[1]Means of three days observation for one cow.

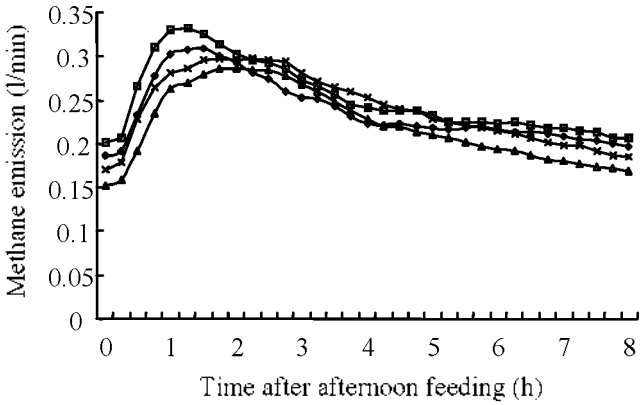

Figure 1. Diurnal changes in methane emission by dairy cows fed OS-0 (◊), OS-2 (□), MS-0 (Δ) and MS-2 (×).

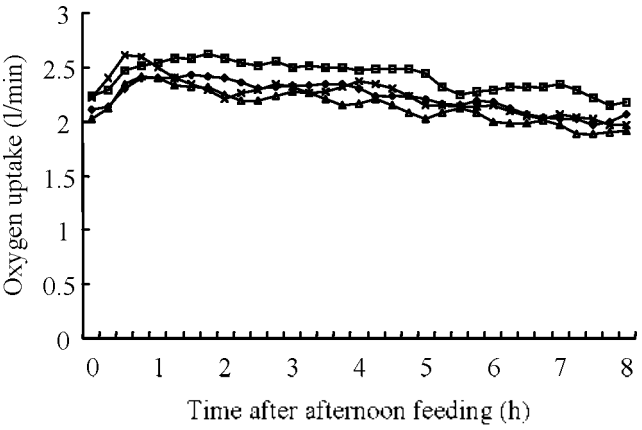

Figure 2. Diurnal changes in oxygen uptake by dairy cows fed OS-0 (◊), OS-2 (□), MS-0 (Δ) and MS-2 (×).

On the other hand, increasing ciliate population by GOS may be a plausible explanation for higher CH_4 production in cows fed OS-2 and MS-2. Bonhomme (1990) found that an addition of readily fermentable carbohydrate to diets fed at maintenance levels causes a proliferation in the ciliate population. Moreover, Stumm et al. (1982) and Tokura et al. (1997) suggested that about 10 to 20% of methanogens are attached on the surfaces of ciliate protozoa and the apparent methane production by ciliates depend primarily on the number of methanogens associated with them. The higher CH_4 emission in cows fed OS diets than those fed MS diets

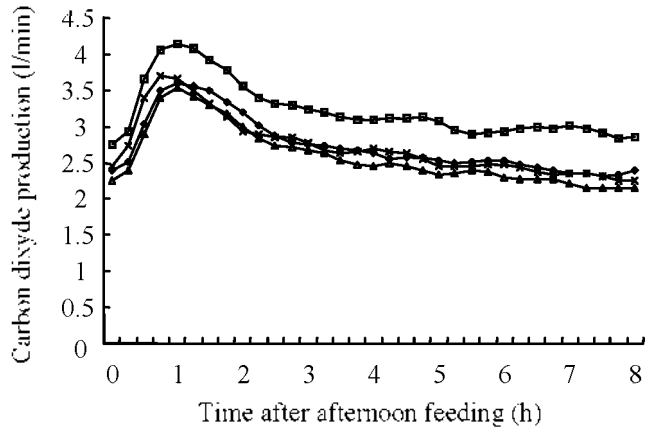

Figure 3. Diurnal changes in carbon dioxide production by dairy cows fed OS-0 (◊), OS-2 (□), MS-0 (Δ) and MS-2 (×).

caused by higher hemicellulose content and higher potentially digestible of hemicellulose in OS. It has been reported that the main substrate of methane is digestible structural carbohydrate (cell wall constituents) such as hemicellulose (Takahashi, 2001). Hemicellulose content in OS and MS was 608 g/kg DM and 560 g/kg DM (Table 1), while potentially digestible hemicellulose was 146 g/kg DM for OS and 40 g/kg DM for MS (Nadeau et al., 1996). Diurnal pattern of CH_4 emission among cows were similar in OS-0, OS-2, MS-0 or MS-2 (Figure 1). The peak CH_4 emission in cows fed OS diets was observed 1 to 1.5 h after feeding, while cows fed MS diets at 1.5 to 2 h after feeding. However, differences in peak CH_4 emission between OS and MS could probably be due to difference in lag time before hemicellulose digestion of both diets. Nadeau et al. (1996) reported a slightly longer time before hemicellulose digestion of alfalfa silage than orchardgrass silage (2.9 vs 2.6 h). Thus, peak CH_4 production in cows fed OS diets was faster than those fed MS diets.

Blood metabolites

Concentration of plasma glucose 6 h after feeding was significantly ($p<0.05$) higher than that 1 h after feeding (Table 5), and their concentration 1 h after feeding was slightly higher in cows fed silage with GOS supplementation compared to without GOS supplementation. Dhiman et al. (1991) pointed out that an increase in the proportion of concentrate in the diet often resulted in higher blood glucose concentration. While, Toharmat et al. (1998) observed that plasma glucose concentration in dairy cows fed at 1.2 times of maintenance plus last 2 month of gestation level of TDN was significantly increased than those fed at maintenance level. Hence, relatively higher plasma glucose 1 h after feeding might be affected by supplementation of GOS. Plasma glucose in the present study was above normal range concentration in lactating and non-lactating cows from 36.5

Table 5. Plasma of glucose, urea-N, total protein and NEFA concentration 1 h and 6 h after feeding of dairy cows fed silages with and without GOS supplementation

	Diets								S.E.[1]	Significance			
	OS-0		OS-2		MS-0		MS-2			Silage	GOS	Int.[2]	Time
	1 h	6 h	1 h	6 h	1 h	6 h	1 h	6 h					
Glucose (mg/dl)	58.2	72.8	60.1	73.7	65.9	69.3	67.7	66.5	2.9	ns[3]	ns	ns	*
Urea-N (mg/dl)	10.6	11.4	11.1	13.4	12.2	13.6	12.0	12.4	0.5	ns	ns	ns	*
Protein (g/dl)	7.4	6.9	7.0	6.9	7.0	6.5	7.1	6.7	0.1	ns	ns	ns	**
NEFA (mEq/l)	0.04	0.08	0.06	0.08	0.07	0.07	0.07	0.06	0.01	ns	ns	ns	ns

[1] SE: Standard error of least-square means.
[2] Interaction of silage and GOS.
[3] Ns: Not significant ($p>0.05$); * $p<0.05$; ** $p<0.01$.

to 53.5 mg/100 ml (Rowlands et al., 1974). Lactate and propionate formed in the rumen from carbohydrates fermentation is absorbed into the bloodstream and converted into glucose by the liver. furthermore their concentration in plasma might serve as an indicator of energy status of dairy cows (Coggins and Field, 1976).

Plasma urea N was increased ($p<0.05$) from 1 to 6 h after feeding, otherwise total protein in plasma was declined ($p<0.01$) 6 h after feeding (Table 5). These results agreed with the previous finding (Coggins and Field, 1976; Gustafsson and Palmquist, 1993) that plasma urea nitrogen concentration increased after feeding and the peak occurred 2 to 4 h after feeding. In the present study, plasma urea nitrogen concentration both 1 h and 6 h after feeding were still in normal concentration as suggested by Rowlands et al. (1974) that normal range of blood urea N concentration in lactating and non-lactating cows were 9.5 to 19.5 mg/100 ml. In addition, plasma total protein and urea N concentration are indicators of protein status of lactating cows (Coggins and Field, 1976).

Plasma NEFA was not influenced by silage, GOS supplementation or sampling time. In the present study, plasma NEFA concentration was relatively constant, ranged 0.04 to 0.08 mEq/l, which is lower than values of previous findings (Coggins and Field, 1976; Toharmat et al., 1998). Pethick and Dunshea (1993) suggested that glycerol and NEFA entry into the plasma pool reflect lypolysis and fat mobilization respectively. However, lower plasma NEFA concentration in the present study suggested to be efficient utilization of dietary energy and low body fat mobilization.

CONCLUSION

This study showed that supplementation of 2% galacto-oligosaccharides in DM fed had no effects on methane emission, nutrient digestibility, energy metabolism or blood metabolites by Holstein cows fed silages. The manipulating effect of galacto-oligosaccharides on these metabolic parameters including methane emission remain to be elucidated quantitatively. In comparison among diets, orchardgrass based silage had higher nutrient digestibility (OM, NDF and ADF) and energy digestibility as a result reducing energy losses in feces. The combination between orchardgrass based silage and alfalfa silage reduced energy loss as methane, thus it is possible to reduce methane emission to environment.

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