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Effects of dietary forage-to-concentrate ratio on nutrient digestibility and enteric methane production in growing goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*)

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Objective: Two experiments were conducted to determine the effects of forage-to-concentrate (F:C) ratio on the nutrient digestibility and enteric methane (CH_4) emission in growing goats and Sika deer.
Methods: Three male growing goats (body weight [BW] = 19.0±0.7 kg) and three male growing deer (BW = 19.3±1.2 kg) were respectively allotted to a 3×3 Latin square design with an adaptation period of 7 d and a data collection period of 3 d. Respiration-metabolism chambers were used for measuring the enteric CH_4 emission. Treatments of low (25:75), moderate (50:50), and high (73:27) F:C ratios were given to both goats and Sika deer.
Results: Dry matter (DM) and organic matter (OM) digestibility decreased linearly with increasing F:C ratio in both goats and Sika deer. In both goats and Sika deer, the CH_4 emissions expressed as g/d, g/kg $BW^{0.75}$, % of gross energy intake, g/kg DM intake (DMI), and g/kg OM intake (OMI) decreased linearly as the F:C ratio increased, however, the CH_4 emissions expressed as g/kg digested DMI and OMI were not affected by the F:C ratio. Eight equations were derived for predicting the enteric CH_4 emission from goats and Sika deer. For goat, equation 1 was found to be of the highest accuracy: CH_4 (g/d) = 3.36+4.71×DMI (kg/d)–0.0036×neutral detergent fiber concentrate (NDFC, g/kg)+0.01563×dry matter digestibility (DMD, g/kg)–0.0108×neutral detergent fiber digestibility (NDFD, g/kg). For Sika deer, equation 5 was found to be of the highest accuracy: CH_4 (g/d) = 66.3+27.7×DMI (kg/d)–5.91×NDFC (g/kg)–7.11×DMD (g/kg)+0.0809×NDFD (g/kg).
Conclusion: Digested nutrient intake could be considered when determining the CH_4 generation factor in goats and Sika deer. Finally, the enteric CH_4 prediction model for goats and Sika deer were estimated.

Keywords: *Capra hircus hircus*; *Cervus nippon hortulorum*; Methane; Greenhouse Gas; Forage-to-concentrate Ratio

INTRODUCTION

Methane (CH_4) production by enteric fermentation in ruminants is recognized as one of the major sources of greenhouse gas emissions worldwide [1]. Besides, the enteric CH_4 represents an energy loss, ranging from 2% to 12% of the gross energy intake, for an animal [2]. Generally, the enteric CH_4 production by ruminants is affected by various dietary factors such as the level of intake [3], carbohydrate type [4,5], forage processing [6], fat addition [7], and ionophore addition [8]. Moreover, the forage-to-concentrate (F:C) ratio in diets affects nutrient digestibility and enteric CH_4 emission in many ruminants [9-11]. Although cattle and buffalo produce maximum greenhouse gases, 4.4% of the total greenhouse gas emissions from the livestock sector is contributed by the goats worldwide [12]. In addition, a large number of Sika deer inhabit East Asian areas or are domesticated in some of those areas [13]. Many studies have been conducted for measuring the emission of enteric CH_4 from dairy cattle, beef cattle, or sheep; however, only a few studies have been conducted for goats and Sika deer.

Mathematical models have been developed for predicting the enteric CH_4 production in ruminants [3,14,15]. Although the models based on databases taken from different studies for enteric CH_4 emission from goats were already developed [16], to our knowledge, the model for enteric CH_4 emission from deer did not exist. Therefore, the objective of the present study was to determine the effects of the F:C ratio on the nutrient digestibility and enteric CH_4 emission from growing goats and Sika deer as well as to derive the equations for CH_4 production.

MATERIALS AND METHODS

Two experiments were conducted to determine the nutrient digestibility and emission of enteric CH_4 and CO_2 in goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*). We performed the experimental procedures in accordance with the Institutional Animal Care and Use Committee of Konkuk University.

Animals, diets, and experimental design

Three growing male goats with initial body weight (BW) of 19.0±0.7 kg and three growing male deer with initial BW of 19.3±1.2 kg were used. Experiments were conducted in an environmentally controlled room (20°C±3°C). Each animal was housed individually in a respiration-metabolism chamber described by Li et al [17]. Experimental diets based on 2% of initial BW (dry matter [DM] basis) were fed daily at 1100 h. Water and mineral blocks were provided at all times. Orts were removed daily and weighed at 1000 h for DM intake calculation. Fecal samples were collected everyday by using the total collection method, dried immediately, and stored at –20°C for subsequent chemical analysis. Three experimental diets were prepared for both goat and deer experiments (Table 1). The dietary treatments included low (25:75), moderate (50:50), and high (73:27) F:C ratios. The experimental design consisted of a 3×3 Latin square design with a diet adaptation period of 7 d and a data collection period of 3 days. Adaption period was according to literature reference [18,19]. The animals were weighed at the beginning of each period.

Table 1. Ingredients and nutrient composition of experiment diets

Items	Forage to concentrate ratio		
	25:75	50:50	73:27
Ingredients (%, DM basis)			
Ground corn	53.3	25.5	-
Soybean meal	21.7	24.5	27.0
Tall fescue, hay	25.0	50.0	73.0
Nutrient composition			
DM (%)	90.6	89.2	87.8
OM (% DM)	93.5	92.8	92.2
CP (% DM)	17.0	17.0	17.0
NDF (% DM)	26.7	42.8	57.7
GE (MJ/kg DM)	18.7	18.7	18.7

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; GE, gross energy.

Chemical analysis

All ingredients and fecal samples were analyzed in duplicate for DM, organic matter (OM), crude protein (CP), and ether extract (EE) as described by AOAC [20]. The contents of neutral detergent fiber (NDF) were analyzed using heat stable α-amylase (Sigma A3306; Sigma Chemical Co., St. Louis, MO, USA) according to the method described by Van Soest et al [21]. Gross energy (GE) was determined using a bomb calorimeter (C5000; IKA, Staufen, Germany).

Gas production measurement

The CH_4 and carbon dioxide (CO_2) production were measured using a respiration-metabolism chamber system [17]. A recovery test was performed before each period using standard CH_4 gas (1.67%, v/v). Inlet and outlet gases were measured by a gas flow meter (GFM57, Aalborg Instruments & Controls Inc., Orangeburg, NY, USA); a sample pump (Columbus Instruments, Columbus, OH, USA) was used to collect gas samples. The gas samples were passed through a desiccant composed of calcium sulfate ($CaSO_4$), before the samples flew into the gas analyzer. Non-dispersive infrared gas analyzer (VA-3000; Horiba Stec Co., Kyoto, Japan) was used to analyze the CH_4 and CO_2 concentrates.

Statistical analysis

The data were analyzed using SAS PROC MIXED (Version 9.2; SAS Institute Inc., Cary, NC, USA). The model considered the diet as the fixed effect and both animals and periods as the random effects. Orthogonal contrasts for linear and quadratic effects were performed with polynomials determined by SAS PROC IML (Version 9.2; SAS Institute Inc., USA). All data were presented as the least squares means. Treatment effects were considered significant at $p<0.05$, and trends were considered at $0.05 \le p<0.10$. The SAS PROC REG (Version 9.2; SAS Institute Inc., USA) was used for estimating the simple and multiple linear equations. Equations were evaluated on the basis of root mean square error (RMSE), adjusted-R^2, and p-value.

RESULTS AND DISCUSSION

The DM and OM digestibility of goats decreased linearly ($p<0.01$) as the F:C ratio increased (Table 2). The DM, OM, and CP digestibility of Sika deer also decreased linearly ($p<0.01$) as the F:C ratio increased. An increase in the F:C ratio decreases the DM and OM digestibility for other ruminants, such as a cow [22] and sheep [23,24], because the forage has a generally higher NDF content than the concentrate. As structural carbohydrates (e.g. NDF) are usually less digestible than non-fiber carbohydrates, the total digestibility decreases with increasing proportions of

Table 2. Effect of forage to concentrate ratio on nutrient digestibility in goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*)

Digestibility (%)	Forage to concentrate ratio			SEM	p-value	
	25:75	50:50	73:27		Linear	Quadratic
Goats						
DM	78.1	68.0	58.5	2.0	< 0.001	0.983
OM	79.8	69.0	58.6	2.0	0.002	0.954
CP	80.5	78.6	77.2	2.1	0.308	0.948
NDF	47.1	45.8	45.5	3.4	0.726	0.906
Sika deer						
DM	76.9	63.8	54.5	0.8	< 0.001	0.194
OM	78.2	64.7	55.0	0.8	< 0.001	0.212
CP	76.8	73.2	72.2	1.3	0.028	0.368
NDF	44.1	38.2	40.1	2.5	0.278	0.253

SEM, standard error of the mean; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber.

forage in the diet [4]. In agreement with previously reported results in other studies on goats [25,26] or deer [27], in the present study, the DM and OM digestibility decreased ($p<0.01$) with increasing F:C ratios. As the NDF digestibility of goats and Sika deer were not significantly affected by the F:C ratios, their DM digestibility decreased with increasing F:C ratios.

In goats and Sika deer, the enteric emission of CH_4 expressed as g/d, g/kg $BW^{0.75}$, % of gross energy intake (GEI), g/kg DMI, and g/kg OMI decreased linearly ($p<0.05$) with increasing F:C ratios (Table 3). However, no difference was observed in enteric CH_4 production expressed as g/kg digested dry matter intake (DDMI) and g/kg digested organic matter intake (DOMI) in both goats and Sika deer. In goats, the CO_2 production expressed as g/kg $BW^{0.75}$ decreased linearly ($p<0.05$) with increasing F:C ratio, and there was a tendency ($p = 0.078$) for a decrease in the CO_2 production expressed as g/d. The emission of enteric CO_2 by Sika deer decreased linearly ($p<0.05$) as the F:C ratio increased. In contrast with the current results of the goats and Sika deer, the high forage diets generally increased the CH_4 production in beef [11] and dairy [9,28] cattle as well as in the modeling [14,15] and batch culture [29] studies. Structural carbohydrate-rich diet causes greater production of enteric CH_4 than non-fiber carbohydrate-rich diet in dairy cows [4] because the diet containing large amounts of non-fiber carbohydrates derives propionate production in the rumen, thereby inhibiting rumen methanogen growth [30]. However, some studies for goats showed that the dietary F:C ratio did not affect CH_4 (g/d) emission [10,31]. According to the morpho-physiological classification of Hofmann [32], goats and Sika deer were intermediate type and concentrate eaters, respectively, whereas cattle and sheep were grass/roughage eaters. As the stomach of concentrate eaters or intermediate types has a lesser capacity, larger opening, faster passage rate, and shorter retention time than grass/roughage eaters [32], more indigested forage contents in the rumino-reticulum pass toward the omasum in concentrate eaters or intermediate types than in grass/roughage eaters. In the current study, the NDF digestibility (NDFD) was similar among the treatments in both goats ($p = 0.726$) and Sika deer ($p = 0.278$), whereas the DM digestibility showed a significant difference ($p<0.001$). For this difference, the low F:C ratio diet may generate more enteric CH_4 than the high F:C ratio diet in goats and Sika deer because large amount of energy sources for rumen microbes was available in the low F:C ratio diet than the high F:C ratio diet. It has been widely recognized that the diversity of rumen bacteria community could vary with animal species

Table 3. Effect of forage to concentrate ratio on enteric methane and carbon dioxide in goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*)

Items	Forage to concentrate ratio			SEM	p-value	
	25:75	50:50	73:27		Linear	Quadratic
Goats						
CH_4 production						
CH_4 (g/d)	10.7	9.2	8.0	0.5	0.008	0.869
CH_4 (g/kg $BW^{0.75}$)	1.2	1.0	0.9	0.1	0.034	0.977
CH_4 (% of GEI)	8.6	7.3	6.0	0.5	0.032	0.934
CH_4 (g/kg DMI)	29.1	24.6	20.2	1.7	0.032	0.934
CH_4 (g/kg DDMI)	37.1	36.4	34.5	2.7	0.474	0.828
CH_4 (g/kg OMI)	31.1	26.5	21.9	1.8	0.035	0.916
CH_4 (g/kg DOMI)	38.9	38.6	37.2	2.8	0.638	0.848
CO_2 production						
CO_2 (g/d)	376	357	337	14	0.078	0.941
CO_2 (g/kg $BW^{0.75}$)	42.3	39.6	36.1	1.4	0.011	0.659
Sika deer						
CH_4 production						
CH_4 (g/d)	10.1	9.6	7.2	1.0	0.002	0.051
CH_4 (g/kg $BW^{0.75}$)	1.1	1.0	0.8	0.04	0.006	0.158
CH_4 (% of GEI)	7.8	7.2	5.7	0.3	0.002	0.162
CH_4 (g/kg DMI)	26.1	24.3	19.0	0.9	0.002	0.162
CH_4 (g/kg DDMI)	33.9	38.1	35.1	1.8	0.654	0.165
CH_4 (g/kg OMI)	27.9	26.1	20.7	1.0	0.002	0.156
CH_4 (g/kg DOMI)	35.7	40.4	37.7	1.9	0.470	0.167
CO_2 production						
CO_2 (g/d)	432	403	341	24	0.003	0.212
CO_2 (g/kg $BW^{0.75}$)	47.4	43.7	37.8	2.9	0.013	0.321

SEM, standard error of the mean; CH_4, methane; BW, body weight; GEI, gross energy intake; DMI, dry matter intake; DDMI, digested dry matter intake; OMI, organic matter intake; DOMI, digested organic matter intake; CO_2, carbon dioxide.

Table 4. Equations for enteric methane emission from goats (*Capra hircus hircus*) and Sika deer (*Cervus nippon hortulorum*)

Items	Equations	Statistical parameters		
		R^2	RMSE	p-value
Goats				
1	CH_4 (g/d) = 3.36(±5.96)+4.71(±14.1)×DMI (kg/d)–0.0036(±0.0021)×NDFC (g/kg)+0.01563(±0.0040)×DMD (g/kg)–0.0108(±0.0070)×NDFD (g/kg)	0.85	0.74	0.059
2	CH_4 (g/d) = 4.81(±6.67)–6.66(±13.7)×DMI (kg/d)–0.0027(±0.0022)×NDFC (g/kg)+0.0119(±0.0036)×DMD (g/kg)	0.76	0.84	0.051
3	CH_4 (g/d) = 19.6(±8.16)–23.7(±20.8)×DMI (kg/d)–0.0030(±0.0037)×NDFC (g/kg)	0.24	1.38	0.445
4	CH_4 (g/d) = 17.8(±7.71)–22.45(±20.2)×DMI (kg/d)	0.15	1.35	0.304
Sika deer				
5	CH_4 (g/d) = 66.3(±24.6)+27.7(±4.48)×DMI (kg/d)–5.91(±2.12)×NDFC (g/kg)–7.11(±3.07)×DMD (g/kg)+0.0809(±0.0888)×NDFD (g/kg)	0.96	0.54	0.004
6	CH_4 (g/d) = 48.3(±14.4)+24.8(±3.09)×DMI (kg/d)–4.30(±1.14)×NDFC (g/kg)–4.73(±1.56)×DMD (g/kg)	0.95	0.53	0.001
7	CH_4 (g/d) = 4.81(±1.91)+20.3(±4.18)×DMI (kg/d)–0.882(±0.216)×NDFC (g/kg)	0.88	0.82	0.002
8	CH_4 (g/d) = 0.820(±2.95)+21.0(±7.51)×DMI (kg/d)	0.53	1.48	0.027

RMSE, root mean square error; CH_4, methane; DMI, dry matter intake; NDFC, neutral detergent fiber concentrate; DMD, dry matter digestibility; NDFD, neutral detergent fiber digestibility.

[33] or geographical region of host animal [34]. As the enteric CH_4 production is correlated with rumen microbial community structure [35], these results which comparing to bovine may be explained by rumen bacteria diversity. In addition, the restriction of experimental diets (2% of initial BW) might affect the energy balance of microorganisms in rumen. Interestingly, the results showed that in both goats and deer, the enteric CH_4 production expressed as g/kg DDMI and g/kg DOMI was not affected by the F:C ratio. Although several studies [36,37] suggested that the nutrient digestibility did not related to enteric CH_4 production, in general, the nutrient digestibility could play an important role in the enteric CH_4 production of ruminants [38]. Therefore, digested nutrient intake, which was available for digestion by rumen microorganisms, could be considered when determining the CH_4 generation factor in goats and Sika deer.

For goats, equation 1, which used the DMI, NDF concentrate (NDFC), DM digestibility (DMD), and NDFD as independent variables, showed the highest accuracy (Table 4; R^2 = 0.85, RMSE = 0.74, and p = 0.059). For Sika deer, equation 5, which used the DMI, NDFC, DMD, and NDFD as independent variables, showed the highest accuracy (R^2 = 0.96, RMSE = 0.54, and p = 0.004). For goats, equation 3 that did not use the digestibility factors as variables showed low accuracy (R^2 = 0.24, RMSE = 1.38, and p = 0.45), whereas, for Sika deer, equation 7 that did not use digestibility factors as variables showed a relatively high accuracy (R^2 = 0.88, RMSE = 0.54, and p = 0.001). The digestibility factors are usually more difficult to measure than the DMI and nutrient concentrate. Therefore, in practice, the model composed without the digestibility is more useful. Although the extant models based on the database organized from different studies on the emission of enteric CH_4 by goats have already been developed [16], to our knowledge, the model for the emission of enteric CH_4 by deer was not available. Thus, although the models for deer were organized from the limited database, these models will partially help to estimate the enteric CH_4 emission from Sika deer.

CONCLUSION

In goats and Sika deer, the F:C ratio decreases the nutrient digestibility and the enteric CH_4 emissions expressed as g/d, g/kg $BW^{0.75}$, % of GEI, g/kg DMI, and g/kg OMI; however, the enteric CH_4 emissions expressed as g/kg DDMI and g/kg DOMI were not affected. Therefore, digested nutrient intake, which was available for digestion by rumen microorganisms, could be considered when determining the CH_4 generation factor in goats and Sika deer. In addition, as the model for enteric CH_4 emission from Sika deer did not exist, the equations that were derived in this study will partially help to estimate the enteric CH_4 emission from Sika deer.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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